For nearly 100 years MACHEREY-NAGEL has been dedicated to products for filtration, water analysis and chromatography. Due to this long tradition we have been strongly involved in the development of chromatography and always one of the pioneers.

Since we introduced CHROMABOND® in 1987 we developed the widest range of phases and products for solid phase extraction based on silica and polymer materials. These build a perfect match with all our products for HPLC, GC and TLC and offer solutions for the whole process of analytics. Aside from all SPE standard products we are specialised on custom made solutions for any analytical problem.

This application guide is based on our own experiences in sample preparation and applications which were kindly provided or published by our customers. We are always interested in ongoing research and developments, and are more than happy to expand this database also with your results and applications. We thank everyone who contributed to this guide. Please feel free to contact us for any questions, ideas, technical requests or feedback.

www.mn-net.com

Contents

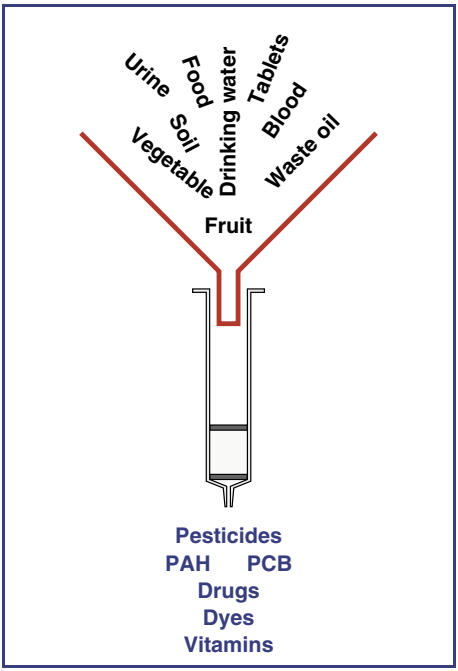
Solid Phase Extraction

Application Gallery

Appendices

Principles of solid phase extraction

Solid phase extraction (SPE) is a powerful method for sample preparation and is used by most chromatographers today. It has capabilities in a broad range of applications such as environmental analyses, pharmaceutical and biochemical analyses, organic chemistry and food analyses.



The advantages of SPE compared to classical liquid-liquid extraction are the low solvent consumption, the enormous time saving and the potential for automation. Additionally, a sample preparation task can often be solved more specifically by using SPE, since different interactions of the analyte with the solid phase (adsorbent) are possible, and methods can be optimised by adjusting chromatographic conditions. SPE offers a multitude of adsorbents for polar, hydrophobic and/or ionic interactions, while liquid-liquid extraction is limited to partition equilibriums in the liquid phase.

The CHROMABOND® columns and CHROMAFIX® cartridges from MN which have been developed especially for SPE provide rapid, economical and effective systems for sample preparation. They can be used to process samples for HPLC, GC, TLC, UV or IR spectroscopy and many more. For the increasingly sensitive chromatographic analyses good sample preparation is essential, because it protects the chromatographic columns, and it allows a greater sensitivity by removal of interfering matrix components. A selective and specific sample preparation thus is a prerequisite for reasonable, economical and sensitive analyses.

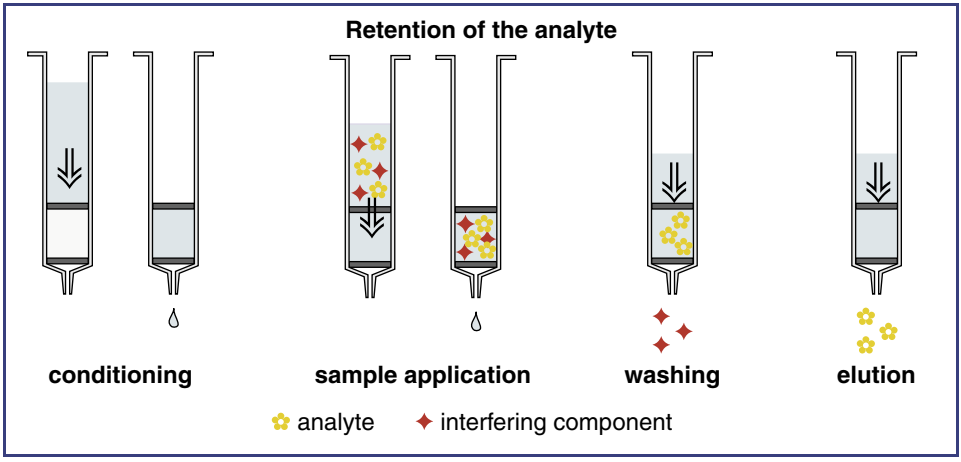
The main objectives of SPE are removal of interfering matrix components and selective concentration and isolation of the analytes. Enrichment can increase the detection sensitivity by a factor of 100 to 5000. Often this step is necessary to reach the concentration limit of detection for the analytes of interest for qualitative or quantitative analyses, i.e. without enrichment often a reliable analysis at trace level is not possible.

In order to maintain the high quality of our CHROMABOND® columns and CHROMAFIX® cartridges and thus to ensure reproducible extraction results, CHROMABOND® adsorbents are subject to defined, stringent control criteria. Our quality control from the starting materials through all intermediate steps up to the final product guarantees consistent quality.

In general, SPE can be used for three important purposes in up-to-date analyses:

- concentration of the analyte
- removal of interfering substances
- changing the matrix of the analyte as needed for subsequent analyses

In most cases these three effects occur together. Since analytes can be either adsorbed on the SPE packing material or directly flow through while the interfering substances are retained, two general separation procedures are possible. The first case is shown in the figure below.

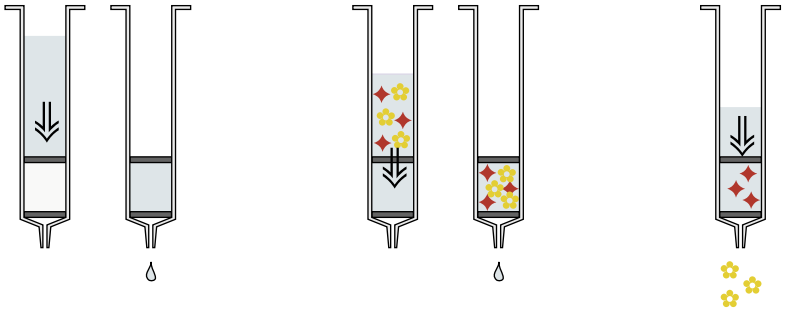


Principles of solid phase extraction

The sample is pressed or drawn through the solid phase, and the analyte molecules are enriched on the adsorbent. Interfering components and solvent molecules (matrix) are not retained. Then remaining interfering components are washed from the adsorbent with a suitable washing solution. Finally, the analyte is removed from the adsorbent by elution with a suitable solvent.

The considerations made above indicate that an optimum SPE presents a poor column-chromatographic separation. If in chromatography substances run at the solvent front, or if substances are adsorbed at the column head, efficient chromatography is not possible using one eluent; one can call this "digital chromatography".

Retention of interfering components



In some cases other interfering components may remain on the adsorbent. Such a strong adsorption of interfering components offers another possibility for the prepurification of difficult matrices, such as waste oils or sludge. If the analytes show no interaction with the adsorbent and if only the interfering components are retained, the solid phase can be used to simply "filter" the sample, as shown in the figure above.

An adsorbed substance can be removed from the adsorbent by a stepwise increase of the elution strength of the eluent (step gradient technique).

However, this method can be utilised efficiently for a preseparation of groups of compounds or a single analyte from the matrix. It is extensively used for clean-up by solid phase extraction.

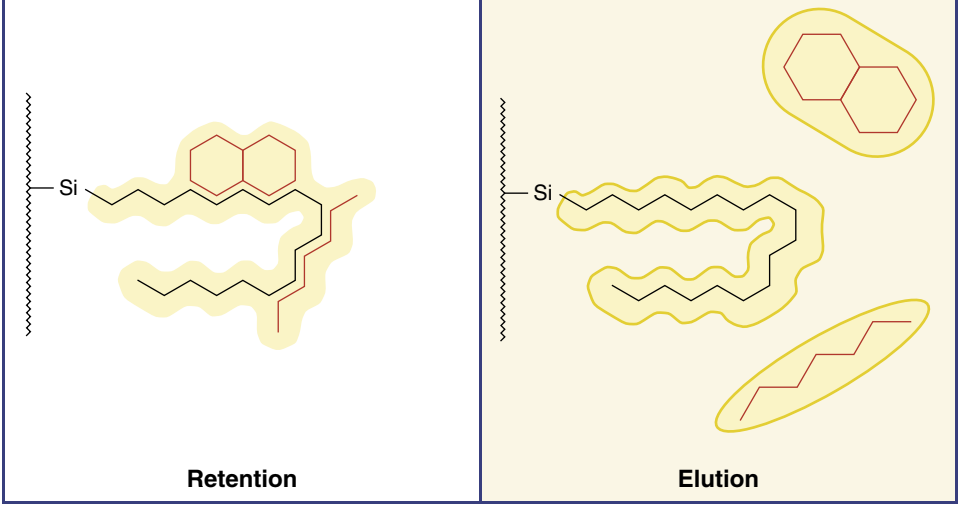
Nonpolar interactions occur between hydrocarbon residues of the functional groups of the adsorbent and the analyte. Since most organic compounds have a nonpolar structure, they can be adsorbed to nonpolar adsorbents via van-der-Waals forces.

Almost all organic compounds have a certain potential for nonpolar interactions. Exceptions are compounds possessing a large number of polar or even ionic groups which shield the nonpolar character of the carbon skeleton (e.g. carbohydrates).

Unmodified silica on the other hand shows no nonpolar interactions. Since the functional groups of most modified silicas are bonded to the silica surface via a hydrocarbon spacer, these modified silicas show a certain degree of nonpolar interactions.

Typical adsorbents with a pronounced nonpolar character are e.g. C18 ec, C18, C18 Hydra and C8 modified silicas. They show a seemingly low selectivity, because their functional groups, the alkyl substituents, can interact with almost all nonpolar analytes. This can be used for the isolation of substance groups of different structure.

Nonpolar interactions on C18 silica



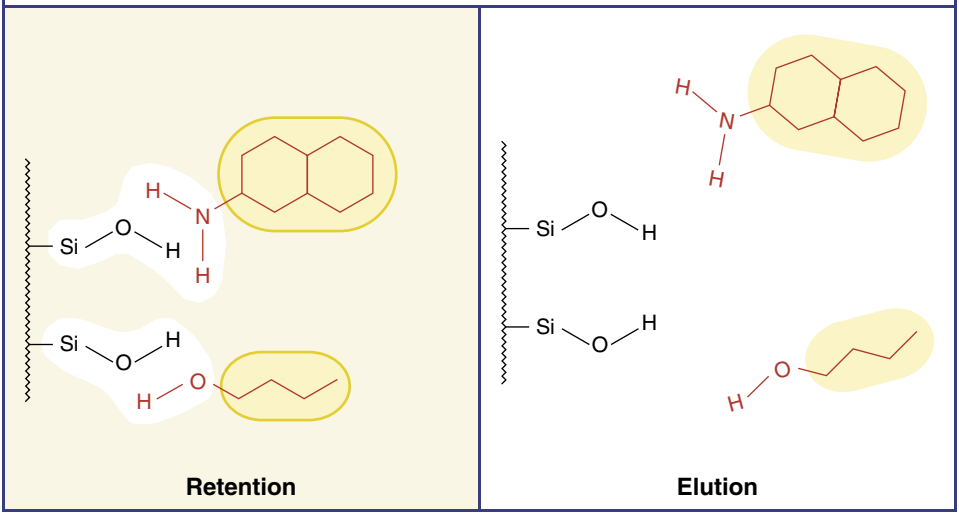
nonpolar (relative)

polar (relative)

Polar interactions include hydrogen bonds, dipole-dipole and π-π interactions, which can occur between many different adsorbents and functional groups of the analytes. Some of these interactions are possible between amino, hydroxyl and carbonyl groups as well as aromatic rings, double bonds and groups with hetero-atoms such as nitrogen, sulphur, phosphorus and oxygen. Typical adsorbents for polar interactions are unmodified silica, CN, NH_2 and OH (diol) modified silicas.

It should be noted, that in SPE the interactions described in this chapter are not found in pure form, but in combination. For example, modified silicas, unless they have been subjected to endcapping (silanisation of residual silanol groups with short-chain silanes), still possess free silanol groups, which can enter into secondary interactions.

Polar interactions on silica



In general, polar compounds are easily adsorbed to a polar adsorbent from a nonpolar environment and are eluted with a polar solvent. The opposite holds true for nonpolar compounds. They are easily adsorbed from a polar environment onto nonpolar surfaces. Elution is achieved by solvents of lower polarity.

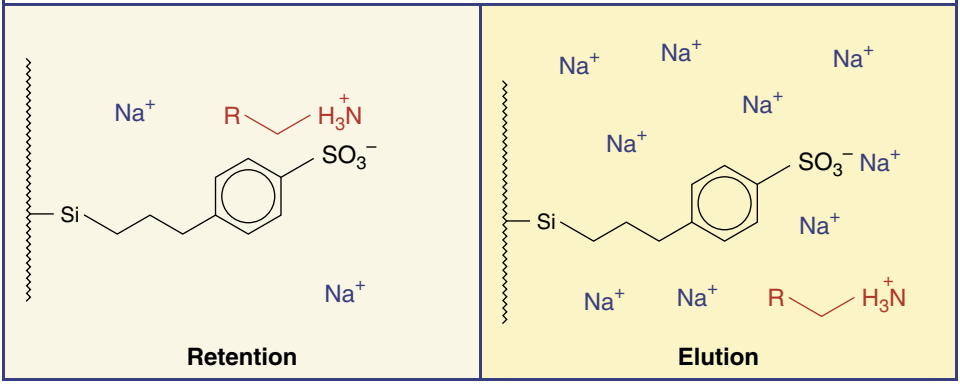
nonpolar (relative)

polar (relative)

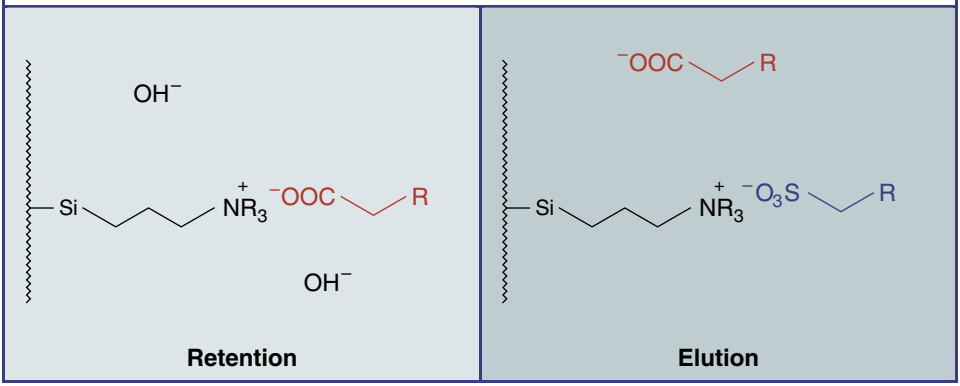
Ionic interactions occur between charged analytes and an adsorbent with a functional group of opposite charge. Cationic groups are present in primary, secondary, tertiary and quaternary amines and inorganic cations, e.g. calcium, sodium, magnesium etc. Examples for anionic groups are carboxylic and sulphonic acids, phosphates and similar groups.

Retention via these ion exchange interactions is enhanced in a matrix of low ionic strength and a counter ion of low selectivity (e.g. acetate, Na^+). For elution a solvent with high ionic strength and high selectivity is preferred (e.g. citrate or Ca^{2+}).

Ionic interactions on SA silica (cation exchange)



Ionic interactions on SB silica (anion exchange)



Selectivity and capacity

Selectivity is the ability of an adsorbent to discriminate between the analyte and other sample components. Thus, in general it describes the ability of the solid phase to adsorb the analyte, while the undesired components are not retained.

The selectivity depends on the chemical structure of the analyte, the properties of the adsorbent, the composition of the sample matrix and the eluent used. Optimum selectivity is achieved via functional groups of the analyte, which are not present in the sample matrix and other interfering components.

The capacity of an adsorbent is defined as the total amount of an analyte, which is adsorbed to a known amount of the adsorbent under optimum conditions. For ion exchangers the capacity is usually given as milliequivalents per gram [meq/g]. For other silica adsorbents capacity values are about 3 – 5 % of the amount of adsorbent. The polystyrene-divinylbenzene based adsorbent resins HR-P and Easy feature an exceptionally high capacity of 30 %. Thus, if the capacity and the amount of analyte to be concentrated are known, one can roughly estimate the amount of adsorbent required.

Summary of SPE phases

For solid phase extraction MN offers adsorbents based on **polymer resins** (Easy, HR-P), **surface-modified silica materials** as well as Florisil®, polyamide, and aluminium oxide. Special phases for defined applications in pharmaceutics, environmental analysis and ion chromatography complete the programme. The following pages describe the properties of all available phases.

Surface-modified silicas are stable in the pH range of about 2 to 8; however, in practical SPE they can often be used in a wider pH range, since cleavage of the functional groups is a function of time, and the adsorbents usually have only short-time contact with the solvents. In addition, the modified silicas are stable in almost all organic solvents. Silicas are hard materials, which show neither swelling nor shrinking, contrary to polyamide and the adsorbent resins HR-P and Easy. They have a mean pore size of 60 Å, which allows adsorption of compounds with molecular weights up to about 5000. Larger molecules can not diffuse into the pores and are thus not retained.

In addition to our **standard phases** the MN programme also comprises **special and combination phases** for SPE. As an example we want to mention the solid phase extraction of polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB).

Especially for extraction of the 16 PAH acc. to EPA from soil samples we have developed the combination phase **CN/SiOH**. This combination utilises the ability of the cyanopropyl phase to

selectively adsorb polycyclic aromatics. For isolation of the 16 PAH acc. to EPA from water our special column **CHROMABOND® C18 PAH** is especially suited, reproducibly allowing recovery rates up to 97%. The **CHROMABOND® NH_2 / C18** combination column is an alternative for the enrichment of PAH especially from humic acid-rich water. The NH_2 phase allows removal of the interfering humic acids.

The column **CHROMABOND® SiOH-H_2SO_4/SA** has been developed for the extraction of PCB from oil according to the German industrial standard DIN 51 527. It contains an upper layer of silica impregnated with H_2SO_4 and a lower layer of a strongly acidic cation exchanger with benzenesulphonic acid groups. This column is used together with the silica column CHROMABOND® SiOH. Both columns are available as Kombi-Kit PCB (Cat. No. 730125). In addition to the DIN procedure there is a method, which is especially recommended for the enrichment of PCB from waste oil samples. The combination phase **SA/SiOH**, which has been developed for this purpose, consists of a cation exchanger with benzenesulphonic acid groups as upper layer and silica as lower layer.

For PCB determination from sludge acc. to the German sludge regulations we have developed the special column **CHROMABOND® NAN**. The column packing is a combination of Na_2SO_4 and silica impregnated with $AgNO_3$.

One of the most important applications for CHROMABOND® and CHROMAFIX® **PS phases** for ion chromatography is the removal of overlapping components. Elimination of interfering components can on the one hand improve the chromatographic separation, if the interfering components overlap the analytes in the chromatogram, on the other hand it also improves lifetime of the chromatographic column, since interfering components can irreversibly cover the column packing. In addition to this simple clean-up of aqueous samples, however, the cartridges can also be used for an enrichment of the analytes.

CHROMABOND® and CHROMAFIX® PS phases are offered in several different chemical modifications. Base material in all cases is a polystyrene-divinylbenzene copolymer of highest purity, which features a very low degree of swelling and is thus very well suited for chromatography. This base material allows a reliable function of the cartridges over the whole pH range. The mean pore diameter of this material is 100 Å, the mean particle size is 100 µm. The most important fields of application for PS-RP, PS-OH^-, PS-H^+, PS-Ag^+ and PS-Ba^{2+} reach from elimination of nonpolar constituents to the removal of specific polar components.

Typical solvents for SPE

Polarity	Solvent	Miscibility with water
nonpolar	Hexane	no
	Isooctane	no
	Petroleum ether	no
	Cyclohexane	no
	Carbon tetrachloride	no
	Chloroform	no
	Methylene chloride	no
	Tetrahydrofuran	yes
	Diethyl ether	no
	Ethyl acetate	poor
	Acetone	yes
	Acetonitrile	yes
	Isopropanol	yes
	Methanol	yes
	Water	yes
polar	Acetic acid	yes

Selection of SPE phases

The scheme on the following page is meant as a first guide to the application of CHROMABOND® columns or CHROMAFIX® cartridges and considers mostly primary interactions. It is only valid for samples with molecular weights below 5000 Dalton to avoid size exclusion effects which might block the pores of the SPE silica.

The solvents recommended for elution should also be considered as a first selection. Applicability of other solvents or solvent mixtures is determined by the polarity required for a separation.

Selection guide for SPE phases and solvents

Sample solubility		Solvent	Sample polarity	Phases recommended for adsorption	Solvents recommended for elution (selection)
soluble in water	not ionic	aqueous	nonpolar	Easy, HR-P C18 ec, C18, C18 Hydra C8, C4, C2, C_6H_5 CN	hexane CH_2Cl_2 acetonitrile alcohols
			moderately polar	SiOH NH_2	$CHCl_3$, CH_2Cl_2 ethyl acetate alcohols water
			polar	CN, OH PA DMA NH_2	$CHCl_3$, CH_2Cl_2 ethyl acetate alcohols water
	ionic	aqueous	cationic	PCA SA PSA PS-H^+	acids salt solutions buffers
			anionic	SB NH_2 DMA PS-OH^-	acids salt solutions buffers
soluble in organic solvents		aqueous	nonpolar	Easy, HR-P C18 ec, C18, C18 Hydra C8, C4, C2, C_6H_5 CN PS-RP	hexane CH_2Cl_2 acetonitrile alcohols
		organic	moderately polar	SiOH NH_2	$CHCl_3$, CH_2Cl_2 ethyl acetate alcohols
		organic	polar	CN, OH PA DMA NH_2	$CHCl_3$, CH_2Cl_2 ethyl acetate alcohols

MN phases for SPE

The large range of analytes to be isolated and the great variability in sample matrices require a large number of phases for solid phase extraction. Combined with different column hardware for different sample sizes, this results in a considerable diversification of our programme for SPE. The following pages provide a brief description of all MN phases for SPE.

Easy

polar, bifunctionally modified polystyrene-divinylbenzene adsorbent resin

- polar modified polystyrene-divinylbenzene copolymer with a weak ion exchanger

  specific surface 650 – 700 m^2/g, particle size 80 µm, pore size 50 Å, pH stability 1 – 14

  due to bifunctional modification much more hydrophilic than conventional polystyrene-divinylbenzene polymers and thus easily wettable with water

- recommended applications:

  drug analysis from urine, blood, serum, plasma
  pharmaceuticals / active ingredients from tablets, creams
  polar herbicides / pesticides from water (acidic, neutral, basic)
  polar phenols from water
  polyaromatic compounds
  polychlorated biphenyls

HR-P

polystyrene-divinylbenzene adsorbent resin

- highly porous polystyrene-divinylbenzene copolymer

  specific surface 1 200 m^2/g, particle size 50 – 100 µm

  very high binding capacity, up to 30% of adsorbent weight (for comparison: silica adsorbents about 3%)

- recommended applications:

  aromatic compounds
  phenols from water
  nitroaromatics from water
  pesticides from water
  PAH from oil

C18 / C18 f (f = fast flow)

octadecyl silica

- base material silica, pore size 60 Å, particle size 45 µm for C18, 100 µm for C18 f (for fast flow), specific surface 500 m^2/g, pH stability 2 – 8

  octadecyl phases, not endcapped, carbon content 14%

  similar to C18 ec, however possesses more free silanols (SiOH), which allow secondary interactions with polar groups of the analytes

- recommended applications:

  nonpolar compounds
  pesticides

  C18 f for viscous samples

C18 ec / C18 ec f (f = fast flow) — octadecyl silica, endcapped

- base material silica, pore size 60 Å, particle size 45 µm for C18 ec, 100 µm for C18 ec f (for fast flow), specific surface 500 m^2/g, pH stability 2 – 8
- octadecyl phases, endcapped, carbon content 14%
- very nonpolar, hydrophobic interactions with a wide variety of organic compounds
- advantageous for clean-up of samples with large structural variations (polarity differences)
- recommended applications:
  - nonpolar compounds
  - aflatoxins, amphetamines, antibiotics, antiepileptics
  - barbiturates, caffeine, drugs, preservatives
  - fatty acids, nicotine, PAH, pesticides, PCB
  - heavy metals, vitamins
- very well suited for desalting of samples
- C18 ec f for viscous samples

C18 Hydra — octadecyl silica for polar analytes

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8
- special octadecyl phase for polar analytes, not endcapped, carbon content 15%
- recommended applications:
  - more polar compounds like pesticides and their polar degradation products
  - phenols
  - phenoxycarboxylic acids
  - nitroaromatics
  - pharmaceuticals

C8 — octyl silica

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8
- octyl phase, not endcapped, carbon content 8%
- similar to C18, however slightly more polar
- secondary interactions with polar compounds are more pronounced due to shorter alkyl chains
- recommended applications:
  - pesticides
  - PCB

MN phases for SPE

C4 — butyl silica

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8
  butyl phase, not endcapped, carbon content 7%
  slightly more polar than C18 or C8
  due to shorter alkyl chains the silica surface is not completely shielded
- recommended applications:
  compounds, which are too strongly retained on C18 or C8
  e.g. analgetics from blood

C2 — dimethyl silica

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8
  dimethyl phase, not endcapped, carbon content 4%
  similar to C4
- recommended applications:
  e.g. antiepileptics from plasma

C_6H_{11} ec — cyclohexyl silica, endcapped

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8
  cyclohexyl phase, endcapped, carbon content 9%
  alternative phase for the mid-polar range
- recommended applications:
  phenols from water
  chloroanilines from waste water
  anthelmintics from tissue

C_6H_5 — phenyl silica

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8
  phenyl phase, carbon content 8%
  polarity similar to C8
  in addition to hydrophobic interactions more selective adsorption is possible by π–π interactions due to the electron density of the phenyl ring
- recommended applications:
  aflatoxins
  caffeine
  phenols

Standard phases

NO_2 nitrophenyl silica

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8

  nitrophenyl phase, carbon content 5.5%

- recommended applications:

  aromatics

NH_2 aminopropyl silica

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8

  aminopropyl phase, carbon content 3.5%

  polar, weak anion exchanger

- recommended applications:

  trace elements
  lipids

DMA dimethylaminopropyl silica

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8

  dimethylaminopropyl phase, carbon content 3.5%

  polar, weak anion exchanger

- recommended applications:

  similar to NH_2 – slightly weaker anion exchanger

CN cyanopropyl silica

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8

  cyanopropyl phase, carbon content 5.5%

  polar to mid-polar

  in addition to weak hydrophobic interactions selective interactions are possible due to the high electron density of the CN group

- recommended applications:

  cyclosporins
  carbohydrates

OH — diol silica

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8

  diol phase, carbon content 5.5%

  polar

  properties similar to SiOH

- recommended applications:

  antibiotics
  prostaglandins

SiOH — unmodified silica

- unmodified, weakly acidic silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8

  very polar

  adsorbs humidity from air, for this reason it should be kept well closed and if necessary dried before use

  due to its high affinity for polar compounds it should not be conditioned with polar (e. g. methanol) or water-containing solvents

- recommended applications:

  aflatoxins
  chloramphenicol
  pesticides
  steroids
  vitamins

Alox A / Alox N / Alox B — aluminum oxide, acidic, neutral, basic

- aluminium oxide, high purity, pore volume 0.90 ml/g, particle size 60 – 150 µm, specific surface 150 m^2/g

- recommended applications:

  together with phase SA for PCB and pesticides

Properties of the individual modifications:

Alox A:	aluminium oxide, acidic	pH value 4 ± 0.3
Alox N:	aluminium oxide, neutral	pH value 7 ± 0.5
Alox B:	aluminium oxide, basic	pH value 9.5 ± 0.3

Standard phases

Florisil®

magnesium silicate

- matrix magnesium silicate (MgO–SiOH 15:85), high purity, particle size 150 – 250 µm

  Florisil® is a product and registered trademark of Floridin Company, USA

- recommended applications:

  organic tin compounds
  aliphatic carboxylic acids
  PCB, PAH

PA

polyamide 6

- matrix polyamide 6, unmodified, high purity, particle size 40 – 80 µm

- recommended applications:

  flavonoids
  PAH

PCA

propylcarboxylic acid cation exchanger based on silica

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8

  propylcarboxylic acid modified silica

  weakly acidic cation exchanger

- recommended applications:

  strong cations

SA

benzenesulphonic acid cation exchanger based on silica

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8

  benzenesulphonic acid modified silica

  strongly acidic cation exchanger
  capacity ~ 0.5 meq/g

  adsorbent with hydrophobic and π–π interactions (benzene ring)

  ion exchange of organic compounds from aqueous matrix

  elution of interesting compounds with solvent systems, which compensate the ionic and nonpolar interactions, e.g. methanolic HCl

- recommended applications:

  amino acids
  chlorophyll
  PCB

PSA propylsulphonic acid cation exchanger based on silica

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8
- propylsulphonic acid modified silica
- very strong cation exchanger capacity ~ 0.7 meq/g
- contrary to the SA phase no π–π interactions
- recommended applications: weak cations

SB quaternary ammonium anion exchanger based on silica

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8
- silica modified with quaternary amine
- strongly basic anion exchanger capacity ~ 0.3 meq/g
- not suited for very strong anions such as sulphonic acids, because these are difficult to elute
- recommended applications: organic acids caffeine saccharin

PS-RP / PS-OH⁻ / PS-H⁺ PS-Ag⁺ / PS-Ba²⁺ phases for RP / ion chromatography

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m²/g, pH stability 2 – 8

  silica modified with quaternary amine

  strongly basic anion exchanger capacity ~ 0.3 meq/g

  not suited for very strong anions such as sulphonic acids, because these are difficult to elute

- recommended applications:

  removal of interfering compounds

  → improves chromatographic separation, if the interfering components overlap with the analyte in the chromatogram

  → improves lifetime of the chromatographic column, since interfering components can irreversibly block the column packing

  → enrichment of the analytes

Properties of the individual modifications:

PS-RP	hydrophobic PS/DVB copolymer	removal of organic interfering components from water
PS-OH⁻	strong PS/DVB anion exchanger, OH⁻ form, capacity 0.6 meq/g	removal or concentration of anions from water increasing the pH value in acidic samples
PS-H⁺	strong PS/DVB cation exchanger, H⁺ form, capacity 2.9 meq/g	removal or concentration of cations from water decreasing the pH value of basic samples
PS-Ag⁺	strong PS/DVB cation exchanger, Ag⁺ form	removal of halide ions from water
PS-Ba²⁺	strong PS/DVB cation exchanger, Ba²⁺ form	removal of sulphate ions from water

Diamino special phase for determination of pesticides in food

- silica with primary and secondary amine function

  removes polar compounds (e.g. organic acids, pigments, sugars) from matrices like fruit or vegetables with low fat content

- recommended applications:

  quick and cheap determination of pesticides in strong matrix contaminated samples by GC (QuEChERS method = **Qu**ick **E**asy **Ch**eap **E**ffective **R**ugged **S**afe)

ABC18

special phase for analysis of acrylamide in food

- octadecyl silica phase with ion exchange functions for acrylamide analysis
- recommended applications:
  clean-up of acrylamide from ultra-heated starch-containing food, such as potato chips and other snacks, french fries, crispbread, cereals etc.

Important note:

Miniumum concentration of acrylamide should be 70 µg/kg.

The procedure includes no concentration step.

Acrylamide and the isotopically labelled form, is carcinogenic, mutagenic and neurotoxic.

Acrylamide is created at temperatures above 100 °C from sugar and proteins, e.g. from potatoes or grain during the process of frying, baking, roasting or grilling. The formation depends on temperature, starting at 120 °C and increasing with more elevated temperatures. In cooked food, no acrylamide is found. During the process of baking or frying, however, a remarkable amount of acrylamide can be formed.

Drug

special silica phase for drug analysis

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8
  special bifunctional modification
- recommended applications:
  enrichment of acidic, neutral and basic drugs from urine or plasma

Tetracycline

special phase for enrichment of tetracyclines

- silica phase with special C18 modification, tested for tetracyclines
  constant recovery rates for the title compounds (every batch individually tested)
- recommended applications:
  tetracyclines from biological samples

Crosslinks

special phase for enrichment of collagen crosslinks

- special cellulose phase for enrichment of collagen crosslinks
- recommended applications: collagen crosslinks in urine

Pyridinoline and deoxypyridinoline are collagen crosslinks occuring in bones and cartilage. If these substances are released, they can be detected in the urine. In cases of increased bone catabolism (e. g. during osteoporosis) the urine concentrations of pyridinoline and deoxypyridinoline are increased.

AOX

AOX from waters with high salt loads (DIN 38409 – H22)

- special PS-DVB phase
- recommended applications: extraction of AOX from waters containing high salt loads / organic pollutants in accordance with DIN 38409 – H22

CN/SiOH

combination phase for PAH analysis

- special combination phase:
  cyanopropyl phase for selective adsorption of polycyclic aromatics via π–π interactions
  unmodified silica phase for removal of polar compounds
- recommended applications: extraction of the 16 PAH according to EPA from soil samples

C18 PAH

octadecyl silica for PAH analysis

- base material silica, pore size 60 Å, particle size 45 µm, specific surface 500 m^2/g, pH stability 2 – 8
  special octadecyl modification for enrichment of PAH, not endcapped, carbon content 14%
- recommended applications: PAH from water

NH_2/C18

combination phase for PAH analysis

- special combination phase:
  aminopropyl phase for removal of interfering humic acids
  octadecyl phase for enrichment of PAH
- recommended applications:
  PAH from water containing humic acids

Na_2SO_4/Florisil®

hydrocarbons from water acc. to DIN H-53 / ISO DIS 9377-4

- special combination phase of sodium sulphate and Florisil®
- recommended applications:
  hydrocarbons from drinking, surface and waste waters

SiOH-H^+/SA

combination phase for PCB analysis

- special combination phase:
  SiOH-H^+: H_2SO_4-impregnated silica phase for oxidation of accompanying compounds to ionic and/or polar compounds
  SA: strongly acidic cation exchanger based on silica with benzenesulphonic acid modification for removal of ionic and sulphur-containing compounds
  This combination column is used together with a SiOH column. Both columns together are available as Kombi-Kit PCB.
- recommended applications:
  extraction of PCB from oil with reference to German industrial standard DIN 51527, part 1

SA/SiOH

combination phase for PCB analysis

- special combination phase:
  SA: strongly acidic cation exchanger based on silica with benzenesulphonic acid modification
  SiOH: unmodified silica for removal of polar compounds
- recommended applications:
  extraction of PCB from waste oil (hexane extract)

NAN

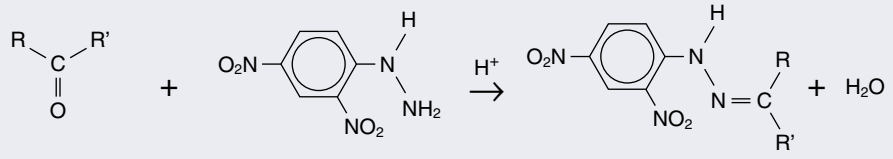
special phase for PCB analysis

- special combination phase:
  $SiOH/AgNO_3$ phase for removal of sulphur, sulphur-containing and polar compounds
  sodium sulphate for removal of trace water
- recommended applications:
  extraction of PCB from sludge

DNPH

special phase for enrichment of carbonyl compounds

- silica impregnated with 2,4-dinitrophenylhydrazine (DNPH)
  capacity about 75 µg formaldehyde, working range 1 – 5000 ppb
  samples can be passed through the cartridge in both directions
  each cartridge is sealed in a laminated aluminium bag
  carbonyl compounds adsorbed as 2,4-dinitrophenylhydrazine derivatives (hydrazones) can be eluted from the cartridges with acetonitrile
- recommended applications:
  carbonyl compounds (aldehydes and ketones) from air
  for enrichment aspirate air through the cartridge with max. 2 l/min



carbonyl compound | 2,4-dinitrophenylhydrazine | 2,4-dinitrophenylhydrazone

Dry

special phase for drying of organic samples

- anhydrous high-purity sodium sulphate which forms Glauber's salt with traces of water
  for removal of larger quantities of water several cartridges can be combined in series
- recommended applications:
  removal of traces of water from organic solutions

CHROMABOND® and CHROMAFIX® method development kits

All individual CHROMABOND® and CHROMABOND® LV columns as well as all CHROMAFIX® cartridges are sealed in units of five columns each to prevent adsorption of contaminants from the environment, e. g. laboratory air.

Only CHROMAFIX® DNPH cartridges are individually sealed in laminated aluminium bags. If you do not know the behaviour of your samples in solid phase extraction, or if you want to optimise the extraction procedure, we recommend our method development kits.

SPE method development kits · ordering information

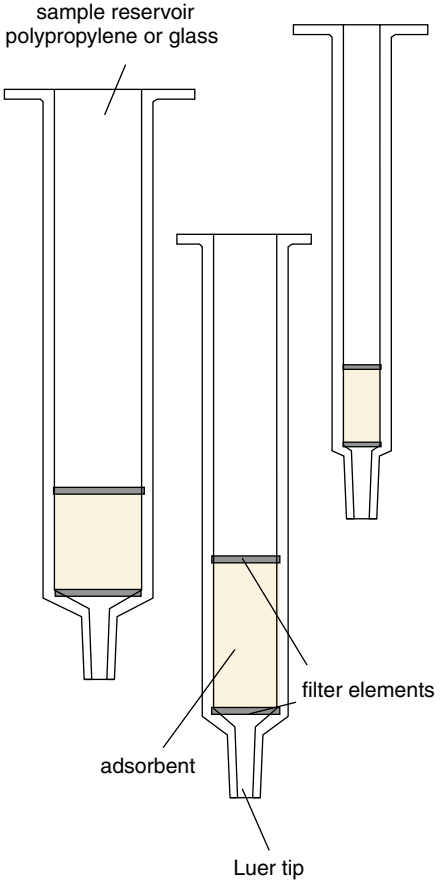
Designation	Contents of the kit	Cat. No.
Investigating the best separation mechanism for a clean-up procedure		
CHROMABOND® standard development kit	10 columns each with 1 ml / 100 mg: C18, C18 ec, C8, Phenyl, NH_2, DMA, OH, CN, SiOH, SA, SB	730110
Selecting the optimum RP phase for a clean-up procedure		
CHROMABOND® RP development kit I	10 columns each with 3 ml / 500 mg: C18, C18 ec, C8, C4 and 10 columns with 3 ml/200 mg HR-P	730197
CHROMABOND® RP development kit II	10 columns each with 1 ml / 100 mg: C18, C18 ec, C8, C4 and HR-P	730207
CHROMAFIX® RP development kit I	10 cartridges each CHROMAFIX® S: C18, C18 ec, C8, C 4 and HR-P	731883
CHROMABOND® RP development kit III	10 columns each with 3 ml / 500 mg: C18, C18 ec, C18 Hydra, C8 and 10 columns with 3 ml / 200 mg HR-P	730490
CHROMABOND® RP development kit IV	10 columns each with 1 ml / 100 mg: C18, C18 ec, C18 Hydra, C8 and HR-P	730491
CHROMAFIX® RP development kit II	10 cartridges each CHROMAFIX® S: C18, C18 ec, C18 Hydra, C8 and HR-P	731886
CHROMABOND® RP development kit V	10 columns each with 3 ml / 500 mg: C_6H_5, NO_2, C_6H_{11} ec, C4, C2	730492
CHROMABOND® RP development kit VI	10 columns each with 1 ml / 100 mg: C_6H_5, NO_2, C_6H_{11} ec, C4, C2	730493
CHROMAFIX® RP development kit III	10 cartridges each CHROMAFIX® S: C_6H_5, NO_2, C_6H_{11} ec, C4, C2	731887

Method development kits

Designation	Contents of the kit	Cat. No.
Selecting the optimum polar phase for a clean-up procedure		
CHROMABOND® polar development kit I	10 columns each with 3 ml / 500 mg: SiOH, Florisil, NH_2, CN, OH	730199
CHROMABOND® polar development kit II	10 columns each with 1 ml / 100 mg: SiOH, Florisil, NH_2, CN, OH	730208
CHROMAFIX® polar development kit	10 cartridges each CHROMAFIX® S: SiOH, Florisil, NH_2, CN, OH	731884
Selecting the optimum ion exchanger for a clean-up procedure		
CHROMABOND® ion exchange development kit I	10 columns each with 3 ml / 500 mg: SA, SB, PS-OH^-, PS-H^+, DMA	730206
CHROMABOND® ion exchange development kit II	10 columns each with 1 ml / 100 mg: SA, SB, PS-OH^-, PS-H^+, DMA	730209
CHROMAFIX® ion exchange development kit I	10 cartridges each CHROMAFIX® S: SA, SB, PS-OH^-, PS-H^+, DMA	731885
CHROMABOND® ion exchange development kit III	10 columns each with 3 ml / 500 mg: SA, PSA, PCA, PS-H^+	730494
CHROMABOND® ion exchange development kit IV	10 columns each with 1 ml / 100 mg: SA, PSA, PCA, PS-H^+	730495
CHROMAFIX® ion exchange development kit II	10 cartridges each CHROMAFIX® S: SA, PSA, PCA, PS-H^+	731888
Phase selection for clean-up procedures for environmental samples		
CHROMABOND® kit for environmental sample preparation	10 columns each with 3 ml / 200 mg HR-P, 6 ml / 1000 mg C18 ec, 6 ml / 2000 mg C18 PAH, 6 ml / 500/1000 mg CN/SiOH, 3 ml / 500/500 mg SA/SiOH	730205

CHROMABOND® polypropylene columns

The polypropylene sample reservoir is compatible with most solvents. The adsorbent is kept in place by two (for combination columns with two phases three) polyethylene filter elements (pore size 20 µm), which are chemically very inert. The figure shows the 3 standard sizes (scale 1:1).

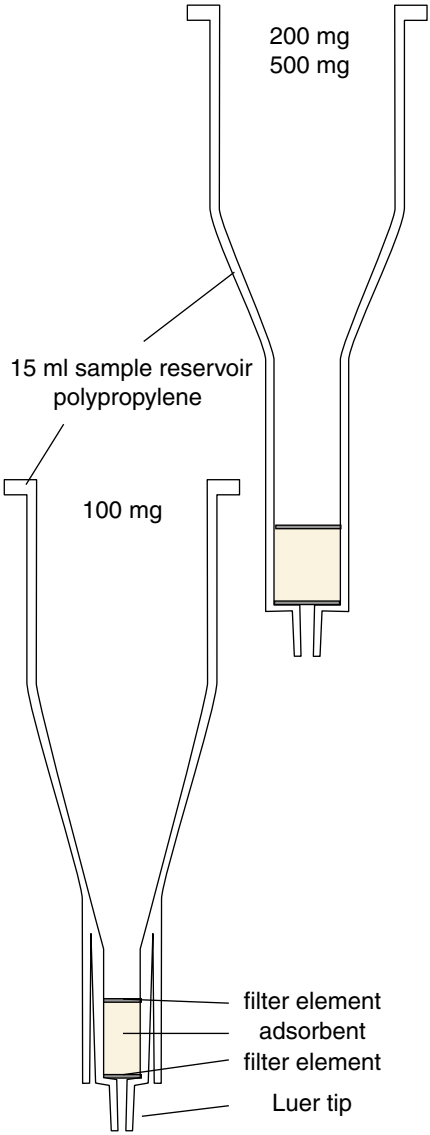


CHROMABOND® glass columns

If it is necessary to exclude any influence from the column material, you can order the complete CHROMABOND® programme in 1, 3 and 6 ml glass columns. CHROMABOND® glass columns are available with all phases described on pages 12 to 23. The catalogue numbers are differentiated by the letter G, e.g. 730001 (polypropylene) and 730001 G (glass). The adsorbent is kept in place by two (for combination columns with two phases three) glass fibre filter elements (nominal pore size 1 µm), which are chemically very inert.

CHROMABOND®
LV columns

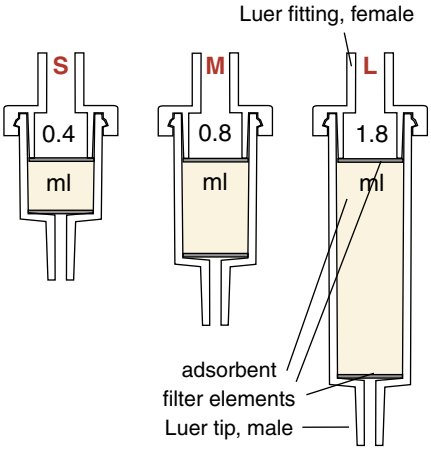
These large volume columns are available with three different sorbent weights (100, 200 and 500 mg) and feature a funnel-shaped reservoir with 15 ml volume. Especially for clinical samples this offers the advantage, that the whole sample (e. g. urine, serum, blood) can be applied to the column in one step. Additionally, CHROMABOND® LV can be directly used in the Zymate® lab robots of Zymark. The figure shows the original size of the LV columns.



CHROMAFIX® cartridges

CHROMAFIX® cartridges are manufactured from polypropylene and allow application of almost all usual solvents. The adsorbent is kept in place by two polyethylene filter elements (pore size 20 µm). As adsorbents we use the same phases as for our CHROMABOND® columns, the basic procedure for sample preparation with these cartridges from conditioning via sample application, washing and elution is the same as with CHROMABOND® columns.

The CHROMAFIX® programme consists of different sizes (S, M, and L) with different sorbent weights. Contrary to CHROMABOND® columns, CHROMAFIX® cartridges have a female Luer tip at the inlet and a male Luer tip as exit, thus these cartridges offer an alternative way of handling samples in SPE. They are especially suited for convenient solid phase extraction of small sample volumes.



CHROMABOND® MULTI 96

SPE is a reliable method for sample preparation. It is often used for enrichment or clean-up of numerous samples prior to analysis. For large numbers of samples MN offers 96-well microtiter plates for SPE.

The method development time is minimal. CHROMABOND® MULTI 96 can be supplied with any of the MN SPE adsorbents. Methods that have been worked out for CHROMABOND® columns or CHROMAFIX® cartridges can be easily transferred to CHROMABOND® MULTI 96.

Simultaneous preparation of 96 samples is economical because of the saving in time and solvent.

Advantages of this high-throughput system are:

- simultaneous preparation of 96 samples; this means a 4-fold increase over traditional 24-position SPE processors
- use of multi-channel pipettors facilitates liquid transfer steps
- readily adaptable to all common automated / robotic handling systems
- minimised dead volume (≤ 40 µl)

CHROMAFIL®

With CHROMAFIL®, rapid purification and removal of particles from liquid samples or gases is very simple: just place the filter on the syringe, and you are ready for filtration. Special manipulations are not required. Contamination of sensitive instrumentation by solid impurities can be avoided, thus increasing lifetime of chromatographic columns an equipment.

Advantages:

- Polypropylene housing
  better solvent stability compared to acrylate and polystyrene filters
- Shells ultrasonically sealed, not glued
  no extractable components from glues
- Filtration in both directions possible, the liquid cannot bypass the membrans
- Luer lock on side of entry
  safe connection on the "high pressure" side
- Luer exit
  standard luer for 3 and 25 mm filters, minispike luer with low dead volume and small OD for 15 mm filters
- Deflector
  the stream of liquid is broken and distributed, and does not directly hit the membane: this prevents rupture of the membrane
- Star-shaped distribution device
  the liquid is evenly distributed to the whole membrane surface: this results in a better utilisation of the total area; the filter is not plugged up rapidly; high flow efficiency
- Colour coded
  filters with 0.2 µm pores have a yellow upper shell, that of filters with 0.45 µm pores is colourless; the different membrane types are distinguished by different colours
- Low dead volume
  ~120 µl for 25 mm ∅, 12 µl for 15 mm ∅, 5 µl for 3 mm ∅

CHROMAFIL® filters are available with pore seizes of 0.2 and 0.45 µm (exceptions: PET filters also 1.2 µm, glass fibre filtes only 1 µm) and filter sizes of 25, 15 and 3 mm diameter. The small diameter filters are especially recommended for very small samples, which require extremely low dead volumes.

Recommended filter size depending on sample volume

sample volume	recommended filter diameter
≤ 1 ml	3 mm
1 – 10 ml	15 mm
10 – 100 ml	25 mm

Technical data

The membrane housing consists of polypropylene (PP). This material is very resistant towards most solvents and has a very low content of extractable substances. Thus it can be used with almost all solvents, acids and bases (see table). The special thick rim of the housing is ideal for use of the filters in laboratory robots (e.g. Benchmate™). Filter inlet and filter exit can be fitted to the CHROMABOND® columns for selective sample preparation with the aid of a special adaptor.

All filters can be autoclaved at 121 °C and 1.1 bar for 30 min.

Application

Depending on your filtration problem you can choose filter membranes made from different materials:

- **Cellulose mixed esters** (MV) – this membrane is recommended for all filtrations in aqueous or polar media.
- **Cellulose acetate** (CA) – this membrane features a very high shape stability in aqueous solutions and a very low binding capacity for proteins. CA filters are available in a sterile and a nonsterile package. For sterile filtration of non-sterile solutions we recommend the CHROMAFIL® Sterilizer. For filtrations under sterile conditions, the proven CHROMAFIL® CA-20/25 S and CA-45/25 S are the filters of choice. All CA filters feature an extremly low binding capacity for proteins (2.9 µg/cm^2 BSA).
- **Regenerated cellulose** (RC) – this hydrophilic membrane features a very low adsorption; it is recommended for filtration of aqueous and organic/aqueous liquids
- **Polyamide** (PA) Nylon – this is a rather hydrophilic membrane; it is recommended for filtration of aqueous and organic/aqueous medium polar liquids.
- **Teflon®** (PTFE) – a hydrophobic membrane; ideal for filtration of nonpolar liquids and gases. It is very resistant towards all kinds of solvents as well as acids and bases. By flushing with alcohol, followed by water, the originally hydrophobic membrane can be made more hydrophilic.
- **Polyvinylidene difluoride** (PVDF) with integrated glass fibre prefilter – compared to PTFE, this membrane also features hydrophilic interactions. It can be used for the filtration of polar and nonpolar solutions.
- **Polyester** (PET) – this fabric-reinforced membrane features an outstanding chemical resistance and is suited for polar as well as nonpolare solvents. This all purpose membrane is recommended for filtration of aggressive media, for dust and aerosol analyses, ultrapurification of solvents etc. It is very well suited for TOC/DOC determination. The membrane is not cytotoxic and does not inhibit the growth of microorganisms and higher cells.
- **Glass fibre** (GF) – The nominal pore size of these filters is 1 µm. They can be used for solutions with high loads of particulate matter or for highly viscous solutions (e. g. soil samples, fermentation broths) either alone or combined with other CHROMAFIL® filters. When membrane filters are combinde with glass fibre filters, they prevent plugging of the membrane. Used alone, glass fibre filters allow higher flow rates than e.g. a 0.45 µm filter.

Chemical compatibility of CHROMAFIL® materials

The following table lists the chemical compatibility of our CHROMAFIL® materials. The chemical compatibility depends on several parameters such as time, pressure, temperature, concentration.
In most cases, CHROMAFIL® filters will have only short contact with a solvent.

In these cases they may be used despite of limited compatibility. For example, a PTFE filter with PP housing does not liberate any UV-detectable substances during filtration of 5 ml THF, although PP shows only limited resistance towards THF.

Solvent	Material								
	MV	CA	RC	PA	PTFE	PVDF	PET	GF	PP
Acetaldehyde	●	●	●	●	●	●	●	●	●
Acetic acid, 100%	●	●	●	●	●	●	●	●	●
Acetone	●	●	●	●	●	●	●	●	●
Acetonitrile	●	●	●	●	●	●	●	●	●
Ammonia, 25%	●	●	●	●	●	●	●	●	●
Benzene	●	●	●	●	●	●	●	●	●
n–Butanol	●	●	●	●	●	●	●	●	●
Carbon tetrachloride	●	●	●	●	●	●	●	●	●
Chloroform	●	●	●	●	●	●	●	●	●
Cyclohexane	●	●	●	●	●	●	●	●	●
Diethyl ether	●	●	●	●	●	●	●	●	●
Dimethylformamide	●	●	●	●	●	●	●	●	●
1,4–Dioxane	●	●	●	●	●	●	●	●	●
Ethanol	●	●	●	●	●	●	●	●	●
Ethyl acetate	●	●	●	●	●	●	●	●	●
Ethylene glycol	●	●	●	●	●	●	●	●	●
Formic acid, 100%	●	●	●	●	●	●	●	●	●
Hydrochloric acid, 30%	●	●	●	●	●	●	●	●	●
Methanol	●	●	●	●	●	●	●	●	●
Methylene chloride	●	●	●	●	●	●	●	●	●
Nitric acid, 65%	●	●	●	●	●	●	●	●	●
Oxalic acid, 10% aq.	●	●	●	●	●	●	●	●	●
Petroleum ether	●	●	●	●	●	●	●	●	●
Phosphoric acid, 80%	●	●	●	●	●	●	●	●	●
Potassium hydroxide, 1 mol/l	●	●	●	●	●	●	●	●	●
2–Propanol	●	●	●	●	●	●	●	●	●
Sodium hydroxide, 1 mol/l	●	●	●	●	●	●	●	●	●
Tetrahydrofuran	●	●	●	●	●	●	●	●	●
Toluene	●	●	●	●	●	●	●	●	●
Trichloroethylene	●	●	●	●	●	●	●	●	●
Urea	●	●	●	●	●	●	●	●	●
Water	●	●	●	●	●	●	●	●	●
Xylene	●	●	●	●	●	●	●	●	●

(Data not guaranteed) ● resistant, ● limited resistance, ● not resistant

PP = polypropylene, MV = cellulose mixed esters, CA = cellulose acetate,
RC = regenerated cellulose, PA = polyamide, PTFE = polytetrafluoroethylene (Teflon),
PVDF = polyvinylidene difluoride, PET = polyester, GF = glass fibre

Main steps of the SPE procedure

1. Conditioning of the adsorbent

Conditioning of the adsorbent is necessary in order to ensure reproducible interaction with the analyte. Conditioning, also called solvation, results in a wetting of the adsorbent and thus produces an environment, which is suitable for adsorption of the analyte. Nonpolar adsorbents are usually conditioned with 2 – 3 column volumes of a solvent, which is miscible with water (MeOH, THF, isopropanol etc.), followed by the solvent in which the analyte is dissolved (pure matrix). Polar adsorbents are conditioned with nonpolar solvents.

After the conditioning step the adsorbent bed must not run dry, because otherwise solvation is destroyed.

2. Sample application (adsorption)

Sample application can be performed with positive or negative pressure with a flow rate of ~3 ml/min.

3. Washing of the adsorbent

Washing of the adsorbent is usually achieved with a special wash solution; however, in some cases it may not be necessary. If the polarity difference between wash solution and eluent is very large, or if both are not miscible, drying of the adsorbent bed after washing is recommended.

4. Elution

Elution with a suitable eluent should not be too fast. The elution speed depends on the column or cartridge dimension and the quantity of adsorbent (about 1 ml/min).

Sample pretreatment

For direct extraction with adsorbents the sample matrix (sample environment) has to fulfil three conditions:

- The matrix has to be liquid, if possible with low viscosity.
- Solids should be removed from the liquid matrix.
- The matrix (sample environment) should be suitable for retention of the analyte.

For solid samples there are different methods to convert the sample into a suitable matrix:

- dissolution of the solid sample in a suitable solvent
- lyophilisation of the sample and dissolution in a suitable solvent
- extraction of the solid sample with a suitable solvent
- homogenisation of the sample in a suitable solvent

In order to find the suitable solvent, one has to consider all desired sample components. Also, the suitable solvent should enhance retention of the analyte. For example, samples with large contents of solids are often homogenised in nonpolar solvents like hexane, while for samples with high water content dissolution in acids, bases, buffers or very polar solvents such as methanol is recommended.

Additionally, SPE allows to alter the properties of the sample matrix. If, for example, natural products are extracted with methanol or acetone, the polarity of the extracts can be increased by dilution with water, in order to enhance nonpolar solid phase extraction on the C18 material.

Sample pretreatment for some difficult matrices

Matrix	Problem	Sample pretreatment
soil, sludge	adsorption of the analyte on the solid matrix	extraction with nonpolar solvents (e.g. hexane) and separation of interfering components on polar adsorbents
crude oil products	very nonpolar matrix	extraction with or dissolution in nonpolar solvents (e.g. hexane) and separation of interfering components on polar adsorbents
wine, lemonade etc.	carbohydrate-containing polar matrix	dilution with water and enrichment on nonpolar adsorbents; for enrichment on ion exchangers pH adjustment with buffers
ointments and creams	differentiation for oil and water based products	oil based: dissolution in nonpolar solvents (e.g. hexane) and separation of interfering components on polar adsorbents water based: dissolution in polar solvents (e.g. methanol, acetone) with subsequent dilution with water, if required; enrichment of the analytes on nonpolar adsorbents
oils, fats, vegetable oils	nonpolar matrix	dissolution in nonpolar solvents (e.g. hexane, petroleum ether) and separation of interfering components on polar adsorbents
cereals	possible fat content	extraction with nonpolar solvents (e.g. hexane) and separation of interfering compounds on polar adsorbents or defat sample with nonpolar solvents and extract with polar solvents (e.g. methanol, acetone), if necessary subsequent dilution with water and enrichment of the analyte on nonpolar adsorbents
fruit, vegetable	heterogeneous matrix, dyes	extraction with polar solvents (e.g. methanol, acetone), if necessary subsequent dilution with water and enrichment of the analyte on nonpolar adsorbents
physiological samples (serum, plasma, blood, urine)	protein content	precipitate proteins and dilute sample with its own volume of water or suitable buffer; enrichment on nonpolar adsorbents
water	humic substances	removal of humic substances on NH_2 modified silica and enrichment of the analyte on nonpolar adsorbents

Standard protocols for CHROMABOND® RP phases

CHROMABOND®
The Simple Way

Standard SPE procedure for **CHROMABOND® C18 ec**
6 ml, 500 mg Cartridges, Cat. No. 730014

Prepare Sample

Condition
6 ml MeOH

Equilibrate
6 ml water

Load Sample
max. 1000 ml

Wash Impurities
3 ml MeOH – water (5:95, v/v)

Elute Analyte
2 x 2 ml MeOH or acetone

CHROMABOND®
The Simple Way

Standard SPE procedure for **CHROMABOND® *Easy***
3 ml, 200 mg Cartridges, Cat. No. 730754

Prepare Sample

Condition
3 ml MeOH

Equilibrate
3 ml water

Load Sample
max. 1000 ml

Wash Impurities
3 ml MeOH – water (5:95, v/v)

Elute Analyte
2 x 2 ml MeOH – acetone

CHROMABOND®
The Simple Way

Standard SPE procedure for **CHROMABOND® HR-P**
3 ml, 200 mg Cartridges, Cat. No. 730108

Prepare Sample

Condition
3 ml MeOH

Equilibrate
3 ml water

Load Sample
max. 1000 ml

Wash Impurities
3 ml MeOH – water (2:98, v/v)

Elute Analyte
2 x 2 ml MeOH – acetone (1:1, v/v)
or CH_2Cl_2

CHROMABOND®
The Simple Way

Standard SPE procedure for **CHROMABOND® PS-RP**
3 ml, 200 mg Cartridges, Cat. No. 730765

Prepare Sample

Condition
3 ml MeOH

Equilibrate
3 ml water

Load Sample
max. 1000 ml

Wash Impurities
3 ml MeOH – water (2:98, v/v)

Elute Analyte
2 x 2 ml MeOH – acetone (1:1, v/v)
or CH_2Cl_2

Standard protocols for CHROMABOND® ion exchangers

CHROMABOND®
The Simple Way

Standard SPE procedure for **CHROMABOND® PS-OH⁻**
3 ml, 200 mg Cartridges, Cat. No. 730396

Prepare Sample
(pH 7 – 11)

Condition
3 ml MeOH

Equilibrate
3 ml water

Load Sample
max. 1000 ml

Wash 1
3 ml 5% NH_3 in water

Wash 2 (Elute 1)*
3 ml MeOH – water (20:80, v/v)

Elute 2
2 x 2 ml MeOH – acetone (1:1, v/v) + 5% AcOH
or
2 x 2 ml MeOH – acetone (1:1, v/v) + 20% NH_3

* In this fraction neutral and basic compounds can be found

CHROMABOND®
The Simple Way

Standard SPE procedure for **CHROMABOND® PS-H⁺**
3 ml, 200 mg Cartridges, Cat. No. 730690

Prepare Sample
(pH 1 – 7)

Condition
3 ml MeOH

Equilibrate
3 ml water

Load Sample
max. 1000 ml

Wash 1
3 ml AcOH – water (5:95, v/v)

Wash 2 (Elute 1)*
3 ml MeOH – water (20:80, v/v)

Elute 2
2 x 2 ml MeOH – acetone (1:1, v/v) + 5% NH_3
or
2 x 2 ml MeOH – acetone (1:1, v/v) + 20% AcOH

* In this fraction neutral and acidic compounds can be found

CHROMABOND®
The Simple Way

Standard SPE procedure for **CHROMABOND® Mix**
3 ml, 200 mg Cartridges

Prepare Sample
(pH 7 – 11)

Condition
3 ml MeOH

Equilibrate
3 ml water

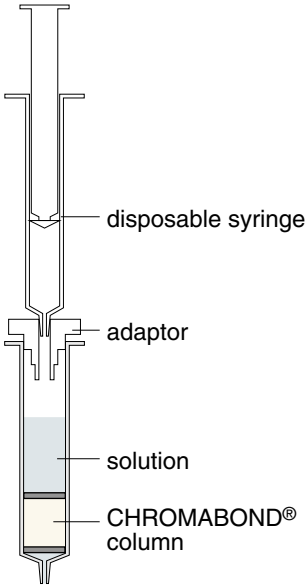
Load Sample
max. 1000 ml

	Com-pounds:
Wash 3 ml MeOH – water (5:95, v/v)	
Elute 1 3 ml MeOH – water (1:1, v/v) + 10% NH_3	polar and basic
Elute 1 3 ml MeOH – water (1:1, v/v) + 10% AcOH	polar and acidic
Elute 1 3 ml MeOH – acetone (1:5, v/v)	nonpolar and neutral

All solvent volumes are based on the amount of adsorbent mentioned (200 or 500 mg) and have to be adjusted according to the size of the cartridge used. The protocols are suggestions for standard procedures and should be optimised (solvents, volumes, amount of adsorbent etc.) for the demands of the investigated sample or compound and for the subsequent analysis (GC, HPLC etc.).

Handling of CHROMABOND® columns and CHROMAFIX® cartridges

For elution either apply pressure at the top of the column or apply vacuum at the column end. For this purpose several procedures are possible as shown in the figures. The adaptor shown in fig. a) can be used for coupling several CHROMABOND® columns of the same or different sizes.



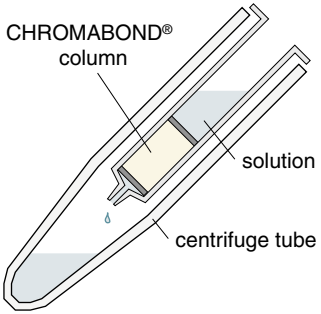
a) Use with a disposable syringe

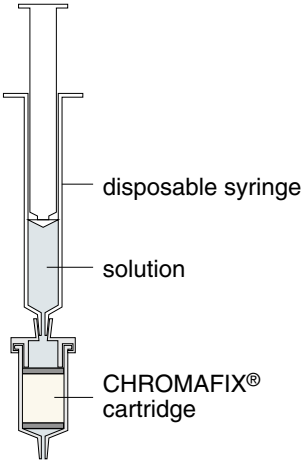
With the aid of a disposable syringe and an adaptor the eluent can be pressed through the CHROMABOND® column.

With the aid of a syringe the required solvents for conditioning, washing and elution as well as the sample itself can be easily pushed through the adsorbent of the CHROMAFIX® cartridges without high pressures.

b) Use in a centrifuge

The same result can be obtained by using the column in a centrifuge tube.

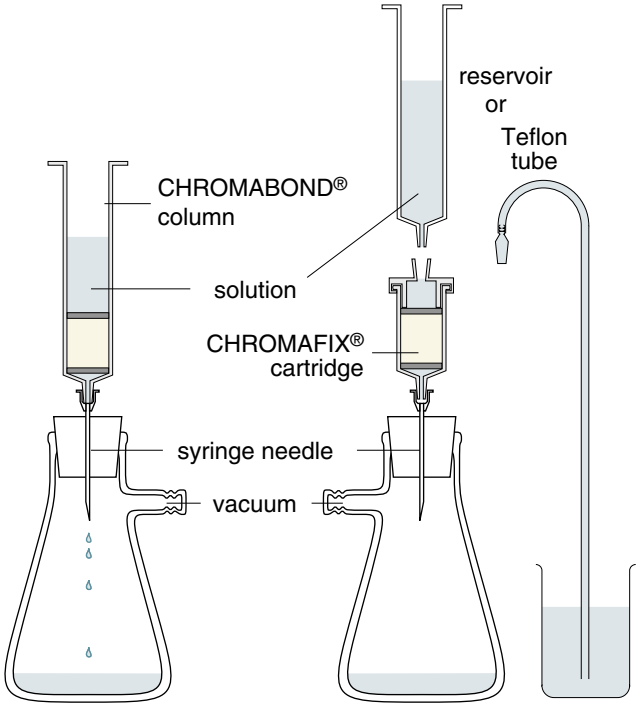




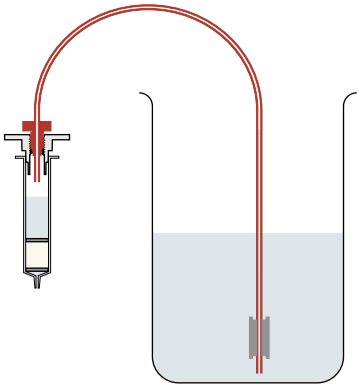
c) Application under vacuum

For drawing the eluent through a column or cartridge it can be placed on an aspirator bottle by means of a syringe needle, or it can be used on the vacuum manifold described below.

CHROMABOND® columns and CHROMAFIX® cartridges can be used with all vacuum systems with Luer fitting.



Handling of large sample volumes



For larger sample volumes MN has developed the CHROMABOND® LV columns, which are available with three different adsorbent weights (100, 200 and 500 mg) and feature a funnel-shaped reservoir of 15 ml volume.

If very large sample volumes are to be extracted, we recommend the CHROMABOND® tubing adaptors, which consist of an adaptor for CHROMABOND® columns and 1 m coloured Teflon tubing with weight. The package contains 4 adaptors with tubes of different colours.

CHROMABOND® vacuum manifolds for simultaneous preparation of up to 12, 16 or 24 samples

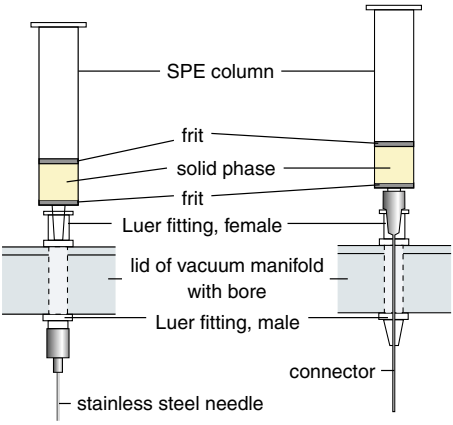
If several samples are to be treated simultaneously, we recommend our vacuum manifolds.

We supply such manifolds for up to 12, 16 or 24 CHROMABOND® columns or CHROMAFIX® cartridges, respectively. The manifolds consist of rectangular glass cabinets (1) with vacuum gauge (3) and a polypropylene lid (2), which can hold the columns or cartridges. The replaceable valves (4) on the lid allow individual vacuum control for each solid phase extraction column, if required.

The cabinet is fitted with a variable rack (5) with exchangeable partitions, which accept a wide variety of vessels like test tubes, measuring flasks, scintillation vials, autosampler vials, plastic vials and many more. With the control valve (6) the vacuum in the chamber can be adjusted and read from the gauge.

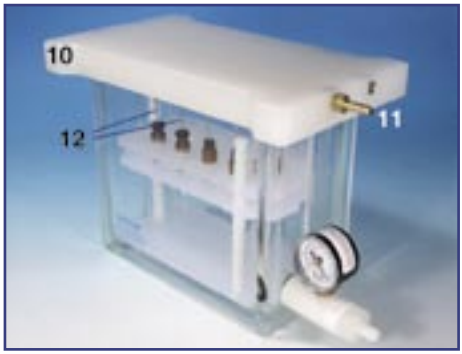
There are several possibilities for applying different sample volumes. Small samples can be applied directly to the CHROMABOND® column. For medium size samples we have developed our CHROMABOND® LV columns (7) with 15 ml sample reservoir. Especially for this column type we offer a vacuum manifold with 16 positions, because with the manifold for 24 columns only every second position can be used. Alternatively, you may use the polypropylene sample reservoirs (30 or 70 ml) from our programme of SPE accessories, which can be fitted onto the CHROMABOND® column with the aid of an adaptor (8). Sample reservoirs fit directly onto the upper Luer fitting of the CHROMAFIX® cartridges. For large sample volumes we recommend our CHROMABOND® tubing adaptors (9), which fit onto the CHROMABOND® columns. The other end of the tubing is placed into the sample, which, by applying vacuum, is continuously drawn into the CHROMABOND® column.

For special applications, which require maximum protection from cross contamination we supply chrome-plated brass valves and stainless steel connectors, the application of which is shown in the figure below. These special stainless steel needles are fitted through the lid; thus the sample only has contact with the inert needle and can flow directly into the receptacle.



Standard configuration (left) compared to the cross-contamination-free elution with stainless steel or PTFE connectors (right)

If the eluate has to be evaporated, this can be performed with the so-called drying attachment (10, see figure below). This special lid has a gas connector on one side (11), from which the gas is fed simultaneously to the 12 or 24 stations (12). Thus 12 or 24 eluates can be evaporated simultaneously by just changing the lid and applying a stream of inert gas, e.g. nitrogen.



For collection of the sample, vials and vial accessories are available on request. Please ask for further information.

CHROMABOND® PP tank for vacuum manifold for 12 columns

This polypropylene tank is a supplement for the 12-column vacuum manifold and allows to collect solvent residues (matrix, washing solutions) which are to be discarded, avoiding any contamination of the vacuum manifold itself. The CHROMABOND® PP tank can be used as follows:

If you want to collect the solvent residues (matrix residues) during solid phase extraction, place the PP tank into the vacuum manifold as shown in the following figure.

The lid is placed onto the glass cabinet (see next figure), and the SPE columns or cartridges are placed onto the valves.

Now the SPE procedure can be started. The sample matrix, which has passed through the adsorbent and is to be discarded, is collected in the PP tank. If the procedure requires washing of the CHROMABOND® columns or CHROMAFIX® cartridges after enrichment, the washing solutions can also be collected in the PP tank.

Before eluting the analyte(s), the lid is taken off and the PP tank is removed from the vacuum manifold. The tank has two small handles on the sides for convenient removal.

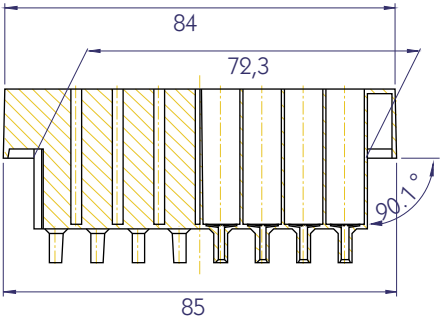
After the PP tank has been removed, the elution can be performed as usual by placing the rack with the eluate flasks into the glass cabinet. When the lid is put back onto the chamber, care has to be taken that the needles on the lower side of the lid are inside the respective flasks.

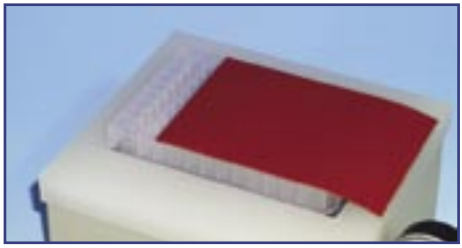
Solvent residues should be thoroughly removed from the PP tank after use. You may rinse the tank and use it again. Application of the PP tank can save valuable time and is very convenient, because it makes cleaning of the vacuum manifold much easier.

Handling of CHROMABOND® MULTI 96

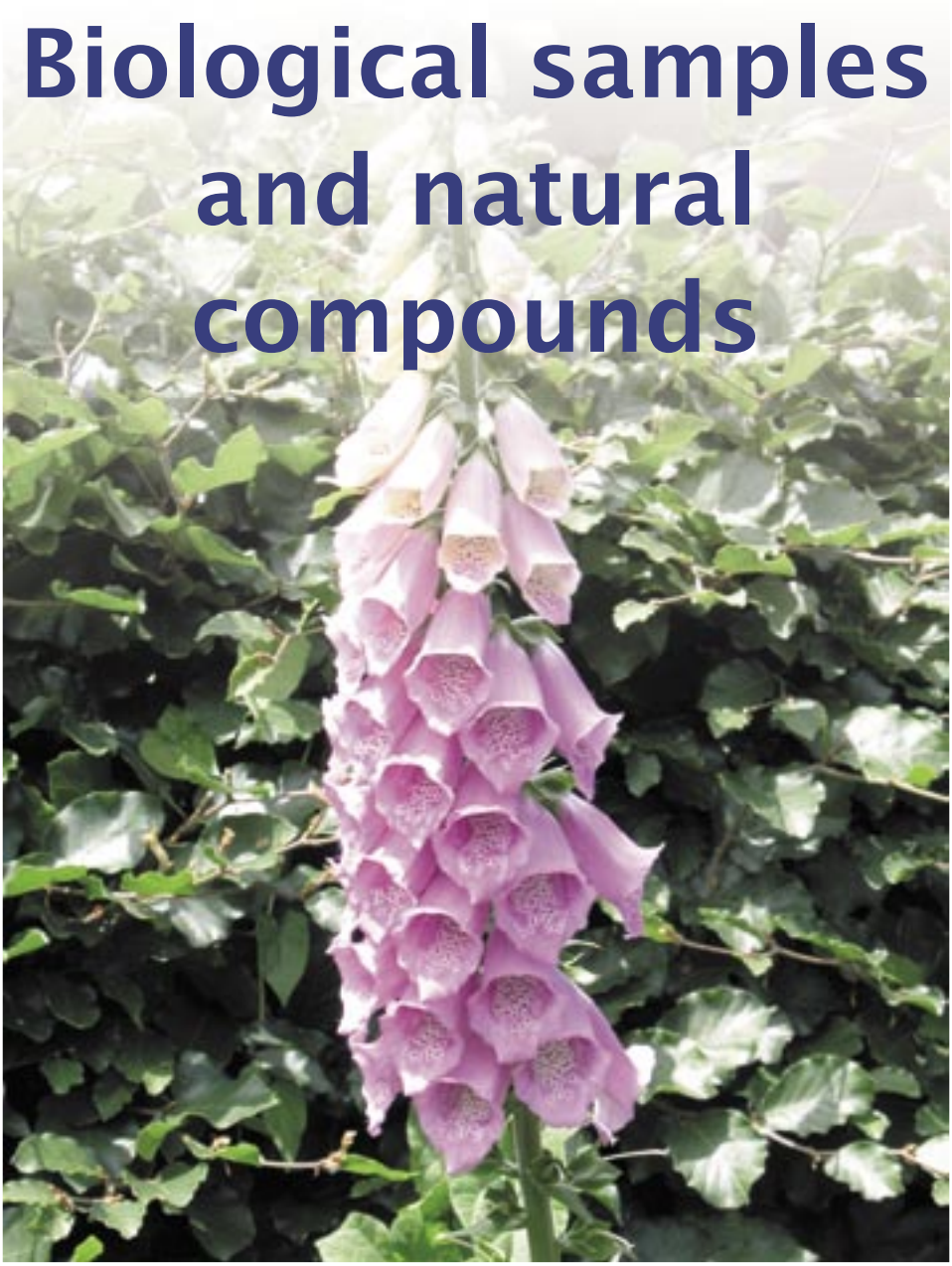
The CHROMABOND® MULTI 96 are particlularly designed for the use in all common robotic workstations or commercially available liquid handling systems. Alternatively, the use of multi-channel pipettors facilitates a manual liquid transfer. The extraction is carried our using the CHROMABOND® MULTI 96 vacuum manifold. With the help of the control valve the vacuum of the manifold can be adjusted leading to an optimum flow rate through the CHROMABOND® MULTI 96 SPE plate.

A reservoir tank and 96-well collection plates (96 x 0.5 or 96 x 2 ml) made of polypropylene can be supplied as accessories. An interesting alternative for collection of the eluates is a collection rack, which can be fitted with twelve 8-well strips of polypropylene tubes (each 1 ml). If you have to work on less than 96 samples, you can seal individual rows of the 96-well plate with a PTFE-covered rubber pad.



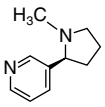


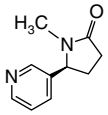
Biological samples and natural compounds



Alkaloids: nicotine and cotinine from plasma

MN Appl. No. 300070


Nicotine


Cotinine

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: mix 1 ml plasma with 1 ml saturated ammonium chloride solution and adjust to pH 10 with aqueous ammonia solution

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 x 200 µl saturated ammonium chloride solution – acetonitrile (9:1, v/v); dry column for 5 min under vacuum

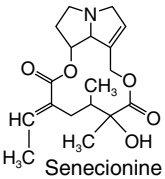
Elution: 2 x 250 µl dichloromethane

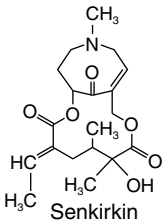
Alkaloids from plant extracts

MN Appl. No. 300080

H. Wiedenfeld, R. Lebada and B. Kopp,
Dtsch. Apothekerzeitung **135** (1995) 17 – 26

Compounds investigated: pyrrolizidine alkaloids from coltsfoot extracts (senecionine, senkirkin)


Senecionine


Senkirkin

Column type:
CHROMABOND® Diol / 3 ml / 500 mg
Cat. No. 730053

Sample pretreatment:
Commercial tea powder:

Dissolve 16.6 g tea powder in 1.5 l boiling water (= 10 cups of tea). Adjust pH to 2.5 – 3 with citric acid and evaporate to 500 ml. Wash aqueous phase several times with ether and CH_2Cl_2. Make ammoniacal and extract with CH_2Cl_2.

Commercial drug (dried coltsfoot leaves, 15 g sample each):
Different extraction methods were compared:

- alcoholic Soxhlet extraction of a powered drug sample
- cold maceration of coarsely cut drug with 1.5 l water during 30 min under stirring
- BGA procedure (German health administration): add 1.5 l boiling water to the coarsely cut drug sample and let stand 15 min
- ÖAB procedure (Austrian pharmacopoeia): wet coarsely cut drug sample with water in a mortar and let stand 5 min; then add 1.5 l boiling water and let stand 30 min

All extracts were processed as described for the tea powder above.

Column conditioning: 3 ml dichloromethane

Sample application: apply dichloromethane extract from the sample pretreatment step

Column washing: 3 ml dichloromethane

Elution: methanol – acetonitrile (1:1, v/v)

For further analysis we recommend GC on an OPTIMA® 5 column.

Pyrrolizidine alkaloids and their *N*-oxides in plant material

MN Appl. No. 302330

G. Hösch et al. Phytochemical Analysis **7** (1996) 284 – 288

Column type:
CHROMABOND® C18 / 3 ml / 500 mg
Cat. No. 730003

Sample pretreatment: approximately 2.5 g plant material are extracted with methanol in a soxhlet apparatus for 6, 16 and 21 h, respectively. After evaporation to dryness the residue is dissolved in 100 ml methanol. An aliquot (8.0 ml) is evaporated to dryness and suspended in 2.5% HCl (3 ml). This suspension was filtered and the filter was washed with 2.5% HCl (1 ml).

Column conditioning: 3 ml methanol

Sample application: slowly force or aspirate the acidic solution from the sample pretreatment through the column

Column washing: 3 ml of water

Elution: 8 ml of 25% methanol; evaporate to dryness under reduced pressure and dissolve in 1.0 ml methanol

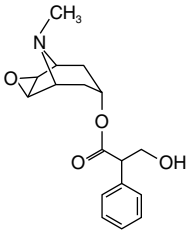
Further analysis: HPLC

Recovery rates: The total recovery of alkaloids decreased with the time of soxhlet extraction. While a PA yield of 0.55% was determined in the plant material after a 6 h extraction, this dropped to 0.49% after 16 h and even to 0.44% after a 21 h extraction. This finding was confirmed using the pure *N*-oxides in the absence of plant material. Recovery of the N-oxides dropped from 99.5 to 84.1% within 24 h of soxhlet extraction.

Isolation of scopolamine from tobacco roots

MN Appl. No. 303410

A. Maslak, Dissertation (2002), Mathematisch-Naturwissenschaftlich-Technische Fakultät der Martin-Luther Universität Halle-Wittenberg, Germany



Column type:
CHROMABOND® C18 / 6 ml / 500 mg
Cat. No. 730004

Sample pretreatment: 5 g ground tobacco roots from 8 weeks old plants of *Nicotiana tabacum* cv. SamsunNN were extracted with 15 ml of 50% MeOH for 30 min and filtered with a 100 µm nylon filter and a cellulose membrane (45 µm). The filtered sample was diluted with water to 10% MeOH.

Column conditioning: 1 column volume methanol, then 1 column volume water

Sample application: slowly force or aspirate the sample extract through the column

Column washing: three times with methanol – water (1:9, v/v)

Elution: 20 ml methanol; evaporate the combined eluates to 1 ml in a vacuum centrifuge

Further analysis: HPLC

Aromatic amines from urine and serum

MN Appl. No. 300090

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: adjust sample to pH 10 with 5 M sodium hydroxide solution

Column conditioning: 1 ml methanol, then 1 ml dist. water

Sample application: slowly force or aspirate 500 µl sample through the column

Column washing: 1 ml dist. water; then dry the column with air for 5 min

Elution: 200 µl ethyl acetate; concentrate eluate in a stream of nitrogen

1,2,3,4-tetrahydroisoquinolines and other potentially neurotoxic compounds

MN Appl. No. 303480

P. Pagel et al., J. Chromatography B, **746** (2000) 283 – 295

Compounds investigated: model compounds for method development (see table below)

Column type:
CHROMABOND® C18 ec / 30 mg
(self-packed by the author, available on request)

Column conditioning: 1 ml methanol, then 1 ml water

Sample application: force or aspirate 500 µl plasma sample or reference solution through the column

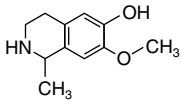
Elution:

Fraction F-1: effluent after load of 500 µl water

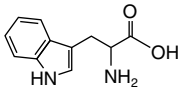
Fraction F-2: effluent after load of additional 500 µl water and application of pressure to remove water completely, subsequently addition of 500 µl methanol to dry the column

Fraction F-3: effluent after load of additional 500 µl methanol

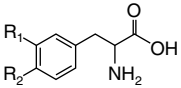
Fraction F-4: effluent after load of additional 500 µl methanol



Salsoline



Tryptophan



Phenylalanine (R_1 = R_2 = H)

Further analysis: HPLC with UV (215 and 240 nm) and fluorescence detection (λ_{ex} 285, λ_{em} 315/340 nm)

Recovery rates:
(in the presence of 1-hexanesulfonic acid sodium salt)

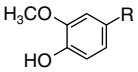
Compound	Recovery [%]					
	F-1	F-2	F-3	F-4	R_1	R_2
DOPA*	<LOD	<LOD	55	60	OH	OH
Tyrosine	<LOD	<LOD	50	50	H	OH
Phenylalanine	<LOD	<LOD	50	50	H	H
Salsoline*	<LOD	<LOD	52	58	–	–
Tryptophan	<LOD	<LOD	55	45	–	–

(*) = spike compounds; LOD = limit of detection

Catecholamine metabolites from urine

MN Appl. No. 300120

Compounds investigated: vanillylmandelic acid (R = CH(OH)-COOH) and homovanillic acid (R = COOH)



Column type:
CHROMABOND® SB (≡ SAX) / 3 ml / 500 mg
Cat. No. 730079

Sample pretreatment: collect 24 h urine (preserved with 0.1 M hydrochloric acid) and store at 4 °C or –20 °C, resp. Prior to extraction dilute sample 1:1 with water and adjust the pH value to 7.5 with 0.5 M sodium hydroxide solution.

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate 1 ml sample through the column

Column washing: 2 x 2.5 ml dist. water

Elution: 2 x 2.5 ml 1.5 M sodium hydroxide solution

Catecholamine metabolites from plasma

MN Appl. No. 300100

Compounds investigated: homovanillic acid

Column type:
CHROMABOND® C8 / 3 ml / 500 mg and
CHROMABOND® SB (≡ SAX) / 1 ml / 100 mg
Cat. Nos. 730023 and 730078

Sample pretreatment: mix 1 ml plasma sample with 50 µl 0.1 M EDTA (if desired, add 30 µl iso-homovanillic acid in 0.01 M hydrochloric acid as internal standard) and add 200 µl 1 M hydrochloric acid. Dilute with 2 ml water.

Column conditioning:
C8: 5 ml methanol, then 5 ml dist. water
SB: 1 ml methanol, then 5 ml 1 M sodium acetate buffer pH 6.0, finally 1 ml dist. water

Sample application: slowly force or aspirate the plasma sample through the C8 column

Column washing: 2 ml water

Elution of C8 column: 2 ml methanol – water (50:50, v/v); dilute eluate with 1 ml 0.1 M sodium acetate buffer pH 6

Sample application: force or aspirate the diluted eluate from the C8 column through the SB column with 0.5 ml/min

Elution of SB column: 2 x 300 µl 1 M hydrochloric acid with 0.5 ml/min

Catecholamines from urine

MN Appl. No. 300110

Column type:
CHROMABOND® SA (≡ SCX) / 3 ml / 500 mg
Cat. No. 730077

Sample pretreatment: mix 1 – 3 ml urine with 2 – 3 drops of a 1 M sodium carbonate solution

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

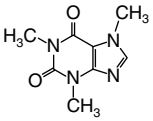
Sample application: force or aspirate pretreated sample through the column

Column washing: 2 column volumes dist. water

Elution: 3 x 500 µl 0.5 M hydrochloric acid; then adjust the eluate to pH 3 with 1 M sodium carbonate solution

Xanthines: caffeine and theophylline from serum

MN Appl. No. 300680



Caffeine

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: mix 1 ml serum with about 1 ml 0.01 M Tris buffer pH 7

Column conditioning: 2 column volumes methanol, then 2 column volumes 0.01 M Tris buffer pH 7

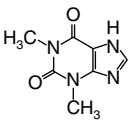
Sample application: slowly force or aspirate the sample through the column

Column washing: 2 column volumes dist. water

Elution: 2 x 300 µl methanol

Xanthines: theophylline from serum

MN Appl. No. 300690



Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: mix 100 µl serum with 100 µl internal standard (20 µg/ml β-hydroxyethyl-theophylline in 0.1 M phosphate buffer pH 4.0)

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 column volumes dist. water

Elution: 2 x 200 µl methanol

Biological samples and natural compounds

Benzalkonium chloride from plasma

MN Appl. No. 301950

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: dilute 2 ml plasma with 4 ml water

Column conditioning: 1 column volume methanol, then 1 column volume water

Sample application: slowly force or aspirate the diluted sample through the column

Column washing: 3 x 3 ml water, then 2 x 3 ml methanol, finally 2 x 3 ml ethyl acetate

Elution: 4 ml methanol – ethyl acetate (1:1, v/v) containing 0.01% ammonium chloride; concentrate eluate in a stream of nitrogen

Removal of chlorophyll from plant cells

MN Appl. No. 300010

Column type:
CHROMABOND® SA (≡ SCX) / 3 ml / 500 mg
Cat. No. 730077

Sample pretreatment: homogenise plant cells 2 min with 100 ml dichloromethane – acetone (3:1, v/v)

Column conditioning: place 0.5 g dry Na_2SO_4 onto the SA packing and condition column with 1 column volume dichloromethane – acetone (3:1, v/v) (special columns containing 500 mg CHROMABOND® SA and 500 mg Na_2SO_4 are available on request)

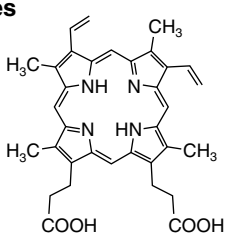
Sample application: slowly force or aspirate 4 ml sample through the column and collect the chlorophyll-free eluate. When chlorophyll is eluted, reduce the sample volume.

1st elution: 0.5 ml dichloromethane – acetone (3:1, v/v), combine the eluate with the chlorophyll-free eluate

2nd elution: if the substance to be determined still remains on the column together with the chlorophyll, elute the desired compounds with a more polar eluent (acetone, acetonitrile, methanol) or an eluent of lower pH value

Porphyrins: protoporphyrin from feces

MN Appl. No. 300500



Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: mix 200 – 250 mg feces with 10 ml 0.5 g/l Tween 40 solution and shake 30 s or sonicate. Mix 0.5 ml of this solution with 2 ml 3.125 M oxalic acid – 0.125 M iron sulphate solution – 0.5 g/l Tween 40 solution and shake thoroughly

Column conditioning: 3 ml 0.05 mol/l HCl, then 2 ml methanol – glacial acetic acid – water (75:2:23, v/v/v)

Sample application: slowly force or aspirate 250 µl of the pretreated sample through the column

Column washing: 3 ml MeOH – HAc – water (75:2:23, v/v/v)

Elution: 5 ml methanol – 30% phosphoric acid (3:1, v/v)

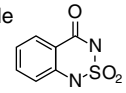
Bacterial cleavage of nitrogen to sulfone bonds in sulfamide and 1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide: formation of 2-nitrobenzamide by *Gordonia* sp.

MN Appl. No. 302530

U. Rein, A. M. Cook, FEMS Microbiology Letters **172** (1999) 107 – 113

Compounds investigated: sulfamate, sulfamide, 1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide, nitrobenzamide-2

Benzothiadiazinone dioxide



Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108

Sample pretreatment: adjust sample to pH 9 with 10 mol/l NaOH

Column conditioning: 2 ml methanol, 2 ml acetonitrile and 2 ml NaOH (c = 10^{-5} mol/l)

Sample application: force or aspirate the sample through the column with 10 ml/min

Column washing: 2 ml dist. water; dry cartridge under vacuum for 5 min

Elution: 3 x 1 ml methanol – acetonitrile (1:1, v/v)

Further analysis: reversed-phase HPLC using a column NUCLEOSIL® 100-5 C_{18} with gradient elution

Cyclic peptides microcystin and nodularin from algal cells and water

MN Appl. No. 302631 / 302632

C. Hummert, et al. Chromatographia **50** (1999) 173 – 180

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Algae samples:

Sample pretreatment: algae samples are filtered through a 0.45 µm glass fiber filter. The filters covered with algae cells and 1.0 ml of a mixture of water – methanol (50:50, v/v) are sonicated for 20 min and finally centrifuged. The supernatant is filtered through a nylon filter with 0.45 µm pore size (Cat. No. 729011).

Column conditioning: 5 ml methanol, 5 ml water (0.05% TFA)

Sample application: slowly force or aspirate 1 ml of algae extract through the column

Water samples:

Column conditioning: 2 ml methanol, 5 ml water (0.05% TFA)

Sample application: slowly force or aspirate 500 ml water sample through the column

For all samples:

Column washing: 10 ml water containing 0.05% TFA, then 5 ml water containing 0.05% TFA – methanol (80:20, v/v)

Elution: 5 ml methanol; evaporate to dryness using rotary evaporation for the main volume and a stream of nitrogen for the last 200 µl. Redissolve in 200 µl methanol – water (50:50, v/v).

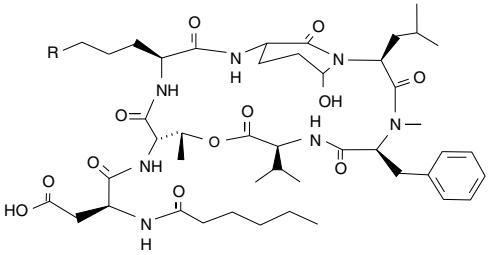
Further analysis: HPLC

Cyanopeptolins from cyanobacteria

MN Appl. No. 300190

C. Martin et al., J. Antibiotics **46** (1993), 1550 – 1556

Compounds investigated: cyanopeptolines A, B, C and D



A: R = NH-C(NH)-NH_2

B: R = CH_2-NH_2

C: R = CH_2-NH-CH_3

D: R = CH_2-N(CH_3)$_2$

Column type:
CHROMABOND® C18 / 6 ml / 1000 mg
Cat. No. 730005

Sample pretreatment: lyophilised cells of *cyanobacterium microcystis* sp. PCC 7806 (25 g dry substance) are extracted with 500 ml MeOH for 2 h at room temperature. After centrifugation the clear supernatant is concentrated to 50 ml using a rotation evaporator and then filled to 500 ml with dist. water.

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate the prepared sample through the column

Column washing: 10 ml dist. water

Elution: force or aspirate 30 ml methanol through the column; concentrate to 3 ml

Pyridinium crosslinks from urine

MN Appl. No. 302070

Compounds investigated: pyridinoline, deoxypyridinoline

Column type:
CHROMABOND® Crosslinks, 3 ml, 300 mg,
Cat. No. 730458

Sample pretreatment: 250 µl urine and 50 µl of an internal standard (e. g. pyridoxine) are hydrolised in 250 µl conc. HCl at about 100 – 105 °C for 12 – 16 h. Then 2.5 ml wash solution (*n*-butanol – glacial acetic acid 80:20, v/v) are added to the hydrolysate.

Column conditioning: 5 ml of the wash solution

Sample application: force or aspirate the pretreated sample through the column. Discard the flow-through.

Column washing: 15 – 25 ml of the wash solution.

Elution: force or aspirate 3 – 5 ml dist. water through the column

Diaminopyridine from urine

MN Appl. No. 300220

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Column conditioning: 1 column volume methanol, then 1 column volume dist. water

Sample application: slowly force or aspirate 1 ml sample through the column

Column washing: 2 ml dist. water, then 2 x 250 µl acetonitrile

Elution: 100 µl of a solution of 100 mg trimethylammonium chloride in acetonitrile – water (80:20, v/v), then 100 µl dist. water

Amino acids from urine

MN Appl. No. 300230

Column type:
CHROMABOND® SA (≡ SCX) / 1 ml / 100 mg
Cat. No. 730076

Sample pretreatment: mix 50 µl urine with 450 µl water and adjust to pH 1.1 with about 5 µl conc. hydrochloric acid

Column conditioning: 5 ml methanol, then 5 ml methanol – 0.1 M hydrochloric acid (1:1, v/v), finally 5 ml 0.1 M hydrochloric acid

Sample application: slowly force or aspirate the sample through the column

Column washing: 700 µl water

Elution: 900 µl 1 M aqueous ammonia solution; then freeze dry, if you do not analyse your sample immediately

Enrichment of viscotoxines from *Viscum Album*

MN Appl. No. 303040

Private communication: S. Jäger, ABNOBA Heilmittel GmbH, Pforzheim, Germany

Column type:
CHROMABOND® PCA / 6 ml / 500 mg
Cat. No. 730483

Sample pretreatment: 2 to 6 ml of *Viscum Album* extracts are diluted in 5 to 15 ml water

Column conditioning: 2 ml methanol, 2 x 2 ml 0.02 M ammonium acetate buffer

Sample application: slowly force or aspirate 2 to 6 ml sample through the column

Column washing: 2 x 2 ml 0.02 M ammonium acetate buffer

Elution: 10 ml 4 M acetic acid buffer

Further analysis: HPLC

Bile acids from serum

MN Appl. No. 300250

Column type:
CHROMABOND® C18 ec / 6 ml / 500 mg
Cat. No. 730014

Sample pretreatment: mix 1 ml serum with 4 ml 0.1 M sodium hydroxide solution and heat 15 min to 64 °C

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 1 column volume dist. water

Elution: 2 x 1 ml methanol

Flavonols and flavones from parsley cell suspensions

MN Appl. No. 302260

J. Hempel et al. Nahrung **43** (1999) 201 – 204

Column type:
CHROMABOND® C18 / 3 ml / 500 mg
Cat. No. 730003

Sample pretreatment: samples of 4.44 g lyophilised cell culture are dispersed in 200 ml methanol – water (70:30, v/v) and mixed for 15 min with an Ultra-Turrax homogeniser. The extract solution is removed by suction on a G3 glass filter covered with a paper filter. The filtrate is evaporated at 35 °C to remove the methanol.

Column conditioning: 3 ml of methanol and 5 ml of water

Sample application: slowly force or aspirate the sample through the column

Column washing: with water

Elution: 5 ml of methanol, evaporate to dryness and suspend in 1 ml of water

Further analysis: HPLC, LC/MS

Flavonoids from tomato peel

MN Appl. No. 300150

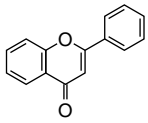
Column type:
CHROMABOND® PA / 6 ml / 500 mg
Cat. No. 730007

Sample pretreatment: extract 0.5 g dried tomato peel 1 h at 70 °C with 30 ml methanol – water (70:30, v/v). Then filter and concentrate to 4 – 5 ml under vacuum.

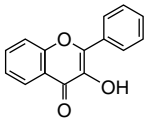
Sample application: slowly force or aspirate the sample through the column

Column washing: 8 ml dist. water

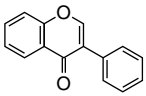
Elution: 6 ml methanol



Flavone



Flavonol



Isoflavone

Analysis of flavonols of *Sedum telephium* L. leaves by capillary electrophoresis and HPLC/MS

MN Appl. No. 302570

S. Sturm et al. Chromatographia **50** (1999) 438 – 433

Column type:
CHROMABOND® C18 / 100 mg / 1ml
Cat. No. 730001

Method A for development of CE and HPLC/MS methods:

Sample pretreatment: dissolve 10 mg lyophilised juice from *S. telephium* leaves in 0.1 ml of formic acid (0.1 mol/l)

Sample application: slowly force or aspirate the sample through the column

Elution: with water containing increasing amounts of MeOH. Flavonol glycosides eluted with 50% MeOH are evaporated to dryness. For analysis the residue is dissolved in 1 ml of a mixture of MeOH, acetone and water (50:25:25, v/v/v).

Method B for quantitative determination:

Sample pretreatment: 2 g of the fresh plant material are frozen with liquid nitrogen, lyophilised and ground. After addition of 1.70 mg of rutin, 500 mg is extracted with a mixture of MeOH, acetone and water (50:25:25, v/v/v) using an Ultra-Turrax T25, centrifuged and filtered. This procedure is repeated 7 times and the solvent of the collected filtrates is evaporated. The residue is dissolved in 5.00 ml formic acid (50 mM).

Sample application: slowly force or aspirate 0.50 ml of the solution through the column

Elution: with water, 10%, 25% and 50% MeOH (2 ml each). The last fraction containing the flavonol glycosides was evaporated and dissolved in 1.00 ml of a mixture of MeOH, acetone and water (50:25:25, v/v/v). A duplicate was prepared in the same way.

Further analysis: LC/MS

Isoflavones from plant tissues

MN Appl. No. 300140

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: extract tissue with ethanol – water (1:1, v/v) at 70 °C; dilute 1 ml of this extract with 1 ml dist. water

Column conditioning: 3 ml methanol, then 5 ml dist. water

Sample application: slowly force or aspirate the diluted extract through the column

Column washing: 3 ml water, then 5 ml water – acetonitrile (3:1, v/v)

Elution: 4 ml methanol

Fatty acids from serum

MN Appl. No. 300270

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: adjust serum sample to pH 3 with formic acid

Column conditioning: 1 column volume methanol, then 1 column volumn water

Sample application: slowly force or aspirate 1 ml adjusted serum through the column

Column washing: 2 ml dist. water; dry in a stream of air or nitrogen

Elution: 2 ml chloroform – methanol (1:1, v/v)

Fatty acids, cholesterol and bile acids from tissue

MN Appl. No. 300280

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg and
CHROMABOND® SB (≡ SAX) / 3 ml / 500 mg
Cat. Nos. 730013 and 730079

Sample pretreatment: saponify tissue with alcoholic potassium hydroxide solution, dilute with water to a 60 – 90% aqueous solution and acidify with 1 M hydrochloric acid

Conditioning of the C18 ec column: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate the pretreated sample through the conditioned column

Column washing: 1 ml water – acetone (8:2, v/v) (reduce volume, if acids or lipids are eluted); then dry the column under vacuum for 3 min

Elution: elute cholesterol and fatty acids with 2 x 500 µl petroleum ether (fraction 1), then elute the bile acids with 2 x 500 µl diethyl ether

Conditioning of the SB column: 2 column volumes petroleum ether

Sample application: apply cholesterol and fatty acid fraction (fraction 1) to the conditioned SB column and collect the eluate

Column washing: 1 – 2 ml petroleum ether, combine this eluate with the eluate from the sample application: cholesterol fraction

Elution: elute fatty acids with 2 x 500 µl acetonitrile – 0.1 M dipotassium hydrogen phosphate buffer pH 3 (7:3, v/v)

Isolation and quantitation of phosphatidylcholine

MN Appl. No. 303450

J. Hradec, P. Dufek, J. Chromatograpy B, **703** (1997) 259 – 262

Column type:
CHROMABOND® C18 / 1 ml / 100 mg
Cat. No. 730001

Sample pretreatment: total lipids from 0.1 ml of serum are extracted with twenty volumes of chloroform – methanol (2:1, v/v). Extracts are evaporated to dryness at 50 °C under a stream of nitrogen and residues are redissolved in 200 µl of chloroform – methanol.

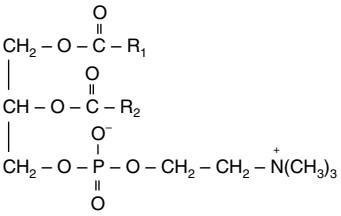
Column conditioning: 10 ml of 40 mM choline in methanol – acetonitrile – acetic acid (20:80:5, v/v/v).

Sample application: slowly force or aspirate the lipid extract through the column

Elution: phosphatidylcholine is eluted using an additional 5 ml of 40 mM choline in methanol – acetonitrile – acetic acid (20:80:5, v/v/v); eluates are collected in conical glass vials and evaporated to dryness at 50 °C under a stream of nitrogen in a Thermovap evaporator. Residues are dissolved in 0.5 ml of ethanol.

Recovery rates:
(added phosphatidylcholine determined using a colorimetric assay)

Added [mg]	Recovery [%]
0.50	97.8
1.00	103.6
1.50	102.0
2.00	103.3
2.50	100.4



Lipid fractions from serum

MN Appl. No. 302970

Private communication: R. Rickert, University of Hamburg, Germany

Compounds investigated: cholesterol esters, triacyl glycerols, phospholipids

Column type:
CHROMABOND® NH_2 / 3 ml / 500 mg
Cat. No. 730033

Sample pretreatment: dilute the lipid extract from 1 ml blood plasma in 1 ml *n*-hexane

Column conditioning: two column volumes of *n*-hexane

Sample application: slowly force or aspirate the diluted lipid extract through the column

Column washing: one column volume of *n*-hexane

Elution:

1st fraction: 3 ml *n*-hexane – chloroform (9:2, v/v) for cholesterol esters

2nd fraction: 4 ml *n*-hexane – diethyl ether (1:1, v/v) for triacyl glycerols

3rd fraction: 8 ml methanol for phopholipids

Isolation of different classes of lipids

MN Appl. No. 300290

Column type:
3 x CHROMABOND® NH_2 / 3 ml / 500 mg
Cat. No. 730033

Column conditioning: 10 ml hexane

Sample application: slowly force or aspirate 5 ml of the lipid-containing chloroform extract through the 1st column

Elution 1: (column 1)

neutral lipids: 1 ml chloroform – 2-propanol (2:1, v/v)

fatty acids: 1 ml diethyl ether – acetic acid (98:2, v/v)

phospholipids: 1 ml methanol

The fraction of the neutral lipids is evaporated, redissolved in hexane and applied to the 2nd column which was conditioned with hexane too.

Elution 2: (column 2)

cholesteryl esters: 1 ml hexane

Since during elution of the triglycerides cholesterol may be eluted too, a third column is placed under the second column during the next elution step.

Elution 3: (through joined columns 2 and 3)

triglycerides: 1 ml hexane – diethyl ether – methylene chloride (89:1:10, v/v/v)

Elution 4: (through separated columns 2 and 3)

cholesterol: 1 ml each of hexane – ethyl acetate (95:5, v/v)

Elution 5: (column 2)

diglycerides: 1 ml hexane – ethyl acetate (85:15, v/v)

monoglycerides: 1 ml chloroform – methanol (2:1, v/v)

Organic acids from plasma

MN Appl. No. 300300

Column type:
CHROMABOND® SB (≡ SAX) / 3 ml / 500 mg glass column
Cat. No. 730079 G

Sample pretreatment: heparinise 1 ml plasma

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 column volumes dist. water; then dry column for 5 min under nitrogen

Elution: 2 x 0.5 ml 0.5 mol/l sulphuric acid

Malondialdehyde from plasma as 2-thiobarbituric acid condensation product

MN Appl. No. 302000

J. Suttnar, Analyt. Biochemistry **249** (1997), 20-23

Column type:
CHROMABOND® C18 ec / 3 ml / 200 mg
Cat. No. 730012

Sample pretreatment: to 200 µl of plasma in a polypropylene Eppendorf tube (1.5 ml) are added 50 µl of a 1% NaOH solution and 250 µl of 10 mmol butylated hydroxytoluene (BHT) in acetonitrile. After thorough mixing the solution is incubated at 60 °C for 30 min in a water bath. After centrifugation at 10.000 g for 5 min, 300 µl of the supernatant are transferred to a polypropylene Eppendorf tube (1.5 ml), and 1.2 ml of 25 mmol 2-thiobarbituric acid (TBA) in a sodium phosphate buffer (0.125 mol, pH 3.0) are added, mixed well, and heated in a boiling water bath for 60 min. Resulting coloured solutions are diluted to 3.5 ml with sodium phosphate buffer (0.125 mol, pH 3.0).

Column conditioning: 2 ml methanol, then 2 ml sodium phosphate buffer (0.125 mol, pH 3.0)

Sample application: slowly force or aspirate the solution from the sample pretreatment through the column

Column washing: 2 x 3 ml water – methanol (95:5, v/v); dry column under vacuum for 20 min

Elution: coloured products are eluted with 800 µl methanol; extracts are concentrated in a stream of nitrogen

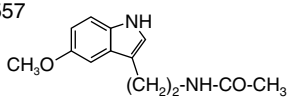
For further analysis we recommend HPLC with column NUCLEOSIL® 100-5 C_{18} (see MN Appl. No. 117430)

Melatonin in human breast cancer tissue

MN Appl. No. 302690

G. J. M. Maestroni, A. Conti, Laboratory investigation **75** (1996) 557

Column type:
CHROMABOND® C18 / 1 ml / 100 mg
Cat. No. 730001



Sample pretreatment: fresh neoplastics tissue was separated from fat tissue by macroscopic analysis of mastectomy or tumorectomy products from 15 breast cancer patients (mean age, 62.8 ± 16.4 years). The time elapsed from surgery to macroscopic examination and tissue sampling never exceeded 50 min. Representative samples of the neoplastic tissue were fixed and embedded in paraffin for histopathologic diagnosis or deep frozen and kept at –80 °C until homogenisation for estrogen and progesterone receptors evaluation and/or melatonin extraction. Both neoplastic and adipose tissues were homogenised and resuspended in 5 ml of perchloric acid 0.1 mol/l. The samples were ultracentrifugated at 100 000 x g for 45 min at 4 °C.

Column conditioning: 80% methanol followed by two washes with distilled water

Sample application: the supernatant from the centrifugation is slowly forced or aspirated through the column

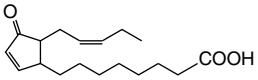
Column washing: methanol – water (10:90, v/v)

Elution: 600 µl of methanol

Further analysis: HPLC

Analysis of 12-oxo-phytodienoic acid enantiomers in biological samples

MN Appl. No. 302310



D. Laudert et al. Analytical Biochemistry **246** (1997) 211 – 217

Column type:
CHROMABOND® NH_2 / 3 ml / 500 mg
Cat. No. 730033

Sample pretreatment: leaf tissue is shock frozen in liquid nitrogen and powdered with mortar and pestle. The powder (10 – 50 g) is then extracted for 2 h at 20 °C in 2 x 25 ml of diethyl ether per gram tissue with stirring.

Sample application: 100 ml of extract are filtered and slowly forced or aspirated through the column

Column washing: 5 ml chloroform – 2-propanol (2:1, v/v)

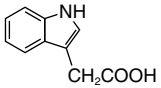
Elution: 10 ml diethyl ether – acetic acid (98:2, v/v); evaporate to dryness under a stream of nitrogen, dissolve in 0.2 ml HPLC solvent, and centrifuge for 2 min at 15 000 x g to remove particles

Further analysis: 0.1-ml aliquots is subjected to HPLC [column NUCLEOSIL® 100-10; eluent *n*-hexane – 2-propanol – acetic acid (98:1.5:0.5, v/v/v), flow rate 1 ml/min; t_R(*cis*-12-oxo-phytodienoic acid) = 15 min]. Appropriate fractions are evaporated to dryness in a stream of nitrogen and methylated with ethereal diazomethane. The dry fractions are finally redissolved in 10 µl $CHCl_3$ and analysed by GC/MS.

12-Oxo-phytodienoic acid and indole-3-acetic acid in jasmonic acid-treated tendrils of *Bryonia dioica*

MN Appl. No. 302200

B. A. Stelmach et al. Phytochemistry **51** (1999) 187–192



Column type:
CHROMABOND® NH_2 / 3 ml / 500 mg
Cat. No. 730033

Sample pretreatment: young leaves (2 – 2.5 g fr. wt) and tendrils (40 organs, approximately 3 – 3.5 g fr.wt) are extracted in 50 ml MeOH at 4 °C overnight. Prior to extraction, an internal standard of ($^2H_5$)*cis*-OPDA (about 250 ng [Stelmach et al., 1998]) and, where appropriate ($^{13}C_6$)-IAA (about 1.75 µg, Cambridge Isotope Laboratories, Andover, MA, isotopic enrichment 99%) is added.

Sample application: extracts are filtered and taken to dryness, redissolved in 20 ml of peroxide-free Et_2O and passed through the columns

Column washing: with 10 ml $CHCl_3$ – isopropanol (2:1, v/v)

Elution: with 12 ml Et_2O – HOAc (49:1, v/v); evaporate to dryness under a stream of nitrogen. Redissolve residues in 0.2 ml HPLC solvent, and centrifuge for 2 min at 15 000 x g to remove particles.

Further analysis: we recommend GC/MS

Determination of 8-iso-prostaglandin $F_{2\alpha}$ from tissue as indicator for oxidative stress

MN Appl. No. 303400

G. Wohler, Dissertation, Department of Chemistry, University of Hamburg, 2001, Germany

Compounds investigated: 8-iso-prostaglandin $F_{2\alpha}$

Column type:
CHROMABOND® C18 / 6 ml / 500 mg
Cat. No. 730004

Sample pretreatment: centrifuge a thawed cell supernatant at 5000 g (4500 U/min) at 4 °C. Mix 2.5 ml of the supernatant with 2.5 ng (3,3',4,4'-$^{2}H_4$)-8-iso-$PGF_{2\alpha}$ as internal standard. Adjust the pH of the sample solution to 2.0 – 3.0 using 5 mol/l formic acid.

Column conditioning: 10 ml methanol, 10 ml 0.05 mol/l formic acid

Sample application: slowly force or aspirate the pretreated sample through the column

Column washing: 10 ml 0.05 mol/l formic acid , then 2 ml heptane

Elution: 2 ml anhydrous ethyl acetate into 4 ml sample vials; concentrate sample to 0.5 ml under a stream of nitrogen, transfer to a silanised conic vial and then evaporate to dryness under a stream of nitrogen; redissolve in 100 µl anhydrous acetonitrile, add 10 µl anhydrous methanol, 10 µl ethyl-diisopropylamine and 10 µl pentafluorobenzylbromide (33% in acetonitrile); close the vial with a PTFE screw cap and derivatise by incubation at 30 °C for 1 h; again evaporate to dryness under a stream of nitrogen. Dissolve the residue in 50 µl anhydrous ethyl acetate and store at – 78 °C for at least 12 hours; finally centrifuge the samples (5 min, 1500 g, 4 °C), transfer the supernatant into another vial, again evaporate to dryness and redissolve the residue in 20 µl anhydrous ethanol.

Further analysis: TLC followed by GC/MS

Tandem mass spectrometric quantification of 8-iso-prostaglandin F_2 and its metabolite 2,3-dinor-5,6-dihydro-8-iso-prostaglandin F_2 in human urine

MN Appl. No. 302250

E. Schwedhelm et al. Journal of Chromatography B, **744** (2000) 99 – 112

Column type:
CHROMABOND® C18 / 3 ml / 500 mg
Cat. No. 730003

Sample pretreatment: (3,3',4,4'-$^{2}H_4$)-8-iso-$PGF_{2\alpha}$ and (1,1'-$^{18}O_2$)-ent-2,3-dinor-5,6-dihydro-8-iso-$PGF_{2\alpha}$ were added as internal standards to 5-ml aliquots of human urine samples resulting in final concentrations of 1 ng/ml. Solid phase extraction (SPE) and all derivatisation steps were performed as described elsewhere for 8-iso-$PGF_{2\alpha}$. Urine samples were acidified to pH 3.

Column conditioning: 10 ml methanol and 3 ml 0.05 mol/l HCOOH

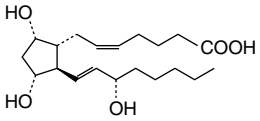
Sample application: slowly force or aspirate the pretreated samples through the column

Column washing: 20 ml of water and 2.5 ml of heptane

Elution: with 2 ml of ethyl acetate

Further analysis: convert analytes to their PFB ester TMS ether derivatives using PFB bromide and BSTFA (100 µl) and standard derivatisation procedures. Reversed-phase HPLC of isoprostanes is performed using a column NUCLEOSIL® 100-5 C_{18}, or GC/MS and GC/tandem MS analyses, or TLC silica gel plates.

Prostaglandin $F_{2\alpha}$



Prostaglandins (PG) from urine

MN Appl. No. 300510

J. Wübert, E. Reder et al., Anal. Chem. **69** (1997) 2143 – 2146

Compounds investigated:
2,3-dinor-6-keto-$PGF_{1\alpha}$ (30 – 1300 pg/ml), $PGF_{2\alpha}$, F_2 isoprostane, PGE_2, PGD_2, 11-dehydro-thromboxane B_2 (3 – 130 pg/ml)

Step 1:

Column type:
CHROMABOND® C18 ec / 6 ml / 1000 mg
Cat. No. 730015

Sample pretreatment: mix 3 ml urine sample with 0.5 ml isopropanol and adjust to pH 3 with 1 mol/l formic acid

Column conditioning: 12 ml methanol, 6 ml dist. water, then 6 ml 0.05 mol/l formic acid

Sample application: force or aspirate the prepared sample through the column

Column washing: 8 ml 1 mol/l formic acid – acetonitrile (3:1, v/v), 4 ml dist. water; then dry the column in a stream of nitrogen

Elution: 4 x 1 ml methanol, then evaporate in a stream of nitrogen, redissolve in *t*-butyl methyl ether, evaporate again; finally dissolve in 100 µl ethyl acetate – formic acid conc. (9:1, v/v), heat to 45 °C for 30 min and dry again in a stream of nitrogen

Step 2: derivatisation with BSTFA with subsequent SPE

Column type:
CHROMABOND® SiOH / 1 ml / 100 mg
Cat. No. 730071

Derivatisation: heat sample from step 1 with 0.5 g methoxyamine hydrochloride in 9.5 ml *N,N*-dimethylformamide at 45 °C for 30 min and evaporate; redissolve in 50 µl acetonitrile, 20 µl *N,N*-diisopropylethylamine and 20 µl pentafluorobenzylbromide solution (33%), heat 25 min to 45 °C and evaporate; then add 50 µl BSTFA and react 2 h at 45 °C and overnight at room temperature; evaporate in a stream of nitrogen and redissolve in 1 ml hexane

Column conditioning: 2 ml dichloromethane, 2 ml dichloromethane – hexane (1:1, v/v), then 2 ml hexane

Sample application: force or aspirate the derivatised sample through the column

Column washing: 2 ml hexane, 2 ml dichloromethane – hexane (1:1, v/v), then 2 ml dichloro-methane

Elution: 2 x 1 ml dichloromethane – methanol (100:1, v/v), evaporate in a stream of nitrogen, redissolve in 50 µl heptane

Recovery rates: > 80% (over the whole procedure)

Prostaglandins from urine and blood

MN Appl. No. 300520

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: adjust 10 ml urine or blood to pH 3 with 1 mol/l formic acid

Column conditioning: 2 column volumes methanol, then 2 column volumes 0.05 mol/l formic acid pH 3

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 x 500 µl 0.05 mol/l formic acid – acetonitrile (3:1, v/v); dry column 5 min under vacuum

Elution: 2 x 500 µl methanol

Leukotrienes and other eicosanoids in biological samples from asthmatic subjects

MN Appl. No. 303320

M. Kumlin, J. Chromatography A, **725** (1996) 29 – 40

Column type:
CHROMABOND® C18 / 3 ml / 200 mg
Cat. No. 730002

Sample pretreatment: acidify 4 ml urine with formic acid, subsequently dilute with an equal volume of methanol, and supplement with H-LTC_4 or H-LTE_4 as internal standard. Samples are left at –20 °C for 1 h followed by centrifugation (400 x g, 5 min)

Sample application: slowly force or aspirate the supernatant from the sample pretreatment through the column

Column washing: 2 ml water, then 2 ml 50% methanol

Elution: 2 ml pure methanol; evaporate to dryness under a gentle stream of nitrogen

Further analysis: HPLC with column NUCLEOSIL® 100-5 C_{18}

Cyclodextrins from plasma or urine

MN Appl. No. 300200

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Column conditioning: 2.5 ml dist. water, then 1.25 ml methanol, again 2.5 ml dist. water

Sample application: slowly force or aspirate 1 ml (or more) plasma or urine through the column

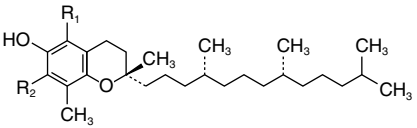
Column washing: 2.5 ml dist. water; then dry column with air

Elution: 1.25 ml dist. water – methanol (2:1, v/v)

Tocopherols from serum

MN Appl. No. 302940

Private communication: H.-U. Melchert and E. Pabel, Robert Koch Institut, Epidemiologie nicht übertragbarer Krankheiten, Umweltmedizin, Pharmakoepidemiologie, Germany



Adsorbent:
CHROMABOND® XTR
Cat. No. 730595.1000

Sample pretreatment: 100 µl serum are mixed with 200 µl methanol to denature the lipid/protein bonds

Liquid-liquid extraction: 5 min after denaturing the sample is added to a Pasteur pipette filled with CHROMABOND® XTR (filling height 4 cm)

Elution: 5 ml *n*-hexane, the extract is evaporated to dryness.

Solid phase extraction: for further purification the lipid extract from the liquid-liquid extraction is applied on a Pasteur pipette filled with silica gel (height 4 cm) using 2 x 100 µl of *n*-hexane

Elution: after waiting 4 min the column is eluted using 4 ml *n*-hexane – diethyl ether (5:1, v/v)

Further analysis: evaporate the extract from the silica column to dryness and either dissolve in 100 µl of HPLC mobile phase for normal-phase HPLC determination or it can be used for trimethylsilyl derivatisation of the different tocopherols for GC/MS. Derivatisation is done by heating the extract with 100 µl N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) for 20 min at 60 °C.

Recovery rates: 98 ± 5%

Compound	Absolute recovery	R_1	R_2
α-Tocopherol	11.62 ± 0.71 mg/l	CH_3	CH_3
β-Tocopherol	0.11 ± 0.01 mg/l	CH_3	H
γ-Tocopherol	0.69 ± 0.05 mg/l	H	CH_3
δ-Tocopherol	0.08 ± 0.01 mg/l	H	H

Isoprene metabolism in oak plants

MN Appl. No. 302640

Private communication: Th. Heuser, Fraunhofer Institut Atmosphärische Umweltforschung, Garmisch Partenkirchen, Germany

Column type:
CHROMABOND® SB / 1 ml / 100 mg
Cat. No. 730078

Sample pretreatment: deep-frosted oak leaves are milled under liquid nitrogen and extracted with urea. The extract is centrifuged before application.

Column conditioning: dist. water for about 30 minutes

Sample application: slowly force or aspirate the leave extract through the column

Column washing: 5 ml dist. water

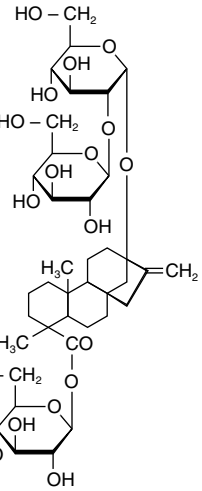
Elution: 2 ml KCl solution (200 mM)

Further analysis: HPLC

Diterpene glycosides from aqueous solutions

MN Appl. No. 302010

L. Bovanová, Z. Lebensm. Unters. Forschung A **207** (1998), 352 – 355



Compound investigated: stevioside

Column type:
CHROMABOND® C18 / 1 ml / 100 mg
Cat. No. 730001

Sample pretreatment:
Leaves of Stevia rebaudiana: 1 g of dried and powdered leaves is extracted three times, each time with 25 ml boiling dist. water for 30 min. The extracts are filtered through a filter paper, pooled and the volume adjusted to 100 ml with dist. water. A portion of this extract is diluted ten times and cleaned using SPE.

Tea: a packet of tea is extracted with 25 ml boiling dist. water for 30 min, cooled, the volume adjusted to 25 ml with dist. water and cleaned using SPE.

Orange juice: cleaned via SPE without any sample pretreatment.

Column conditioning: 3 column volumes methanol, then 3 ml dist. water

Sample application: slowly force or aspirate 0.5 ml sample through the column

Column washing: 3 ml dist. water, then 5 ml acetonitrile – water (20:80, v/v)

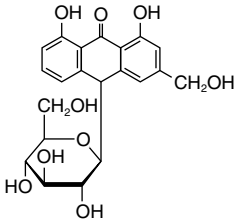
Elution: 1 ml methanol – acetonitrile (50:50, v/v)

For further analysis we recommend HPLC with column NUCLEOSIL® 100-5 C_{18}.

Anthraquinone glycosides: aloin from feces

MN Appl. No. 302340

A. Koch, J. Pharm. Biomed. Anal. **14** (1996) 1335 – 1338



Column type:
CHROMABOND® NH_2 / 3 ml / 500 mg
Cat. No. 730033

Sample pretreatment: collected feces are rapidly frozen and placed for drying under low pressure into a Christ Alpha 1-4 Freezing and Drying unit. The dried feces (20 g) are extracted with methanol several times in an ultrasonic bath and concentrated to 30 ml.

Column conditioning: methanol

Sample application: slowly force or aspirate 0.5 ml sample through the column

Column washing: 1 ml methanol

Elution: 1 ml methanol

Further analysis: the eluate (1 ml) is concentrated to 0.5 ml and analysed by HPLC and HPTLC

Biological samples and natural compounds

Nucleoside bases from aqueous solutions

MN Appl. No. 300440

Column type:
CHROMABOND® SA (≡ SCX) / 3 ml / 500 mg
Cat. No. 730077

Sample pretreatment: adjust sample to pH 4

Column conditioning: 1 column volume *n*-hexane, then 1 column volume methanol, finally 1 column volume dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 1 column volume water of pH 4; dry column under vacuum for 3 – 4 min

Elution: 2 x 500 µl phosphate buffer pH 7

Nucleosides from plasma and urine

MN Appl. No. 300450

Compounds investigated: 2',3'-didesoxyinosine

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: centrifuge sample

Column conditioning: 1 column volume methanol, then 2 column volumes dist. water

Sample application: force or aspirate 1 ml sample through the column with about 500 µl/min

Column washing: 2 ml dist. water (about 1 ml/min)

Elution: 2 ml methanol (500 µl/min)

Nucleosides from aqueous solutions

MN Appl. No. 300460

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: adjust sample to pH 7

Column conditioning: 2 column volumes methanol, 1 column volume dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 1 column volume dist. water; dry column 3 – 4 min under vacuum

Elution: 3 x 500 µl acetonitrile

For state-of-the-art purification and isolation of nucleic acids please ask for our catalogue BIOANALYSIS

Nucleotides from aqueous solutions

MN Appl. No. 300470

Column type:
CHROMABOND® SB (≡ SAX) / 3 ml / 500 mg
Cat. No. 730079

Sample pretreatment: adjust sample to pH 7

Column conditioning: 1 column volume *n*-hexane, then 1 column volume methanol, finally 1 column volume dist. water

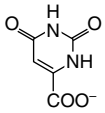
Sample application: slowly force or aspirate the sample through the column

Column washing: 1 column volume dist. water;
dry column 3 – 4 min under vacuum

Elution: 2 x 500 µl 0.1 mol/l hydrochloric acid; for acid sensitive nucleotides 2 x 500 µl 0.1 mol/l aqueous sodium carbonate solution

Orotate from urine

MN Appl. No. 300480



Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: filter urine sample through a 0.2 µm CHROMAFIL® filter A-20/25 (Cat. No. 729006)

Column conditioning: 1 column volume acetonitrile – dist. water (6:4, v/v), then 1 column volume dist. water

Sample application: slowly force or aspirate the sample through the column, collect the eluate

Elution: 2 x 500 µl water, combine with the above eluate and adjust to pH 1.5 – 2.0 with conc. hydrochloric acid

PAH and PCB from blood, serum and plasma

MN Appl. No. 301350

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: mix 1 ml sample with 1 ml water – *n*-propanol (85:15, v/v) and centrifuge

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water – *n*-propanol (85:15, v/v)

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 x 500 µl dist. water – *n*-propanol (85:15, v/v); dry in a stream of air or nitrogen

Elution: 2 x 250 µl dichloromethane

PCB and organochlorine pesticides from serum

MN Appl. No. 301410

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: dilute 2.5 ml serum with 2.5 ml dist. water – 1-propanol (85:15, v/v), mix intensely and centrifuge

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water – 1-propanol (85:15, v/v)

Sample application: force or aspirate the clear supernatant of the pretreated sample through the column

Column washing: 2 x 500 µl dist. water – 1-propanol (85:15, v/v); dry in a stream of air or nitrogen

Elution: 4 x 250 µl *n*-hexane

PCB and pesticides from adipose tissue

MN Appl. No. 301420

Column type:
CHROMABOND® SA (≡ SCX) / 3 ml / 500 mg and
CHROMABOND® ALOX / 6 ml / 1000 mg
Cat. Nos. 730077 and 730139

Sample pretreatment: dissolve 0.5 g melted, filtered fat at 50 °C in 5 ml *n*-hexane. Precipitate fat 5 min in an ice bath at 0 °C. Warm solution to ambient temperature without disturbing the precipitated fat.

Column conditioning: force or aspirate 1 column volume *n*-hexane each through the SA and the ALOX columns; then pour 1 ml *n*-hexane into the ALOX column and place the SA column onto the ALOX column using the adaptor

Sample application: force or aspirate 0.5 ml of the clear supernatant from the sample pretreatment through both columns; collect the eluate

Column washing: force or aspirate 3 x 0.5 ml *n*-hexane through the columns; collect the eluates

Elution: remove the SA column and elute the ALOX column with 5 ml cyclohexane – dichloromethane (85:15, v/v) – combine the eluate with the other eluates and concentrate

PCB and organochlorine pesticides from animal fats

MN Appl. No. 301430

Column type:
CHROMABOND® SA (≡ SCX) / 3 ml / 500 mg
Cat. No. 730077

Sample pretreatment: dissolve 0.5 g precipitated fat in 5 ml *n*-hexane at 50 °C; precipitate fat in an ice bath (5 min); warm solution to ambient temperature without disturbing the precipitated fat; use the clear supernatant

Column conditioning: 1 column volume *n*-hexane

Sample application: pour 0.5 ml sample through the column, collect the eluates

Elution: 3 x 500 µl *n*-hexane, combine eluate with the eluate from the sample application; if necessary, concentrate eluates in a nitrogen stream and redissolve in 100 µl *n*-hexane

PCB and organochlorine pesticides from animal fats
MN Appl. No. 301440

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: dissolve 0.5 g melted fat in 1 ml ethyl acetate at 50 °C, add 4 ml absolute methanol and precipitate fat in an ice bath (2 min); warm solution to ambient temperature without disturbing the precipitated fat

Column conditioning: 1 column volume *n*-hexane, then 1 column volume methanol

Sample application: force or aspirate 0.5 ml of the clear supernatant through the column

Column washing: 500 µl acetonitrile – water (1:1, v/v); dry column under vacuum for 10 min

Elution: 3 x 500 µl *n*-hexane; concentrate eluate in a stream of nitrogen and redissolve in 100 µl *n*-hexane

Pesticides from animal oils
MN Appl. No. 301450

Column type:
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. No. 730073

Sample pretreatment: mix 0.5 g oil with *n*-hexane

Column conditioning: 2 column volumes *n*-hexane

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 x 2 ml *n*-hexane – 2-propanol (95:5, v/v)

Elution: 2 x 1.5 ml dichloromethane – *n*-hexane – acetonitrile (50:48:2, v/v/v)

Pesticides from plant and animal materials
MN Appl. No. 301720

Compounds investigated: organochlorine pesticides aldrin, *p,p'*-DDT, dieldrin, heptachlor, lindane, methoxychlor (for formulas see page 173)

Column type:
CHROMABOND® C18 ec / 6 ml / 500 mg
Cat. No. 730014

Sample pretreatment: homogenise 10 g sample with 100 ml methanol and filter. Dilute solution with water to a methanol content < 40% (maximum volume 250 ml).

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: force or aspirate 250 ml aqueous methanolic extract through the column with 30 ml/min

Column washing: 1 column volume dist. water; dry column 10 min under vacuum

Elution: 3 x 500 µl *n*-hexane

For formulas of PAH, PCB and pesticides see structure index from page 239

Biological samples and natural compounds

Pesticides: atrazine from tissues

MN Appl. No. 301600

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: homogenise tissue in methanol, filter and dilute with the 10-fold volume of dist. water

Column conditioning: 1 column volume methanol, then 1 column volume dist. water

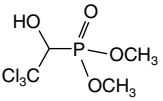
Sample application: slowly force or aspirate the sample through the column

Column washing: 1 column volume dist. water

Elution: methanol

Pesticides: insecticide dipterex® from serum

MN Appl. No. 301680



Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: mix 1 ml serum with 1 ml 0.1 M HCl

Column conditioning: 1 column volume water, 1 column volume 0.1 M HCl

Sample application: slowly force or aspirate the sample through the column

Column washing: 1 ml 0.1 M HCl, 3 ml methanol – water (1:9, v/v)

Elution: 2 x 1 ml methanol – water (1:1, v/v) with 3 ml/min

Polyethylene glycol 400 from plasma or urine

MN Appl. No. 300490

C. Fakt, M. Ervik, J. Chromatography **700** (1997) 93 – 100

Column type:
CHROMABOND® C18 / 3 ml / 500 mg
Cat. No. 730003

Sample pretreatment: mix 1 g centrifuged plasma or urine sample with 50 µl 1,16-hexadecanediol (IS)

Column conditioning: force or aspirate 5 ml methanol, then 5 ml dist. water – methanol (99:1, v/v) through the column

Sample application: apply sample to the column; rinse sample vessel with dist. water – methanol (99:1, v/v) and add rinsing liquid to the column; slowly force or aspirate the sample through the column

Column washing: force or aspirate 4 ml dist. water – methanol (99:1, v/v), then 4 ml hexane through the column

Elution: 10 ml dichloromethane – isopropanol (85:15, v/v); evaporate in a gentle stream of nitrogen and redissolve with 200 µl toluene and 50 µl heptafluorobutyric acid anhydride (HFBA)

Recovery rate: 93% (1.0 – 500 µmol/l, RSD 2.7; n = 4)

Pharmaceuticals and drugs

Automatic extraction of indomethacin from human plasma

MN Appl. No. 302400

P. Hubert, J. Crommen, Journal of Liquid Chromatography **13** (1990) 3891 – 3907

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: the plasma sample is centrifuged at 3000 rpm during 10 min. 2 ml of plasma are introduced into a vial placed on the appropriate rack of the auto-sampler. After this manual operation, the automatic procedure is started.

Column conditioning: column holder located above the drain cuvette (front position): 2.0 ml methanol, then 2.0 ml phosphate buffer pH 7.4; flow rate 3.0 ml/min; air volume 0.1 ml

Sample application: 1.0 ml of plasma; flow rate 0.18 ml/min; air volume 1.0 ml

Column washing: 2.0 ml phosphate buffer pH 7.4; flow rate 1.5 ml/min; air volume 1.0 ml. Then flush packing with 1.0 ml air.

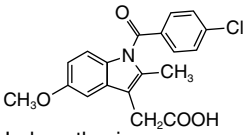
Elution: column holder pushed over the collection rack: 0.25 ml methanol; flow rate 1.5 ml/min; air volume 1.0 ml. The eluate is collected in the tube positioned under the column.

Dilution: 0.3 ml phosphate buffer pH 7.4; flow rate 1.5 ml/min; air volume 1.0 ml. The column holder is then replaced in its position.

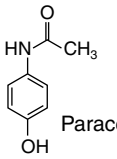
Further analysis: HPLC

Recovery rate: absolute recovery was 96%

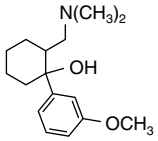
Analgetics



Indomethacin



Paracetamol



Tramadol

Analgetics: paracetamol from serum

MN Appl. No. 300700

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: mix 1 ml serum with 1 ml 0.1 M Tris buffer, which was adjusted to pH 7 with 1 mol/l hydrochloric acid

Column conditioning: 2 column volumes methanol, then 2 column volumes 0.1 M Tris buffer, adjusted to pH 7 with 1 mol/l hydrochloric acid

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 x 250 µl water

Elution: 2 x 200 µl methanol

Direct determination of tramadol glucuronides in human urine by HPLC with fluorescence detection

MN Appl. No. 302600

P. Overbeck et al., J. Chromatography B, **732** (1999) 185 – 192

Sample pretreatment: mix 0.5 ml of urine with 0.5 ml of 1.0 M ammonium sulphate adjusted to pH 9.8 with ammonia

Step 1:

Column type:
C18 / 1 ml / 100 mg
Cat. No. 730001

Column conditioning: 2.0 ml methanol, 1.0 ml water, and 1.0 ml of 1.0 M ammonium sulphate adjusted to pH 9.8 with ammonia

Sample application: slowly force or aspirate the pretreated sample solution through the column

Column washing: 1 ml 1 M ammonium sulphate and 1 ml water

Elution: 2 ml of 1.0 M phosphoric acid – methanol (7:3, v/v)

Step 2:

Column type:
CHROMABOND® SA / 3 ml / 500 mg
Cat. No. 730077

Column conditioning: 3 ml methanol, 2 ml water, and 1 ml of a mixture of 0.1 M phosphoric acid – methanol (7:3, v/v)

Sample application: force or aspirate the eluate (2 ml) from the C18 cartridge through the cation exchange cartridge followed by 1 ml of 0.1 M phosphoric acid – methanol (7:3, v/v), 1 ml 0.1 M acetic acid, and 2 ml methanol

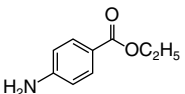
Elution: 2 ml of 3% ammonia in methanol; evaporate to dryness under a gentle stream of nitrogen. Add 50 µl of internal standard solution. After evaporation of the solvent dissolve residue in 1.0 ml of mobile phase. Inject 50 µl for HPLC.

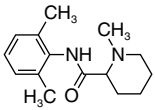
Recovery rates:
(diasteromers of *O*-demethyltramadol glucuronide after extraction from blank urine)

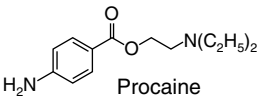
Compound	Concentration [µg/ml]	Average recovery [%]
1*R*,2*R*-enantiomer	0.035	91.9
	0.086	93.2
	0.539	89.7
	1.081	94.9
	2.158	88.3
	4.317	82.3
1*S*,2*S*-enantiomer	0.042	97.5
	0.105	89.8
	0.654	87.6
	1.312	93.7
	2.620	87.8
	5.240	81.0

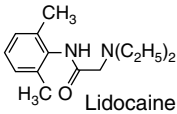
Pharmaceuticals and drugs

Anesthetics


Benzocaine


Mepivacaine


Procaine


Lidocaine

Anesthetics from serum

MN Appl. No. 300710

Compounds investigated: benzocaine, mepivacaine, procaine

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Column conditioning: 1 column volume methanol, then 1 column volume dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 8 ml water – methanol (3:1, v/v)

Elution: 500 µl methanol

Anesthetics: lidocaine and metabolites from serum

MN Appl. No. 300720

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate 500 µl serum through the column

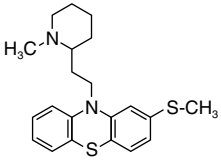
Column washing: 3 column volumes dist. water – methanol (3:1, v/v)

Elution: 2 x 200 µl methanol

Tranquilisers: thioridazine from serum

MN Appl. No. 301110

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011



Sample pretreatment: mix 500 µl serum with 500 µl 0.2 M phosphoric acid (pH about 2.3), shake and let stand 5 min

Column conditioning: 1 ml methanol, then 1 ml dist. water

Sample application: slowly pour 400 µl pretreated sample through the column

Column washing: 1 ml H_2O, 1 ml acetonitrile – water (1:1, v/v)

Elution: acetonitrile – methanol – buffer A (1:2:1, v/v/v), pH 4.1 [buffer A: 4.5 ml 85% phosphoric acid and 4.5 ml triethylamine filled up to 1000 ml with water (pH 2.2)]

Sedative / hypnotic drugs from urine

MN Appl. No. 301070

Compounds investigated: amobarbital, barbital, caffeine, diazepam, glutethimide, meprobamate, methaqualone, 4-methylprimidone, methyprylon, nordiazepam, oxazepam, phenacetin, phenobarbital, secobarbital (for structures not shown below see index from page 239)

Column type:
CHROMABOND® C18 ec / 6 ml / 500 mg
Cat. No. 730014

Sample pretreatment: adjust 20 ml urine to pH 7 with 2 ml 0.5 M phosphate buffer

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

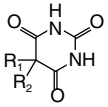
Sample application: slowly force or aspirate the sample solution through the column

Column washing: 3 ml dist. water

Elution: 2 x 500 µl acetone – chloroform (1:1, v/v)

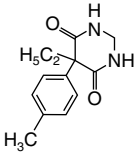
Sedative / hypnotic drugs

Barbiturates

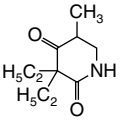


Compound	R_1	R_2
Amobarbital	C_2H_5	$(CH_2)_2$-$CH(CH_3)_2$
Barbital	C_2H_5	C_2H_5
Phenobarbital	C_2H_5	C_6H_5
Secobarbital	$CH(CH_3)$-C_3H_7	CH_2-CH=CH_2

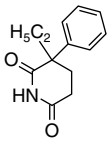
4-Methylprimidone



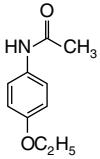
Methyprylon



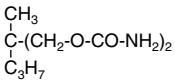
Glutethimide



Phenacetin



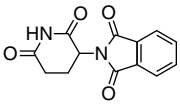
Meprobamate



Sedative / hypnotic drugs from urine

MN Appl. No. 301080

Compounds investigated: thalidomide analogues



Column type:
CHROMABOND® C18 ec / 3 ml / 200 mg
Cat. No. 730012

Sample pretreatment: stabilise urine (100 – 500 µl) with twice its volume of hydrochloric acid – potassium chloride buffer pH 2 (0.2 M HCl – 0.2 M KCl – water [5.3:25:69.7, v/v/v])

Column conditioning: 2 ml methanol, then 5 ml dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 ml hydrochloric acid – potassium chloride buffer pH 2, then 3 ml buffer – methanol (85:15, v/v)

Elution: 2 ml methanol – water (7:3 or 6:4, v/v) pH 7.0

Barbiturates from urine

MN Appl. No. 300950

Column type:
CHROMABOND® C18 ec / 6 ml / 500 mg
Cat. No. 730014

Sample pretreatment: adjust 20 ml urine to pH 7 with 0.5 M potassium phosphate buffer

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: force or aspirate the sample through the column with 4 ml/min

Column washing: 1 column volume dist. water

Elution: 2 x 500 µl acetone – chloroform (1:1, v/v)

Barbiturates from serum

MN Appl. No. 300940

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: mix 1 ml sample with 1 ml 0.01 M hydrochloric acid

Column conditioning: 2 column volumes methanol, then 2 column volumes 0.01 M hydrochloric acid

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 x 500 µl water; dry column for 5 min under vacuum

Elution: 2 x 250 µl methanol – dichloromethane (1:1, v/v)

Liquid-liquid extraction of heterocyclic pharmaceuticals from aqueous solutions

MN Appl. No. 302120

Column type:
CHROMABOND® XTR / 70 ml / 14.5 g
Cat. No. 730507

Sample pretreatment: add 1 ml of a spiked solution (10 mg chlorpromazine and methaqualone in 100 ml acetonitrile) to 9 ml of an aqueous sample solution. Transfer 1 ml of this solution to 19 ml aqueous NH_3 solution (pH 9).

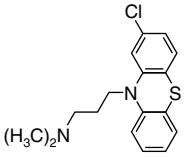
Sample application: apply the ammoniacal sample solution to the column and allow the solution to be soaked up for 10 min

Elution: elute with 30 ml dichloromethane – isopropanol (85:15, v/v) and evaporate the eluate to dryness with a rotation evaporator. Rinse the flask with four times 125 µl acetonitrile each and transfer the combined solutions into a HPLC vial. Fill up with 500 µl phosphate buffer (50 mmol NaH_2PO_4, pH 2.5).

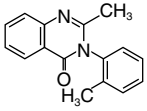
Further analysis: HPLC with column NUCLEOSIL® 100-5 C_{18} HD.

Recovery rates:

Chlorpromazine: 91%



Methaqualone: 92%



Nonpolar pharmaceuticals from polar syrupy liquids

MN Appl. No. 300040

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: dilute syrup with so much dist. water, that it can be readily forced or aspirated through the column

Column conditioning: 1 column volume methanol, then 1 column volume dist. water

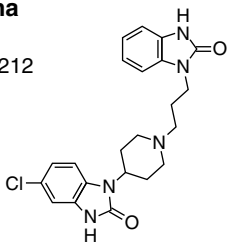
Sample application: slowly force or aspirate sample through the column

Column washing: 0.5 column volume water; dry column under vacuum

Elution: 3 x 1.5 ml *n*-hexane (or methanol, diethyl ether)

Drugs: domperidone in human plasma

MN Appl. No. 303350



M. Kobylinska, K. Kobylinska, J. Chromatography B, **744** (2000) 207 – 212

Column type:
CHROMABOND® CN / 3 ml / 500 mg
Cat. No. 730063

Sample pretreatment: to 1 ml of plasma in a glass tube are added 20 µl of methanol, 50 µl of an aqueous internal standard solution of cisapride (4 µg/ml) and 0.1 ml of 0.1 M HCl. The plasma is mixed for 10 s with a hand vortex mixer and centrifuged at 2000 g for 10 min.

Column conditioning: 2 ml of water, then 1 ml of 0.1 M HCl

Sample application: the solution is passed slowly through the column by mild suction (<1 ml/min)

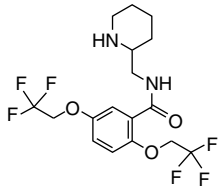
Column washing: 2 ml of water, then 1 ml of acetone, finally dry column under vacuum for 10 min

Elution: a 1 ml aliquot of a mixture consisting of methanol (100 ml), triethylamine (0.03 ml), and acetic acid (0.3 ml) is applied to the column, allowed to pass through the column by gravity and finally drained completely by centrifugation at 2000 g for 1 min; the eluent is evaporated to dryness under an air stream at 50 °C

Further analysis: HPLC

Antiarrhythmic drug flecainide from plasma

MN Appl. No. 300740



Column type:
CHROMABOND® C8 / 1 ml / 100 mg
Cat. No. 730021

Sample pretreatment: mix 1 ml plasma with 1 ml water and 200 µl 0.2 M sodium carbonate solution

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

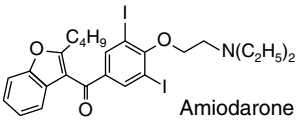
Sample application: slowly force or aspirate the sample through the column

Column washing: 2 column volumes dist. water, then 2 x 1 ml acetonitrile

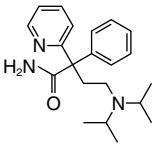
Elution: 500 µl methanol, leave solvent in the column packing for 1 min, then elute

Pharmaceuticals and drugs

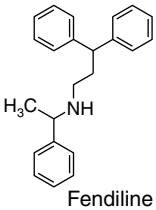
Antiarrhythmic drugs



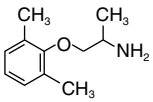
Amiodarone



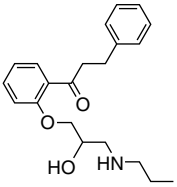
Disopyramide



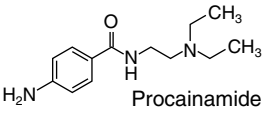
Fendiline



Mexiletin

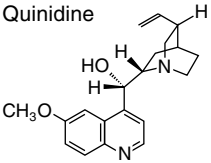


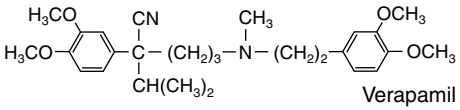
Propafenone



Procainamide

Quinidine





Verapamil

Antiarrhythmic drugs and metabolites in serum

MN Appl. No. 302190

E. Brandsteterová, A. Ferencová, Chem. Listy **93** (1999) 249 – 253

Compounds investigated: mexiletin, propafenone, 5-hydroxypropafenone, verapamil, norverapamil, D617, D620, fendiline

Column type:
CHROMABOND® C18 / 3 ml / 200 mg
Cat. No. 730002

Column conditioning: 3 ml methanol, then 3 ml water

Sample application: slowly force or aspirate 1 ml serum through the column

Column washing: 2 ml water, then 1 ml acetonitrile

Elution: methanol containing 0.1 – 0.5% triethylamine; evaporate the eluate to dryness and dissolve the residue in 200 µl mobile phase

Further analysis: 20 µl portions of the redissolved extract are analysed by HPLC: column NUCLEOSIL® C_{18} with aqueous 35% acetonitrile containing 0.2% triethylamine as mobile phase (1 ml/min) and detection at 245 nm.

Recovery rates: 81.4 – 90.7% for 0.06 – 0.43 µg/ml of drugs added to serum

Antiarrhythmic drugs from serum

MN Appl. No. 300730

Compounds investigated: *N*-acetylprocainamide, quinidine, despropyldisopyramide, disopyramide, lidocaine, procainamide

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: mix 200 µl serum with 200 µl 0.1 M sodium carbonate solution and add 50 µl internal standard, if desired (15 µg/ml 2-*p*-chlorodisopyramide in 0.01 mol/l HCl)

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 3 column volumes dist. water – MeOH (9:1, v/v)

Elution: 2 x 200 µl of a solution of 100 mM acetic acid and 50 mM diethylamine in methanol

Antiarrhythmic drugs: amiodarone from serum

MN Appl. No. 302610

Private communication: Mr. Nicholls, Zentralklinikum Augsburg, Inst. f. Labormedizin u. Toxikologie, Dept. Dr. I. Renk, Germany

Column type:
CHROMABOND® CN / 3 ml / 500 mg
Cat. No. 730063

Sample pretreatment: mix 1 ml thawed calibrator, control or patient serum with 100 µl ISTD (trifluoperazine 500 ng/100 µl) and 1 ml water

Column conditioning: 1 column volume methanol, then 1 column volume water; DO NOT USE VACUUM

Sample application: apply the whole prepared sample to the column without vacuum

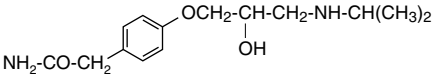
Column washing: 2 column volumes water, then 2 x 1 ml methanol – water (50:50, v/v) without vacuum; dry the columns by centrifugation (3 – 5 min at 1000 rpm and 30 s >3000 rpm)

Elution: 1 ml of the elution reagent (mixture of 100 ml diisopropyl ether, 80 ml propanol-2 and 20 ml ammonim hydroxide solution 25%); evaporate to dryness in a gentle stream of nitrogen; add 200 µl of the mobile phase “TAD-Clozapin” (mixture of 1500 ml AAS water, 3 ml phosphoric acid 85% suprapure, 6 ml triethylamine, 800 ml acetonitrile, adjusted to pH 4.2 with phosporic acid) to the sample tube and vortex thoroughly for 30 s. The dissolved sample is centrifuged for at least 5 minutes. Use conical HPLC sample vials for the liquid fraction.

Further analysis: HPLC

Beta-blockers: atenolol from plasma

MN Appl. No. 300960



Column type:
CHROMABOND® CN / 1 ml / 100 mg
Cat. No. 730061

Column conditioning: 2 x 1 ml acetonitrile, then 2 x 1 ml dist. water

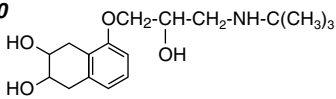
Sample application: slowly force or aspirate 1 ml plasma through the column

Column washing: 500 µl water, then 500 µl acetonitrile

Elution: 2 x 250 µl 0.05 M disodium hydrogen phosphate – acetonitrile (7:3, v/v) with 4 mM triethylamine, adjusted to pH 4 with orthophosphoric acid

Beta-blockers: nadolol from serum

MN Appl. No. 300970



Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: mix 1 ml serum with 1 ml 0.1 M sodium acetate

Column conditioning: 2 column volumes methanol, then 2 column volumes 0.1 M sodium acetate

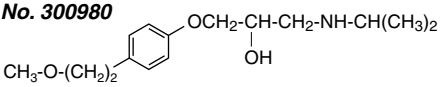
Sample application: slowly force or aspirate 2 ml sample through the column

Column washing: 2 x 250 µl water – 0.1 M sodium acetate (7:3, v/v), then 200 µl *n*-hexane; dry column under vacuum for 5 min

Elution: 2 x 250 µl methanol

Beta-blockers: metoprolol from plasma

MN Appl. No. 300980



Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Column conditioning: 2 x 1 ml acetonitrile, then 2 x 1 ml dist. water

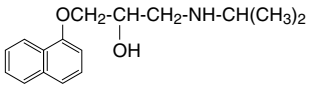
Sample application: slowly force or aspirate 1 ml plasma through the column

Column washing: 2 x 500 µl dist. water – acetonitrile (9:1, v/v)

Elution: 500 µl acetonitrile – 0.1 M hydrochloric acid (1:1, v/v)

Beta-blockers: propranolol from serum

MN Appl. No. 300990



Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: mix 2 ml serum with 500 µl water and adjust to pH 7

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 x 250 µl dist. water – acetonitrile (95:5, v/v)

Elution: 2 x 250 µl methanol or acetonitrile – methanol (60:40, v/v)

Sympathomimetics: amphetamines from biological samples

MN Appl. No. 301090

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: adjust sample to pH 10 with 0.1 M aqueous ammonia solution

Column conditioning: 2 column volumes methanol, then 2 column volumes 0.1 M aqueous ammonia solution

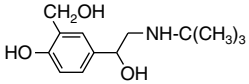
Sample application: slowly force or aspirate 2 ml sample through the column

Column washing: 2 x 500 µl water – acetonitrile (9:1, v/v); dry column under vacuum for 5 min

Elution: 2 x 500 µl ethyl acetate

β-Sympathomimetics: salbutamol from calves urine

MN Appl. No. 301100



Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: mix 4 ml urine with 4 ml phosphate buffer pH 7.6 (buffer: 3.58 g KH_2PO_4 + 7.26 g Na_2HPO_4, filled to 1 l with H_2O and adjusted to pH 7.6 with 1 mol/l NaOH)

Column conditioning: 9 ml methanol, 6 ml water, 2 ml phosphate buffer pH 7.6

Sample application: slowly force or aspirate the sample through the column

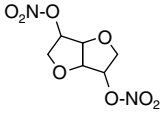
Column washing: 9 ml dist. water, then 6 ml water – methanol (4:1, v/v), finally 500 µl methanol; then dry column under vacuum

Elution: 9 ml methanol; concentrate eluate in a stream of nitrogen at 60 °C

Vasodilators from blood or plasma

MN Appl. No. 301120

Compounds investigated: isosorbide dinitrate



Column type:
CHROMABOND® C18 ec (or Phenyl) / 3 ml / 500 mg
Cat. No. 730013 (or 730084)

Sample pretreatment: immediately after sampling cool 9 ml blood to 0 °C in a pre-cooled test tube containing 1 ml of a 3.8% sodium citrate solution and centrifuge at 4 °C. Use the supernatant plasma (if necessary, store at – 20 °C)

Column conditioning: 2 ml methanol, then 2 ml dist. water

Sample application: slowly force or aspirate the sample through the column

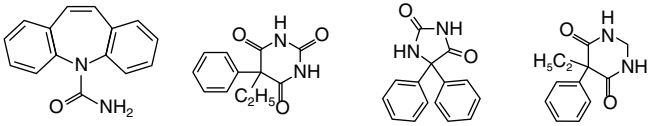
Column washing: 2 x 1 ml water; vacuum dry column 5 min; rinse with 50 µl *n*-hexane, again dry under vacuum

Elution: 1 ml methanol

Antiepileptics from serum

MN Appl. No. 300860

Compounds investigated: carbamazepine, phenobarbital, phenytoin (=dilantin), primidone



Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: mix 500 µl serum with 500 µl internal standard (500 µg/ml 4-methylprimidone in citrate buffer pH 4)

Column conditioning: 2 column volumes methanol, 2 column volumes water

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 column volumes dist. water

Elution: 2 x 100 µl acetone

Antiepileptics from serum

MN Appl. No. 300870

Compounds investigated: carbamazepine, dilantin (= phenytoin), phenobarbital, primidone

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: mix 500 µl serum with 100 µl 0.1 M phosphate buffer pH 3.5 and 100 µl internal standard (200 µg/ml 5-*p*-methylphenyl-5-phenylhydantoin)

Column conditioning: 2 column volumes methanol, 2 column volumes water

Sample application: slowly force or aspirate the sample through the column

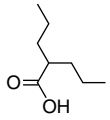
Column washing: 3 column volumes dist. water

Elution: 2 x 200 µl methanol

Antiepileptics: valproic acid from serum

MN Appl. No. 300880

Compounds investigated: valproic acid = 2-propylpentanoic acid



Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: mix 500 µl serum with 500 µl internal standard (100 µg/ml cyclohexane carboxylic acid in 0.7 M phosphoric acid)

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

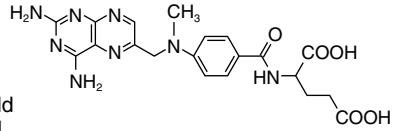
Sample application: slowly force or aspirate the sample through the column

Column washing: 4 column volumes water

Elution: 2 x 200 µl acetone

Antineoplastic agents: methotrexate from plasma

MN Appl. No. 300900



Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: mix 500 µl plasma with 50 µl aqueous ascorbic acid solution (10 g/l); if desired, add internal standard and 500 µl 5% aqueous acetic acid

Column conditioning: 5 ml methanol, then 5 ml dist. water

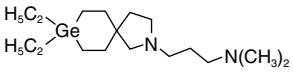
Sample application: slowly force or aspirate the sample through the column

Column washing: 2 ml 10 mM ammonium formiate buffer (pH 3.5) containing 1 g/l ascorbic acid

Elution: acetonitrile – 1 g/l ascorbic acid in 10 mM phosphate buffer pH 6 (5:95, v/v)

Antineoplastic agents: spirogermanium from serum

MN Appl. No. 300910



Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: dilute 1 ml serum with 1 ml dist. water

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

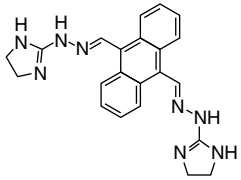
Sample application: slowly force or aspirate the sample through the column

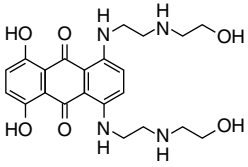
Column washing: 2 x 500 µl water, then 2 x 250 µl *n*-hexane; dry column for 5 min under vacuum

Elution: 2 x 250 µl methanol

Antineoplastic agents: bisantrene and mitoxantrone from plasma

MN Appl. No. 300890





Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

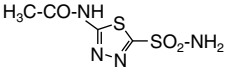
Sample application: slowly force or aspirate 1 – 2 ml plasma through the column

Column washing: 2 column volumes dist. water

Elution: 2 x 200 µl 0.5 M methanolic hydrochloric acid

Diuretics: acetazolamide from plasma

MN Appl. No. 301040



Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Column conditioning: 2 column volumes methanol, then 2 column volumes 0.1 M hydrochloric acid

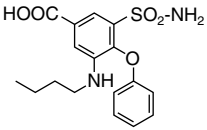
Sample application: apply 100 µl plasma to the column (if desired, add 50 µl propazolamide, 4 µg/ml, as internal standard), let stand 1 min, then force or aspirate sample through the column, let stand 2 min

Column washing: 1 column volume 0.1 M hydrochloric acid, 1 column volume dist. water, finally 200 µl methanol

Elution: 300 µl methanol

Diuretics: bumetanide from urine

MN Appl. No. 301050



Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Column conditioning: 1 column volume methanol, then 1 column volume methanol – water (5:95, v/v), finally 1 ml buffer pH 5 (0.1 M potassium dihydrogen phosphate – 1.34 M potassium chloride)

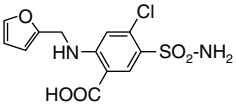
Sample application: slowly force or aspirate 1 ml urine through the column

Column washing: 3 x 2 ml methanol – water (5:95, v/v); dry column under vacuum

Elution: 2 ml methanol

Diuretics: furosemide from plasma and urine

MN Appl. No. 301060



Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: work in subdued daylight, use amber glass vessels; mix 180 µg desmethylnaproxene (as internal standard) with 500 µl plasma or 50 µl urine, 450 µl 0.075 M phosphate buffer pH 6.8 and 1.5 ml 50% aqueous urea solution. Shake briefly and let stand 10 min. Add 2 ml (1.5 ml for urine) 0.01 M potassium citrate buffer pH 3.0. Shake briefly again.

Column conditioning: 3 column volumes methanol, then 3 column volumes 0.01 M potassium citrate buffer pH 5.0

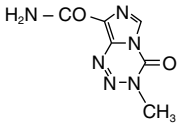
Sample application: slowly force or aspirate pretreated sample through the column

Column washing: 10 ml 0.01 M K-citrate buffer pH 5.0

Elution: 1 ml methanol – 0.01 M aqueous sodium hydrogen carbonate solution (1:1, v/v)

Antitumor drug temozolomide from plasma and urine

MN Appl. No. 300930



L.A. Decosterd et al., J. Chromatography **667** (1995) 291 – 300

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: biological samples are immediately stabilised with 1 M HCl (10 + 1), frozen and stored at –20 °C. 253 µl of acidified plasma or urine are mixed with 115 µl internal standard solution.

Column conditioning: 2 x 1 ml methanol, then 2 x 1 ml 0.5% acetic acid

Sample application: 160 µl of the prepared sample are applied to the column under light vacuum

Column washing: cartridges are allowed to stand for 1 min, then washed with 750 µl of 0.5% acetic acid and finally dried under vacuum for 5 min

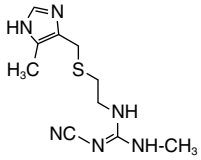
Elution: 1.25 ml methanol; the eluate is evaporated under a stream of nitrogen at room temperature, redissolved in 200 µl 0.5% acetic acid and centrifuged

Further analysis: HPLC

Recovery rates: 86 – 90% temozolomide from plasma, 103 – 105% temozolomide from urine

Cimetidine from plasma

MN Appl. No. 301030



Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Column conditioning: 1 column volume methanol, then 1 column volume dist. water

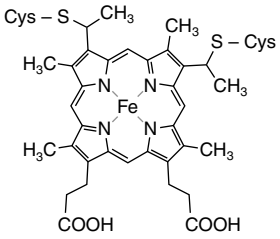
Sample application: slowly force or aspirate 1 ml plasma through the column

Column washing: 2 x 1 ml dist. water

Elution: 3 x 250 µl methanol

Cytochrome C from eye drops

MN Appl. No. 300210



Column type:
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. No. 730073

Column conditioning: 1 column volume dist. water

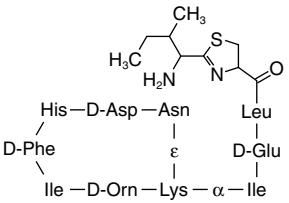
Sample application: slowly force or aspirate the sample through the column

Column washing: small amount of dist. water

Elution: 50 mM phosphate buffer pH 7.0 containing 1% (w/v) sodium dodecylsulphate – acetic acid (9:1, v/v)

Antibiotics: bacitracin from ointments

MN Appl. No. 300750



Column type:
CHROMABOND® OH (Diol) / 3 ml / 500 mg
Cat. No. 730053

Sample pretreatment: suspend 200 mg ointment in 2 ml dichloromethane, shaking and warming slightly

Column conditioning: 1 column volume dichloromethane

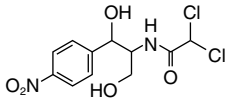
Sample application: slowly force or aspirate whole suspension from sample pretreatment through the column

Column washing: 2 x 1 ml dichloromethane; then dry column under vacuum for 3 min

Elution: 2 x 1 ml 0.1 mol/l hydrochloric acid

Antibiotics: chloramphenicol from animal tissues

MN Appl. No. 300760



Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: mix 0.2 – 2 ml ground tissue with 2 ml 0.025 M disodium hydrogen phosphate buffer (pH 6.8) or 4 ml tissue fluid and heat with 1 ml 0.1 M phosphate buffer (0.1 M potassium dihydrogen phosphate and 0.1 M disodium hydrogen phosphate). Add 200 µl β-glucuronidase solution (Sigma NRG 3510), incubate 90 min at 37 °C, extract 2 times with 10 – 15 ml ethyl acetate in a mixer and centrifuge. Evaporate extract to 1 ml and mix with 4 ml of a 4% aqueous sodium chloride solution. Flush solution with nitrogen

Column conditioning: 4 column volumes methanol, then 4 column volumes dist. water

Sample application: slowly force or aspirate the pretreated solution through the column

Column washing: 1 ml dist. water, then 2 ml dist. water – methanol (4:1, v/v)

Elution: 3 ml water – methanol (1:1, v/v)

Antibiotics: cyclosporin from blood

MN Appl. No. 300780

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: haemolyse and centrifuge blood sample

Column conditioning: 2 column volumes ethanol – dist. water (95:5, v/v), then 1 column volume dist. water

Sample application: slowly force or aspirate 1 ml pretreated blood sample through the column

Column washing: 5 ml acetonitrile – dist. water (1:1, v/v)

Elution: 250 µl ethanol – water (95:5, v/v); then mix the eluate with 200 µl water and 500 µl *n*-hexane and centrifuge: cyclosporin is in the lower phase

Antibiotics: cyclosporin from blood

MN Appl. No. 300790

Column type:
CHROMABOND® CN / 3 ml / 500 mg
Cat. No. 730063

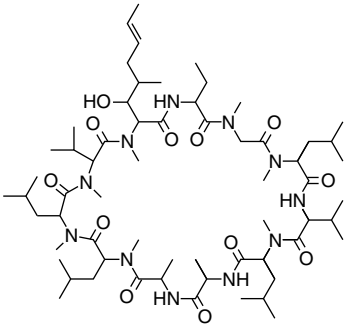
Sample pretreatment: mix 1 ml heparinised blood with 2 ml water – acetonitrile (7:3, v/v). Stir and centrifuge after 5 min.

Column conditioning: 1 column volume acetonitrile, then 1 column volume water – acetonitrile (8:2, v/v)

Sample application: slowly force or aspirate the sample through the column

Column washing: 0.5 M acetic acid – acetonitrile (8:2, v/v), then 0.5 M acetic acid – acetonitrile (6:4, v/v)

Elution: acetonitrile



Antibiotics: cyclosporin from serum

MN Appl. No. 300800

Column type:
CHROMABOND® CN / 3 ml / 500 mg
Cat. No. 730063

Sample pretreatment: mix 1 ml serum with 1 ml water

Column conditioning: 2 column volumes methanol, 2 column volumes water

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 x 500 µl water – acetonitrile (7:3, v/v); then dry column for 5 min under vacuum

Elution: 2 x 500 µl methanol

Antibiotics: gentamycin from liquid manure or urine

MN Appl. No. 300820

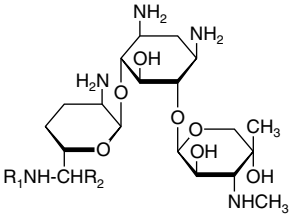
Column type:
CHROMABOND® SA (≙ SCX) / 3 ml / 500 mg
Cat. No. 730077

Sample pretreatment: dilute sample with 0.1 N phosphate buffer pH 6.5 and centrifuge

Column conditioning: 1 column volume methanol – water (1:1, v/v), then 1 column volume 0.1 N phosphate buffer pH 6.5

Sample application: slowly force or aspirate 500 µl sample through the column

Column washing: 0.1 N phosphate buffer pH 6.5, methanol – water (1:1, v/v)



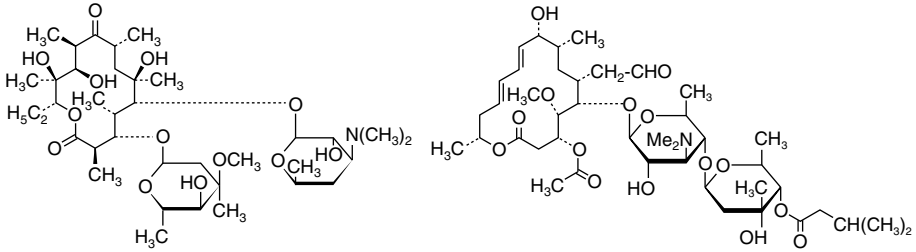
Gentamycin C_1: $R_1 = R_2 = CH_3$
Gentamycin C_2: $R_1 = R_2 = H$

Elution: force or aspirate 2 x 250 µl OPA (*o*-phthalaldehyde) – mercaptoethanol reagent through the column, let eluate stand 5 min, then elute into the same vessel using 1 ml methanol

Antibiotics from serum and urine

MN Appl. No. 300810

Compounds investigated: erythromycin and josamycin



Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: vigorously mix 1 ml sample with 1 ml acetonitrile, centrifuge 5 min at 1600 g and mix the clear supernatant with 5 ml dist. water

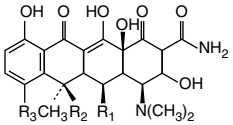
Column conditioning: 5 ml acetonitrile, then 5 ml dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 20 ml water, then 4 – 5 ml acetonitrile – water (1:1, v/v); thoroughly dry the column under vacuum

Elution: 2 – 3 x 500 µl acetonitrile – 0.05 M phosphate buffer pH 5.8 (3:2, v/v)

Antibiotics: tetracyclines



Compound	R_1	R_2	R_3
Oxytetracycline	OH	OH	H
Tetracycline	H	OH	H
Chlorotetracycline	H	OH	Cl
Doxycycline	OH	H	H

Antibiotics: tetracyclines from muscle and kidney tissues

MN Appl. No. 300830

Column type:
CHROMABOND® SA (≡ SCX) / 3 ml / 500 mg
Cat. No. 730077

Sample pretreatment: mix 10 g ground tissue with 25 ml ethyl acetate, stir and sonicate 10 min below 40 °C. After filtration, repeat extraction twice with 25 ml ethyl acetate each. Rinse tissue with 25 ml ethyl acetate. Mix the combined filtrates with 0.5 ml acetic acid and fill up to 100 ml with ethyl acetate.

Column conditioning: 2 x 3 ml *n*-hexane; dry column; then 2 x 3 ml ethyl acetate with 0.5% acetic acid. Do not let the column run dry!

Sample application: slowly force or aspirate the pretreated sample through the column; dry column 10 min with air; for 10 min pass ammonia vapours through the column packing

Column washing: 10 ml methanol

Elution: 5 ml 10% triethanolamine in methanol

Tetracyclines from musculature

MN Appl. No. 302030

Private communication: Mr. Lippold, Chemisches Landesuntersuchungsamt (Chem. Research Agency) Freiburg, Germany

Compounds investigated: tetracycline, oxytetracycline, chlorotetracycline (100 – 500 mg/kg)

Column type:
CHROMABOND® Tetracycline / 6 ml / 500 mg
Cat. No. 730315

Sample pretreatment: Weigh 10 g of a cut-up sample in a centrifuge glass and add 93 g succinate buffer pH 4 (5.0 g succinic acid anhydride in 1 l dist. water, pH adjusted with 1 M NaOH). Mix intensively (Ultra-Turrax, 2 min), homogenise in an ultrasonic bath (3 min), and centrifuge 15 min at 5000 g. Aspirate 50 ml of the supernatant through a Cu-loaded chelating sepharose column. Wash the column with 10 ml dist. water, 30 ml methanol and 2 x 10 ml dist. water, finally elute (4 ml/min) with 50 ml EDTA-succinate buffer (37.2 g Titriplex III · H_2O in 1 l succinate buffer).

Column conditioning: 1 column volume methanol, 1 column volume dist. water, then 1 column volume EDTA-succinate buffer (see above)

CAUTION: DO NOT LET THE COLUMN RUN DRY!

Sample application: force or aspirate 50 ml of the eluate from the sample pretreatment through the CHROMABOND® column

Column washing: 2 ml dist. water (removal of Cu ions), 1 ml *n*-hexane

Elution: with 7.5 ml methanol into a 25-ml tapered flask. Add 1 ml of an ethylene glycol – methanol mixture (22 g ethylene glycol filled up to 100 ml with methanol) and evaporate to dryness with a rotation evaporator (max. 40 °C). Fill up the residue to 400 ml with 0.1 M McIlvain-EDTA buffer (52.5 g citric acid · H_2O, 44.5 g $Na_2HPO_4 \cdot H_2O$ and 93 g Titriplex III dissolved in 2.5 l dist. water, adjusted to pH 4 with NaOH).

Further analysis: HPLC with column 250 x 4 mm NUCLEOSIL® 100-5 C_{18} HD (MN Appl. No. 110710 at *www.mn-net.com*)

Recovery rates: tetracycline, chlorotetracycline ~ 50 – 70%, oxytetracycline ~ 60 – 80%

Enrichment of quinolones and tetracyclines

MN Appl. No. 302470

Private communication: Chemisches und Veterinäruntersuchungsamt (Chem. and Veterinary Research Agency) Sigmaringen, Germany

Compounds investigated: enrofloxacin, ciprofloxacin, difloxacin, danofloxacin, marbofloxacin, sarafloxacin, piromidic acid, nalidixic acid, oxolinic acid, flumequine, tetracyclines

Column type:
CHROMABOND® C18 ec / 6 ml / 500 mg
Cat. No. 730014

Column conditioning: 6 ml methanol, then 6 ml water and 6 ml McIlvaine EDTA buffer pH 4

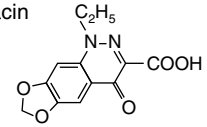
Sample application: 35 ml defatted extract in McIlvaine EDTA buffer

Column washing: 5 ml water, then 1 ml hexane, aspirate to dryness

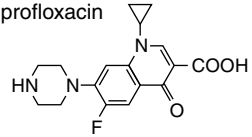
Elution: 12 ml methanol

Quinolone antibiotics

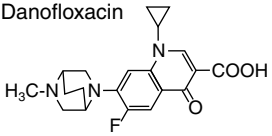
Cinoxacin



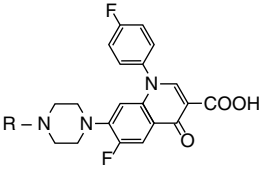
Ciprofloxacin



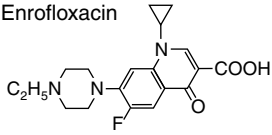
Danofloxacin



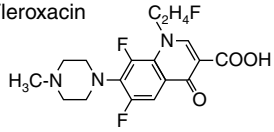
Difloxacin (R = CH_3)
Sarafloxacin (R = H)



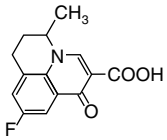
Enrofloxacin



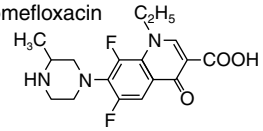
Fleroxacin



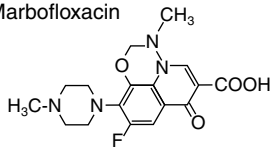
Flumequine



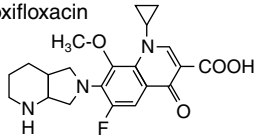
Lomefloxacin



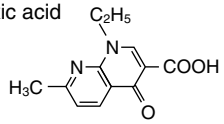
Marbofloxacin



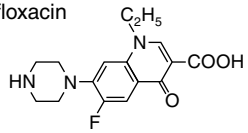
Moxifloxacin



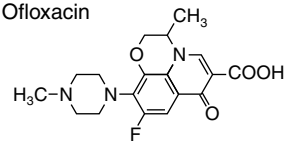
Nalidixic acid



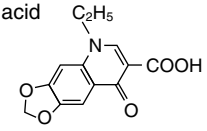
Norfloxacin



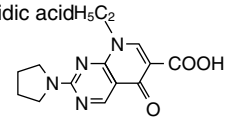
Ofloxacin



Oxolinic acid



Piromidic acid



Determination of (fluoro)quinolones from blood and surface water samples

MN Appl. No. 303740

M. Ferdig, A. Kaleta, T. D. T. Vo, W. Buchberger; Journal of Chromatography A **1047** (2004) 305 – 311

Compounds investigated: moxifloxacin, lomefloxacin, norfloxacin, ciprofloxacin, ofloxacin, enrofloxacin, oxolinic acid, flumequine, fleroxacin

Column type:
CHROMABOND® Tetracycline / 6 ml / 500 mg, Cat. No. 730315
CHROMABOND® C8 / 3 ml / 200 mg, Cat.-No. 730022

Sample pretreatment: in the case of blood samples, 1 ml of EDTA-treated human blood is thoroughly mixed with 2 ml of protein precipitation solution consisting of 0.1 M $ZnSO_4$ in methanol – water (30:70, v/v). After centrifugation (4000 rpm, 10 min) the liquid layer is diluted with 50 ml of water containing 100 mg EDTA disodium salt adjusted to pH 4.00 with acetic acid. In case of surface water, 500 ml sample are filtrated through a 0.1 µm filter. Afterwards, 1 g EDTA disodium salt is added and the pH is adjusted to pH 4.00 with acetic acid.

Column conditioning: ethyl acetate, methanol, aqueous 0.2% EDTA (pH 4.00)

Sample aspiration: slowly force or aspirate sample through the column

Column washing: twice with water containing 0.2% EDTA (pH 4.00)

Elution: 2 ml methanol – water (75:25, v/v) and 2 ml methanol

Comparison of recovery rates for CHROMABOND® *Easy* and other SPE phases

MN Appl. No. 302780

Column type:
CHROMABOND® *Easy* / 500 mg / 3 ml, Cat. No. 730759,
CHROMABOND® HR-P / 500 mg / 3 ml, Cat. No. 730117
CHROMABOND® C18 ec / 500 mg / 3 ml, Cat. No. 730013

Column conditioning: a) 2 ml methanol, than 2 ml dist. water, b) no conditioning

Sample application: slowly force or aspirate the sample (100 – 200 µg/compound in 200 ml water) through the column; in procedure b) the column is allowed to run dry

Column washing: 10 ml water

Elution: slowly aspirate 10 ml methanol/THF (1:1, v/v) through the column

Recovery rates: [%] for the complete procedure (a) and procedure (b) without column conditioning and with running dry

Phase	Ciprofloxacin [200 µg/l]	Doxepin [200 µg/l]	Cinoxacin [100 µg/l]
Easy (a)	91	100	87
Easy (b)	86	100	83
HR-P (a)	91	88	94
HR-P (b)	53	51	86
C18 ec (a)	71	77	78
C18 ec (b)	1	1	1

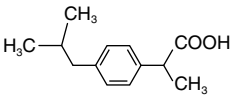
Recovery of antibiotics and other drugs

MN Appl. No. 302770

Compounds investigated: ciprofloxacin, doxepin, cinoxacin, ibuprofen, doxycycline, caffeine, paracetamol

Column type:
CHROMABOND® *Easy* / 3 ml / 500 mg
Cat. No. 730759

Ibuprofen:



Column conditioning: 5 ml methanol, 5 ml water

Sample application: slowly force or aspirate 200 ml sample through the column

Column washing: 10 ml water

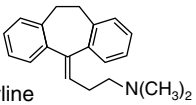
Elution: 2 x 5 ml MeOH – THF (1:1, v/v)

Recovery rates:

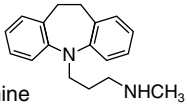
Compound	Concentration [µg/l]	Recovery [%] conditioned	unconditioned
Ciprofloxacin	200	97	96
Doxepin	200	98	98
Cinoxacin	100	90	87
Ibuprofen	200	96	88
Doxycycline	200	90	80
Caffeine	150	92	100
Paracetamol	50	100	96

Tricyclic antidepressants

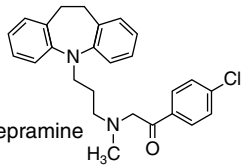
Amitriptyline



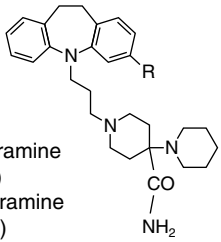
Desipramine



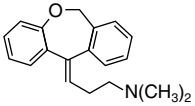
Lofepramine



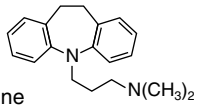
Carpipramine (R = H)
Clocapramine (R = Cl)



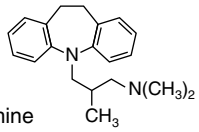
Doxepin



Imipramine



Trimipramine



Antidepressant drugs: dibenzazepines from serum

MN Appl. No. 300840

Column type:
CHROMABOND® CN / 1 ml / 100 mg
Cat. No. 730061

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water, finally 2 column volumes 0.1 M Tris buffer, adjusted to pH 3 with hydrochloric acid

Sample application: apply to the column without vacuum:
1) 1 column volume 0.1 M Tris buffer, adjusted to pH 3 with hydrochloric acid
2) sample
3) 1 column volume 0.1 M Tris buffer, adjusted to pH 3 with hydrochloric acid
then slowly force or aspirate liquid through the column

Column washing: 1 column volume 0.1 M Tris buffer, adjusted to pH 3 with hydrochloric acid, then 1 column volume dist. water

Elution: 2 – 3 ml methanol – 25% aqueous ammonia solution (99.5:0.5, v/v)

Tricyclic antidepressants from urine, plasma, blood

MN Appl. No. 300850

Compound investigated: amitriptyline · HCl, carpipramine · HCl, chlorimipramine · HCl, clocapramine · HCl, desipramine · HCl, imipramine · HCl, lofepramine · HCl, trimipramine · HCl

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: mix 1 ml sample with 1 ml 1 M sodium hydrogen carbonate solution and 2 ml water (centrifuge, if necessary)

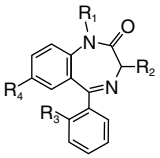
Column conditioning: 10 ml chloroform – 2-propanol (9:1, v/v), then 10 ml acetonitrile, finally 10 ml dist. water

Sample application: force or aspirate sample through the column with 3 – 5 ml/min

Column washing: 10 ml dist. water; then dry column with air

Elution: 3 ml chloroform – 2-propanol (9:1, v/v)

Benzodiazepines



Compound	R_1	R_2	R_3	R_4
Aminoflunitrazepam	CH_3	H	F	NH_2
Diazepam	CH_3	H	H	Cl
Flunitrazepam	CH_3	H	F	NO_2
Lorazepam	H	OH	Cl	Cl
Lormetazepam	CH_3	OH	Cl	Cl
Nordazepam	H	H	H	Cl
Oxazepam	H	OH	H	Cl

Detection of benzodiazepines and other psychotropic drugs in human hair by GC/MS

MN Appl. No. 302280

M. Yegles et al., Forensic Sci Int **84** (1997) 211 – 218

Column type:
CHROMABOND® C18 ec / 3 ml / 200 mg
Cat. No. 730012

Sample pretreatment: to reduce external contaminants the hair is washed with warm water (5 min) and two times with acetone (1 min). After drying with warm air, the hair is cut into segments of 3 cm and the different segments are pulverised in a ball mill. 2 ml of acetate buffer (pH 4) are added to 3 050 mg of the pulverised hair with the deuterated standards. The samples are hydrolysed with 70 µl of β-glucoronidase – arylsulfatase for 2 h at 40 °C. After centrifugation, the supernatant is removed and 2 ml distilled water are added again, shaken, centrifuged and the supernatant removed.

Column conditioning: 6 ml of methanol, then 3 ml dist. water

Sample application: the two buffer fractions are passed trough the column

Column washing: 3 ml of water, followed by 3 ml of 0.6 M $NaHCO_3$ and 3 ml of dist. water

Elution: columns are dried and eluted with 2 ml of acetone – dichloromethane (3:1, v/v); the eluant is evaporated to dryness under a stream of nitrogen at 40 °C and reconstituted in 50 µl of ethyl acetate.

Further analysis: GC/MS

Limits of detection:

Compound	[ng/mg]
Oxazepam	0.20
Diazepam	0.01
Nordazepam	0.10
Amino-flunitrazepam	0.02
Lorazepam	1.0
Lormetazepam	2.0
Carbamazepine	0.07
Amitriptyline	1.0

Benzodiazepines: diazepam from serum

MN Appl. No. 301000

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: adjust sample to pH 9.6 with 0.1 M sodium borate buffer

Column conditioning: 2 column volumes methanol, then 2 column volumes sodium borate buffer pH 9.6

Sample application: slowly force or aspirate 2 ml sample through the column

Column washing: 2 x 250 µl 0.1 M sodium borate buffer pH 9.6 – acetonitrile (9:1, v/v)

Elution: 2 x 200 µl methanol

Benzodiazepines from serum

MN Appl. No. 301010

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: mix 500 µl serum with 100 µl 0.1 M sodium carbonate solution (if desired, add 2 µg/ml nitrazepam as internal standard)

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 column volumes dist. water, then 50 µl methanol

Elution: 2 x 200 µl methanol

Benzodiazepines from urine

MN Appl. No. 301020

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: adjust 10 ml urine to pH 10.5 with 0.05 mol/l sodium carbonate solution

Column conditioning: 2 column volumes methanol, then 2 column volumes 0.05 mol/l sodium carbonate solution

Sample application: slowly force or aspirate the sample through the column

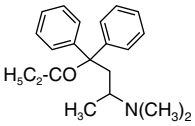
Column washing: 2 x 500 µl 0.05 mol/l sodium carbonate solution – acetonitrile (85:15, v/v)

Elution: 2 x 500 µl methanol

Enantioselective separation of methadone and its main metabolite in human hair by LC/MS

MN Appl. No. 302270

P. Kintz et al., Journal of Forensic Science **42** (1997) 291 – 295



Column type:
CHROMABOND® C18 / 3 ml / 200 mg
Cat. No. 7300112

Sample pretreatment: hair samples, weighing at least 100 mg, are cut as close as possible to the skin from the posterior vertex. To eliminate external contaminants, the hair is washed with warm water (5 min) and acetone (1 min) and then dried in a stream of warm air. To 60 mg of pulverised hair in a ball mill, are added 6 ml acetate buffer (pH 4) and 300 ng of the deuterated standards (methadone-d_3 and EDDP-d_3). The sample is hydrolysed with 180 µl β-glucoronidase – arylsulfatase for 1.5 h at 40 °C. The extract is then neutralised with $NaHCO_3$.

Column conditioning: twice 3 ml methanol and 3 ml dist. water

Sample application: slowly force or aspirate the sample through the column

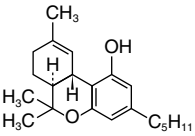
Column washing: consecutively with 3 ml dist. water, 3 ml $NaHCO_3$ (5%) and 3 ml dist. water; dry column by passing air through for 10 min and centrifuging at 4000 units/min for 15 min

Elution: 2 ml acetone – dichloromethane (3:1, v/v); evaporate the eluate to dryness under a stream of nitrogen at 60 °C, and then reconstitute it in 50 µl of methanol

Further analysis: HPLC, LC/MS

Drugs: tetrahydrocannabinol from plasma

MN Appl. No. 301200



Column type:
CHROMABOND® C18 ec / 3 ml / 200 mg
Cat. No. 730012

Sample pretreatment: mix 1 ml plasma with 2 ml 8 M aqueous urea solution, stir vigorously, add 2 ml methanol, stir vigorously (if necessary, preclean urea solution with a conditioned column CHROMABOND® C18 ec / 3 ml / 500 mg)

Column conditioning: 2 ml methanol, then 2 ml dist. water

Sample application: force or aspirate the sample through the column in 2 – 3 min, rinse with 2 ml water – methanol – 8 M aqueous urea solution (1:2:2, v/v/v)

Column washing:
1) 2 ml methanol – water (1:1, v/v)
2) 1 ml 0.2 M hydrochloric acid
3) 1 ml methanol – water (1:1, v/v)
4) 1 ml 0.01 M sodium hydroxide solution
5) 3 ml methanol – water (1:1, v/v)
then centrifuge 10 min to remove the remaining liquid

Elution: 500 µl diethyl ether

Drugs: Δ^9-carboxy-tetrahydrocannabinol from urine

MN Appl. No. 301190

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: heat 5 ml urine with 0.5 ml 10 mol/l KOH solution to 55 °C for 15 min and cool. Add 1 ml glacial acetic acid and mix well

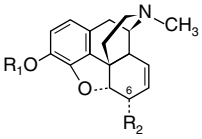
Column conditioning: 2 column volumes methanol, 2 column volumes 0.01 mol/l HCl

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 x 500 µl acetonitrile – 0.01 mol/l hydrochloric acid (6:4, v/v); then dry column under vacuum for 5 min

Elution: 2 x 500 µl *n*-heptane – ethyl acetate (85:15, v/v)

Morphine alkaloids



Compound	R_1	R_2
Morphine	H	OH
6-Monoacetylmorphine	H	O-CO-CH_3
Codeine	CH_3	OH
Heroin	CO-CH_3	O-CO-CH_3

Drugs: opiates from blood and serum

MN Appl. No. 301170

M.J. Bogusz et al., J. Chromatography **683** (1996) 177 – 188

Compounds investigated: codeine, 6-monoacetylmorphine and morphine

Column type:
CHROMABOND® Drug / 3 ml / 200 mg
Cat. No. 730168

Sample pretreatment: mix 1 ml blood or serum with 1 ml borate buffer 0.05 M, pH 8.5, vortex and centrifuge for 5 min at 12 000 x g.

Column conditioning: 5 ml methanol, 5 ml water and 5 ml borate buffer 0.05 M, pH 8.5

Sample application: force or aspirate the clear supernatant from the sample pretreatment through the column in about 5 min

Column washing: 2 ml dist. water, 1 ml acetate buffer 0.1 M, pH 4.0, then 2 ml methanol; dry column under vacuum for 5 min

Elution: elute with freshly prepared dichloromethane – isopropanol – concentrated ammonia (80:20:2, v/v/v) under gravity force.

Further analysis: we recommend GC/MS on an OPTIMA® 1 fused silica column (10 m x 0.25 mm ID), temperature programme 1 min at 150 °C, 20 °C/min to 250 °C, 5 min at 250 °C or HPLC on a 250 x 4.6 mm ID column NUCLEOSIL® 100-5 C_{18} AB, eluent acetonitrile – 5 mM citrate buffer pH 5.0 containing 20 mM Li perchlorate (12:88, v/v), flow rate 0.8 ml/min.

Morphine and its metabolites from plasma

MN Appl. No. 303190

Private communication: Mr. Schmidt, Pharmakologie, Uniklinik Frankfurt, Germany

Column type:
CHROMABOND® *Easy* / 1 ml / 30 mg
Cat. No. 730751

Column conditioning: 1 ml MeOH, then 1 ml water

Sample application: slowly force or aspirate 700 – 800 µl blood plasma through the column

Column washing: aqueous solution pH 9.3, then let the column run dry

Elution: 1 ml MeOH

Simultaneous determination of morphine, 6-monoacetylmorphine, codeine and dihydrocodeine in plasma and whole blood

MN Appl. No. 302500

A. Geier et al., Int. J. Legal Med. **109** (1996) 80 – 83

Column type:
CHROMABOND® C18 ec / 3 ml / 200 mg
Cat. No. 730012

Sample pretreatment: 10 µl internal standard (10 mg/l methaqualon in ethanol) is added to 1 ml plasma or whole blood. The samples are vortexed and blood samples are centrifuged for 15 min at 12500g in 1.5 ml Eppendorf tubes. The supernatant is decanted in 10 ml glass tube and dissolved in 9 ml borate buffer pH 9.2.

Column conditioning: 2 x 1 ml methanol, 2 x 1 ml water and 1 ml borate buffer pH 9.2 (flow rate 1 – 2 ml/min)

Sample application: samples are aspirated through the columns with approximately 1 ml/min

Column washing: 1 ml water, then 1 ml 20% methanol in water (v/v) using mild vacuum, finally the column is dried under strong vacuum for several minutes

Elution: 1 ml methanol with a flow rate of 1 ml/min

Further analysis: GC/MS after derivatisation

Recovery rates:

Compound	Concentration [µg/l]	Recovery [%]
Morphine	1000	93.1 ± 7.4
6-Monoacetylmorphine	100	68.0 ± 6.7
Codeine	500	77.0 ± 8.3
Dihydrocodeine	500	67.9 ± 8.4

Drugs from blood

MN Appl. No. 301160

Compounds investigated: codeine, 6-monoacetylmorphine and morphine

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: mix 1 g blood with internal standard (50 ng [$^2H_3$]morphine, 50 ng [$^2H_3$]codeine and 10 ng [$^2H_3$]MAM in 0.5 ml ethanol) and shake vigorously. Cool 10 min in an ice bath, then add 6.5 ml ice-cooled 0.1 M sodium carbonate buffer (pH 9). Centrifuge 10 min with 5 900 x g at 6 °C.

Column conditioning: 1 column volume methanol

Sample application: slowly force or aspirate the clear supernatant from the sample pretreatment through the column

Column washing: 3 ml dist. water; dry column 10 min under vacuum

Elution: elute with 1.3 ml dichloromethane – acetone (1:1, v/v) into silanised vessels

Analysis of hair for cocaine

MN Appl. No. 303500

E. J. Cone et. al., International Research on Standards and Technology (1995) 91– 120; NIH Publication No 95-3727

Column type:
CHROMABOND® C18 / 3 ml / 200 mg
Cat. No. 730002

Sample pretreatment: Segmental analysis often is used in hair analysis in an attempt to correlate time of ingestion with location of drug along the hair shaft. Approximately 100 mg of hair can be obtained by gripping a bundle of hair with the circumference of a pencil and then pulling gently to remove any loose strands of hair in the resting stage that are easily shed. The remaining strands are grasped firmly while the hair is cut as close to the scalp as possible. The root ends of the hair sample are aligned, and the sample is cut into 1-cm segments corresponding to approximately 1 month's growth. 5 – 10 mg hair are washed with 1 ml methanol for 15 min at 37 °C followed by three 30-minute washes with phosphate buffer (pH 6) at 37 °C removing any externally bound drug. Approximately 10 mg hair are placed in a screw-capped silanised glass centrifuge tube (10 mm wide x 100 mm deep) with 2.6 ml digest buffer (1 ml 1 M Tris HCl buffer, 20 ml 10 percent sodium dodecyl sulfate, and 79 ml deionised water) and with 0.4 ml 0 4 M dithiothreitol in 10 mM sodium acetate buffer and then was vortexed and incubated for 2 hours at 40 °C. Then 55 µl proteinase K solution (10 mg/ml or 136 units/ml) is added; the sample is vortexed again and incubated overnight at 40 °C.

Column conditioning: 6 ml methanol, then 3 ml water

Sample application: slowly force or aspirate the digested hair sample through the column

Column washing: 3 ml water, 3 ml 0.25 mol/l acetic acid, and then 3 ml water; then the column is dried by passing air through for 10 min and then centrifuged at 4.000 rpm for 15 min

Elution: three times with 500 µl of three parts acetone to one part dichloromethane

Further analysis: GC/MS

Determination of markers of illicit heroin in urine samples

MN Appl. No. 303660

F. Musshoff, J. Trafkowski, B. Madea, J. Chromatography B **811** (2004) 47 – 52

An automated SPE device was used: RapidTrace (Zymark, Idstein, Germany)

Compounds investigated: acetylcodeine (AC), codeine (COD), codeine-6-glucuronide (C6G), 6-acetylmorphine (6AM), morphine (MOR), morphine-3-glucuronide (M3G), morphine-6-glucuronide (M6G), noscapine (NOS), papaverine (PAP)

Column type:
CHROMABOND® C18 ec / 3 ml / 200 mg
Cat.-No. 730012

Sample pretreatment: urine samples are thawed, a volume of 1000 µl is combined with 1920 µl pH 9 buffer solution and spiked with 40 µl of two internal standard solutions. After mixing on a rotary shaker, the 3 ml samples are extracted automatically.

Column conditioning: 2 ml MeOH, 2 ml bidestilled water and 2 ml pH 9 buffer solution (CertiPur® boric acid buffer)

Column washing: 2 ml pH 9 buffer solution

Elution: two fractions are collected in one vial. The first fraction is collected with 0.7 ml of methanol, the second fraction with 0.7 ml methanol – acetic acid (9:1, v/v).

The eluates are evaporated to dryness and reconstituted in 100 µl of the HPLC mobile phase (water – acetonitrile [98:2, v/v], 5 mM ammonium acetate) and put into the ultrasonic bath for 5 min in order to improve dissolution.

Further analysis: LC-MS-MS

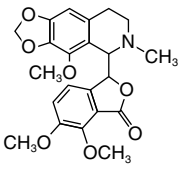
Recovery rates:

Compound	Abs. recovery [%] at high concentration	Abs. recovery [%] at low concentration	LOD[a] [ng/ml]	LOQ[b] [ng/ml]
AC	52.28	55.09	0.35	1.16
COD	90.96	92.43	1.74	5.5
C6G	96.45	80.11	3.04	10
6AM	59.44	59.44	2.83	9.86
MOR	98.28	97.41	3.4	12.38
M3G	39.75	45.5	5	17.82
M6G	46.76	52.78	7.4	26.1
NOS	84.61	70.06	0.48	1.56
PAP	59.75	50.28	0.1	0.33

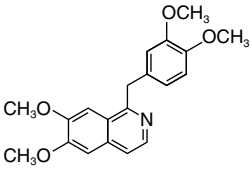
a) limit of detection, b) limit of quantitation

Papaverine alkaloids

Noscapine

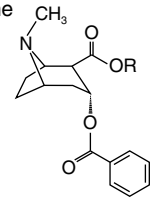


Papaverine



Tropane alkaloids

Benzoylecgonine
R = H
Cocaine
R = CH_3



Influence of sample preparation on analytical results: drug analysis (GC/MS) on hair snippets versus hair powder using various extraction methods

MN Appl. No. 302290

H. P. Eser et al., Forensic Sci Int. **84** (1997) 271 – 279

Column type:
CHROMABOND® C18 ec / 3 ml / 200 mg
Cat. No. 730012

Sample pretreatment: hair samples from five persons with a known history of drug abuse are collected at autopsy. The hair strands are washed in 10 ml ethanol – methanol (9:1, v/v) at room temperature for 3 min. Then the proximal 4-cm segments of each strand are divided longitudinally into 10 portions. Five portions of a particular hair sample are cut into pieces of approximately 1 – 3 mm in length, the other five portions are pulverised for 10 min in a ball mill.

1st method: to 20 mg of hair 2 ml of acetate buffer (pH 4), 100 ng of internal standard and 60 µl of arylsulfatase/b-glucuronidase are added, the mixture is incubated at 42 °C for 1.5 h

2nd method: to 20 mg of hair 2 ml of 8 M aqueous urea solution and 100 ng of internal standard are added and incubated at room temperature overnight

Column conditioning: water

Sample application: after incubation, the mixtures are neutralised with $NaHCO_3$ and applied to the preconditioned column

Column washing: 3 ml dist. water, 3 ml of 5% $NaHCO_3$ and again with 3 ml of dist. water

Elution: acetone – dichloromethane (3:1, v/v)

Further analysis: GC/MS

Recovery rates:
(for the 1st method)

Compound	Snippets [ng drug/mg hair]	Powder [ng drug/mg hair]
Cocaine	0.1	0.5
Benzoylecgonine	0.2	0.8
Morphine	0.4	1.6
6-Monoacetylmorphine	0.7	2.8
Codeine	0.2	0.8
Dihydrocodeine	0.1	0.9

Cocaine and its metabolites, benzoylecgonine and ecgonine methyl ester, in hair

MN Appl. No. 302350

M. R. Möller et al., J. Analytical Toxicology **16** (1992) 291 – 296

Column type:
CHROMABOND® C18 / 3 ml / 200 mg
Cat. No. 730002

Sample pretreatment: strands of hair are fixed with strings so that the hair cannot shift. They are washed with warm water (5 min) and acetone (1 min) to eliminate external contaminations. The samples are then dried in a stream of warm air. The strands of hair are cut into 2-cm segments which are separately pulverised in a ball mill. To 10 – 30 mg of pulverised hair, 2 ml phosphate buffer (pH 7.6), and 100 ng of deuterated standards (cocaine, benzoylecgonine, and ecgonine methyl ester) are added. The sample is hydrolised with 75 µl β-glucuronidase – arylsulfatase for 2 h at 40 °C. After centrifugation, the supernatant is removed to a clean vessel and 2 ml phosphate buffer are again added to the residue, shaken, and centrifuged. The two buffer fractions are combined. 1 ml of a 0.1M K_2HPO_4 solution is added to adjust the pH to 8.

Column conditioning: 6 ml methanol, then 3 ml water

Sample application: the sample is tranferred to the column

Column washing: 3 ml H_2O, 3 ml 0.25 mol/l acetic acid and 3 ml H_2O; dry by passing air through the column (10 min) and centrifuge at 4000 rpm (15 min)

Elution: 3 x 500 µl acetone – dichloromethane (3:1, v/v); evaporate the eluent to dryness under a stream of nitrogen at 60 °C. The residue obtained is derivatised with 100 µl pentafluoropropionic anhydride and 70 µl pentafluoropropanol for 30 min at 60 °C. The mixture is again evaporated to dryness using a stream of nitrogen at 60 °C.

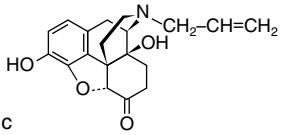
Further analysis: GC/MS

Recovery rates:

Compound	Recovery [%]
Cocaine	90
Benzoylecgonine	75
Ecgonine methyl ester	50

Drugs: narcotic antagonist naloxone from plasma

MN Appl. No. 301210



Column type:
CHROMABOND® CN / 1 ml / 100 mg
Cat. No. 730061

Sample pretreatment: centrifuge plasma 15 min at 1000 g. Mix 700 µl plasma with 375 µl water, 25 µl 0.4 M pentanesulphonic acid and, if desired, with internal standard

Column conditioning: 1 ml acetonitrile, then 1 ml dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 3 x 1 ml dist. water; dry column 1 min with air

Elution: 1 ml 5 mM pentanesulphonic acid monohydrate in acetonitrile – water – 85% orthophosphoric acid (18:82:0.0045, v/v/v)

Drugs from blood serum

MN Appl. No. 302020

W. Weinmann, M. Renz, C. Pelz, P. Brauchle, S. Vogt, S. Pollak, Blutalkohol **35** (1998), 1 – 9

Compounds investigated: benzoylecgonine, amphetamine, codeine, morphine

Column type:
CHROMABOND® Drug / 3 ml / 200 mg
Cat. No. 730168

Sample pretreatment: 0.1 ml blood serum are mixed with 1.4 ml of a 0.1 mol KH_2PO_4 buffer (pH 6) and centrifuged

Column conditioning: 2 ml methanol, then 2 ml 0.1 mol KH_2PO_4 buffer (pH 6)

Sample application: slowly force or aspirate the supernatant from the sample pretreatment through the column

Column washing: 2 ml 0.1 mol KH_2PO_4 buffer (pH 6), then 1 ml 0.1 mol acetic acid, then 2 ml methanol; finally dry the column first by centrifugation (2 min, 4000 U/min), then under vacuum for 10 min

Elution: 1.5 ml dichloromethane – 2-propanol – 25% ammonia solution (80:20:2, v/v/v)

Further analysis: we recommend HPLC with column 250 x 2 mm NUCLEOSIL® 100-5 C_{18} AB (application 110240) or GC/MS after derivatisation with perfluoropropanoic acid anhydride – pentafluoropropanol, e. g. with column OPTIMA® 5 MS, 0.25 mm film, 30 m x 0.25 mm ID (Cat. No. 726220.30)

Drugs from urine and blood

MN Appl. No. 301140

Compounds investigated: amphetamines, barbiturates, cannabinoids, cocaine, opiates

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: adjust 10 ml urine to the proper pH value with hydrochloric acid or ammonia and centrifuge. Cannabinoids can be extracted from blood without pH adjustment.

Barbiturates:	pH 7
Active components in general (TDx negative Btm urines for cocaine):	pH 7 – 8
Spec. bases such as amphetamines and opiates:	pH 8 – 9

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water pH 7

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 column volumes water (if necessary, adjust pH with hydrochloric acid or ammonia as indicated above); dry column under vacuum for 5 min

Elution: aspirate 750 µl eluent into the column packing, after 1 min elute and flush with another portion of 750 µl eluent

Eluents: acetone for cannabinoids from blood; acetone – chloroform (1:1, v/v) for barbiturates, active components in general, bases and amphetamines

Fast screening for drugs of abuse by SPE combined with FIA-ionspray-MS-MS

MN Appl. No. 302240

W. Weinmann et al., J. Anal. Toxicol. **22** (1998) 319 – 328

Compounds investigated: amphetamine, benzoylecgonine, codeine, morphine in serum, urine or hair samples

Column type:
CHROMABOND® Drug / 3 ml / 200 mg
Cat. No. 730168

Sample pretreatment: for method evaluation, 1-ml serum samples spiked with deuterated standards (25 ng in 50 µl methanol) are diluted with 1 ml phosphate buffer (pH 6). Aliquots of real-case samples (serum or urine, 0.1 – 2 ml) are spiked with deuterated standard mixture (200 ng each) and diluted with phosphate buffer.

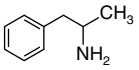
Column conditioning: methanol and phosphate buffer

Column washing: 2 ml deionised water, 0.1 ml acetic acid and 2 ml methanol

Elution: 1.5 ml of dichloromethane – 2-propanol – 25% NH_4OH (80:20:2, v/v/v); evaporate the eluate to dryness

Recovery rates:
(determined by GC/MS analysis, drugs spiked to 8 ng/ml)

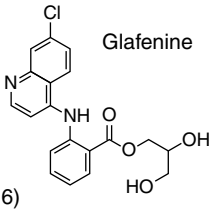
Compound	Recovery [%]
Amphetamine	92 ± 4.3
Benzoylecgonine	86 ± 3.5
Codeine	88 ± 4.2
Morphine	87 ± 2.3



Amphetamine

Serum extraction methods for systematic toxicological analysis with mass spectra libraries

MN Appl. No. 303050



Glafenine

C. Müller et al., J. Chromatography B, **773** (2000) 47 – 52

Compounds investigated: codeine, glafenine

Column type:
CHROMABOND® Drug / 3 ml / 200 mg
Cat. No. 730168

Sample pretreatment: blank serum (1 ml) is mixed with 1 ml KH_2PO_4 (pH 6)

Column conditioning: 2 ml methanol, then 2 ml KH_2PO_4

Sample application: slowly force or aspirate the sample solution through the column

Column washing: 1 ml 0.1 M acetic acid, then 0.1 ml methanol

Elution:
1st (acidic/neutral) fraction: 1.5 ml acetone – dichloromethane (1:1, v/v)
2nd (alkaline) fraction: 1.5 ml of a mixture of dichloromethane – 2-propanol – NH_4OH 25% (80:20:2, v/v/v)
evaporate eluates to dryness and redissolve with 100 µl eluent each (1 mM ammonium formate, 0.1% formic acid pH 3.1 – acetonitrile [1:1, v/v])

Further analysis: LC/MS of 20 µl of each extract

Drugs from urine

MN Appl. No. 301150

Compounds investigated: acid, neutral and basic drugs of abuse

Column type:
CHROMABOND® Drug / 3 ml / 200 mg
Cat. No. 730168

Solutions:

A) 0.1 M Na_2HPO_4: 14.2 g Na_2HPO_4 per litre H_2O
B) 0.1 M NaH_2PO_4: 13.8 g NaH_2PO_4 per litre H_2O
C) phosphate buffer pH 6: 1.7 g Na_2HPO_4 and 12.14 g NaH_2PO_4 dissolved in 1000 ml water and adjusted to pH 6 with A) or B)
D) 1.0 M acetic acid: 57.2 g glacial acetic acid per litre H_2O

Sample pretreatment: mix 5 ml urine sample intensively with 2 ml 0.1 M phosphate buffer (C) and if necessary adjust pH value to 6 with 0.1 M NaH_2PO_4 solution (B)

Column conditioning: carefully aspirate or force 3 ml methanol, 3 ml dist water and 1 ml phosphate buffer (C) through the column

Sample application: slowly aspirate or force sample through the column (about 1 to 2 ml/min)

Column washing: 3 ml dist. water and then 1 ml 1.0 M acetic acid (D); dry column thoroughly by applying vacuum for about 5 min

Aspirate or force 2 ml *n*-hexane through the column

Elution: (acid and neutral drugs)
slowly aspirate or force 3 ml n-hexane – ethyl acetate (1:1, v/v) through the column, if necessary evaporate eluate in a stream of nitrogen and fill to 100 µl with ethyl acetate

Column washing: aspirate or force 3 ml methanol through the column, then dry for about 5 min

Elution: (basic drugs)
slowly aspirate or force 2 ml methanol – NH_4OH 25% (98:2, v/v) through the column; add 3 ml water and 250 µl chloroform to the eluate and mix well; centrifuge for phase separation; analyse (lower) chloroform phase

Basic drugs: pentacaine and stobadin from serum

MN Appl. No. 302410

V. Marko, K. Radová, J. Liquid Chromat. **14** (1991) 1645 – 1658

Column type:
CHROMABOND® C18 / 3 ml / 200 mg
Cat. No. 730002

Sample pretreatment: 1 ml of serum or water is spiked with 1 µg pentacaine or stobadin

Column conditioning: 2 ml methanol, then 1 ml water

Sample application: slowly force or aspirate the sample through the column

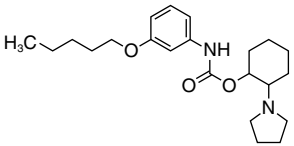
Column washing: 1 ml water, the residual water is displaced from the column under mild pressure

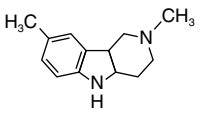
Elution: three 1-ml portions followed by one 2-ml portion of methanol or acetonitrile; after the methanolic elution, 1 ml of 5% triethylamine in methanol was used to elute the drugs completely. Individual portions of the eluate are collected into 3-ml conic vials containing each 1 µl of a suitable internal standard. The solvent is evaporated to dryness at 55 °C under nitrogen, 250 µl of ethyl acetate are added to the dry residue and the vials are agitated on a Vortex for 10 s.

Further analysis: GC; pentacaine and its internal standard are methylated before analysis, stobadin is analysed directly

Recovery rates:

Compound	Recovery [%]
Pentacaine:	97.8
Stobadin:	92.1





Liquid-liquid extraction of alkaloids from aqueous solutions

MN Appl. No. 302110

Column type:
CHROMABOND® XTR / 70 ml / 14.5 g
Cat. No. 730507

Sample pretreatment: add 1 ml of a spiked solution (10 mg each of codeine and quinine in 100 ml water) to 9 ml of an aqueous sample solution. Transfer 1 ml of this solution to 19 ml aqueous NH_3 solution (pH 9).

Sample application: apply the ammoniacal sample solution to the column and allow the solution to be soaked up for 10 min

Elution: 30 ml dichloromethane – isopropanol (85:15, v/v). Evaporate the eluate to dryness with a rotation evaporator. Rinse the flask four times with 250 µl acetonitrile – water (8:2, v/v) each and transfer the combinded solutions into a HPLC vial.

Further analysis: HPLC, e.g. with column NUCLEOSIL® 100-5 C_{18} HD (see MN Appl. No. 110160 at *www.mn-net.com*).

Recovery rates: (structures see pages 95 and 118, resp.)

Codeine: 92%

Quinine: 94%

Steroids from hydrocortisone ointment
MN Appl. No. 300530

Column type:
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. No. 730073

Sample pretreatment: extract 1 g ointment with 2 x 10 ml *n*-hexane – ethyl acetate (1:1, v/v); dilute the extract to 50 ml with *n*-hexane – ethyl acetate (1:1, v/v)

Column conditioning: 2 ml *n*-hexane – acetone (8:2, v/v)

Sample application: slowly force or aspirate 1 ml sample through the column

Column washing: 2 ml *n*-hexane – acetone (8:2, v/v); dry 3 min under vacuum

Elution: 2 x 500 µl methanol

Steroids and peptide hormones from plasma
MN Appl. No. 300540

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: mix plasma with an equal volume of 0.5 M pyridine acetate pH 5.0

Column conditioning: 5 ml 0.5 M pyridine acetate pH 5.0

Sample application: slowly force or aspirate the sample through the column

Column washing: 10 ml 0.5 M pyridine acetate pH 5.0

Elution:

Elute peptide hormones with 2 x 0.5 ml 1-propanol – 0.5 M pyridine acetate pH 5.0 (2:8, v/v)

Elute steroids with 2 x 0.5 ml 1-propanol – 0.5 M pyridine acetate pH 5.0 (4:6, v/v)

Steroids from urine, serum and plasma
MN Appl. No. 300550

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: for bound steroids hydrolyse 5 ml sample with 750 µl conc. HCl for 30 min in silanised glass vessels; free steroids do not require any sample pretreatment.

Column conditioning: 2 column volumes methanol, 2 column volumes dist. water

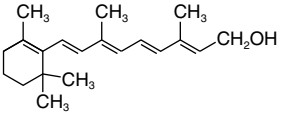
Sample application: slowly force or aspirate 5 ml urine or 2 ml serum or plasma through the column

Column washing: 2 ml water – acetone (8:2, v/v) or 12 – 13 ml water; dry column 3 min under vacuum

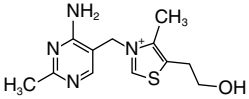
Elution: after washing with water – acetone 2 x 500 µl methanol suffice; after washing with plain water 12 – 13 ml methanol are required for steroid elution. The methanolic solution can then be concentrated at 40 °C in a stream of nitrogen.

Vitamins

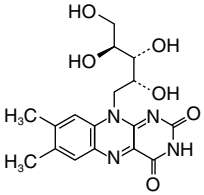
Vitamin A (retinol)



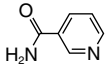
Vitamin B_1 (thiamine)



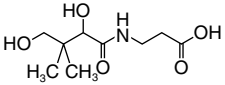
Vitamin B_2 (riboflavin)



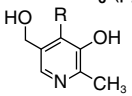
Vitamin B_3 (nicotinamide)



Vitamin B_5 (pantothenic acid)

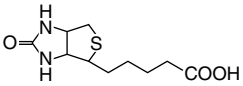


Vitamin B_6 (pyridoxine, R = CH_2OH)

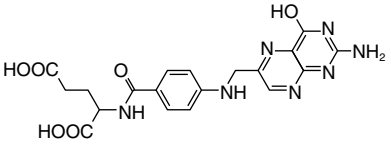


R = $CH_2 - NH_2$: pyridoxamine
R = CHO: pyridoxal

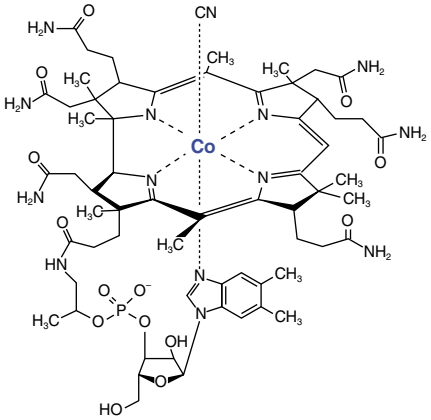
Vitamin B_7 = Vitamin H (biotin)



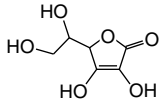
Vitamin B_{11} (folic acid)



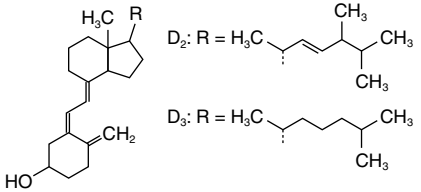
Vitamin B_{12} (cyanocobalamin)



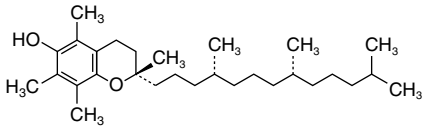
Vitamin C (ascorbic acid)



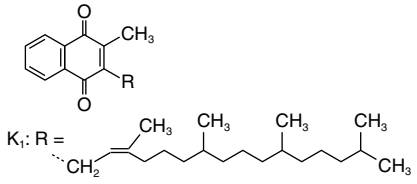
Vitamin D_2 (calciferol) **and D_3** (cholecalciferol)



Vitamin E (α-tocopherol)



Vitamin K



Vitamin D_2 from multi-vitamin tablets

MN Appl. No. 300610

Column type:
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. No. 730073

Sample pretreatment: in a 250 ml flask mix internal standard (vitamin D_3), up to 5 tablets and 40 ml dimethyl sulfoxide and sonicate 30 min at 40 – 50 °C. Add 40 ml methanol – water (1:1, v/v) and cool to ambient temperature. Add 80 ml *n*-hexane and shake 30 min. After centrifugation evaporate a portion of the hexane phase at 30 °C under nitrogen and redissolve the residue in 4 ml *n*-hexane

Column conditioning: 2 column volumes *n*-hexane

Sample application: slowly force or aspirate the pretreated sample through the column

Elution: 8 x 1 ml *n*-hexane – ethyl acetate (85:15, v/v), analyse individual fractions for their vitamin content

Water-soluble vitamins from aqueous solutions

MN Appl. No. 300620

Compounds investigated: niacinamide, pyridoxine, riboflavin, thiamine

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: in an amber glass bottle mix 50 ml sample with a riboflavin content < 6 mg with 0.5 ml acetic acid and 0.1 g heptane-1-sulphonic acid sodium salt. Flush bottle with nitrogen and heat to 55 °C for 5 min shaking occasionally; cool down rapidly.

Column conditioning: 1 column volume methanol, then 1 column volume of a solution of 0.5 ml acetic acid and 0.1 g heptane-1-sulphonic acid sodium salt in 50 ml water

Sample application: force or aspirate 2 ml sample solution through the column

Column washing: 2 x 250 µl of a solution of 0.5 ml acetic acid and 0.1 g heptane-1-sulphonic acid sodium salt in 50 ml water; dry column 1 min under vacuum

Elution: 3 x 500 µl methanol, analyse immediately

Please note: the solution of acetic acid and heptane-1-sulphonic acid sodium salt has to be prepared fresh daily

Vitamins A, D and E from tablets

MN Appl. No. 300560

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: dissolve sample in 5 ml 0.1 mol/l hydrochloric acid (if necessary, heat to max. 50 °C for 2 – 3 min). After cooling add 5 ml 2-propanol and centrifuge

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate up to 5 ml sample through the column

Column washing: 2 x 500 µl 2-propanol – water (1:1, v/v), then 1 ml water – methanol (9:1, v/v)

Elution: 2 x 500 µl methanol – acetonitrile (1:1, v/v)

Vitamin D_3 metabolites from serum

MN Appl. No. 300580

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: dilute serum with twice its volume of ethanol and centrifuge, use the supernatant

Column conditioning: 2 column volumes methanol, 2 column volumes water

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 x 500 µl methanol – 0.01 mol/l hydrochloric acid (7:3, v/v)

Elution: 2 x 500 µl dichloromethane

Vitamin K from liver

MN Appl. No. 300630

Column type:
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. No. 730073

Sample pretreatment: homogenise 1 g liver tissue with 5 ml 66% 2-propanol, add 6 ml *n*-hexane and shake. Dry 5 ml of the upper phase under exclusion of light and reduced pressure; redissolve in 2 ml *n*-hexane.

Column conditioning: 10 ml *n*-hexane with 4% diethyl ether, then 10 ml *n*-hexane

Sample application: slowly force or aspirate the pretreated sample through the column

Column washing: 10 ml *n*-hexane

Elution: 5 ml *n*-hexane with 4% diethyl ether

Further analyses: TLC on plates SIL G-25 UV_{254} with petroleum ether – diethyl ether (85:15, v/v) as developing solvent or HPLC with column 250 x 4 mm NUCLEOSIL® 100-5 C_{18}.

Vitamin B_{12} from multi-vitamin preparations

MN Appl. No. 300600

Column type:
CHROMABOND® C18 ec / 6 ml / 1000 mg
Cat. No. 730015

Sample pretreatment: pulverise tablet(s) with a content of 1 – 15 µg vitamin B_{12} and shake with 0.05 M aqueous sodium dihydrogen phosphate solution at ambient temperature (more than 0.2 µg vitamin B_{12}/ml). Mix oily preparations with some chloroform and extract as above. Filter or centrifuge and use the clear aqueous phase

Column conditioning: 2 column volumes methanol, then 1 column volume 0.05 M sodium dihydrogen phosphate solution

Sample application: slowly force or aspirate 10 ml clear sample solution (2 – 5 µg vitamin B_{12}) through the column

Column washing: 1 column volume water; the column must not run dry!

Elution: 2 ml ethanol – 0.05 M sodium dihydrogen phosphate (1:1, v/v), then 3 ml dist. water

Interfering dyes can be removed using a CHROMABOND® SB (≡ SAX) column (3 ml / 500 mg) on top of the C18 ec column.

Hydroxyvitamin D_3 metabolites from plasma

MN Appl. No. 300590

Compounds investigated: hydroxyvitamin D_3 metabolites [24,25-$(OH)_2$-vitamin D_3, 1,25-$(OH)_2$-vitamin D_3 and 25-(OH)-vitamin D_3] (5 – 500 ng/ml)

Step 1:

Column type:
CHROMABOND® C18 / 3 ml / 500 mg
Cat. No. 730003

Sample pretreatment: intensely extract 2 ml plasma with 2 ml acetonitrile, centrifuge and mix the supernatant in the ratio 35:65 with 400 mM phosphate buffer (pH 10.5)

Column conditioning: 3 ml methanol; dry column, then aspirate 3 ml 400 mM phosphate buffer (pH 10.5) through the column

Sample application: slowly force or aspirate the sample through the column

Column washing: force or aspirate 3 ml 400 mM phosphate buffer (pH 10.5), then 3 ml methanol – dist. water (70:30, v/v) through the column

Elution: 3 ml methanol – dist. water (90:10, v/v); evaporate in a stream of nitrogen and redissolve in 1 ml isopropanol – hexane (1:99, v/v)

Step 2:

Column type:
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. No. 730073

Column conditioning: force or aspirate 3 ml isopropanol – hexane (1:99, v/v) through the column

Sample application: slowly force or aspirate the eluate from step 1 through the column

Column washing: force or aspirate 10 ml isopropanol – hexane (3:97, v/v) through the column

Elution: 5 ml isopropanol – hexane (25:75, v/v); evaporate in a stream of nitrogen and redissolve in 100 µl methanol

Further analyses: we recommend HPLC

Recovery rates: 94 – 101%

Vitamin K_1 from nutrient solutions

MN Appl. No. 300640

Column type:
CHROMABOND® C18 ec / 6 ml / 3 g (special)

Column conditioning: 15 ml methanol, then 15 ml dist. water

Sample application: Slowly pour 15 ml nutrient solution through the column

Column washing: 20 ml water; dry column in a stream of air (e.g. water jet pump) for about 30 min

Elution: 20 ml methylene chloride, elute slowly and evaporate

Further analysis: we recommend HPLC with column 250 x 4 mm NUCLEOSIL® 120-5 C_{18}, mobile phase acetonitrile – water (40:60, v/v), 1.0 ml/min, UV detection at 248 nm.

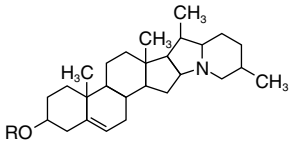
For isolation of vitamins from food please see pages 135 – 137

Food and beverages

Alkaloids from potatoes and potato products

MN Appl. No. 300060

Compounds investigated: α-solanine and α-chaconine



α-Solanine R = glu / rham — gal

α-Chaconine R = rham / rham — glu

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: homogenise 5 g sample with 30 ml methanol for 2 min and filter. Wash residue with 10 ml methanol. Fill up to 50 ml with methanol. Mix 5 ml of this extract with 8 ml water (for potato starch, evaporate 50 ml to about 5 ml in a rotation evaporator and add 8 ml water).

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 5 ml dist. water – methanol (6:4, v/v)

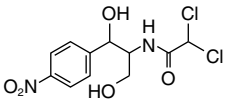
Elution: 15 ml methanol; evaporate to dryness and redissolve in 1 ml methanol

For products with high lipid contents such as potato chips, potato salad etc. mix 1 ml of the concentrated eluate with 19 ml acetonitrile and pour through a CHROMABOND® NH_2 column (3 ml / 500 mg), which was conditioned with 2 column volumes methanol and 2 column volumes acetonitrile. Wash with 5 ml acetonitrile and elute with 10 ml methanol. Evaporate as above.

For further analysis of the glycoalkaloids we recommend HPLC on column NUCLEOSIL® NH_2, mobile phase acetonitrile – 20 mM potassium dihydrogen phosphate buffer (75:25, v/v), flow rate 0.7 ml/min, 30 °C, UV detection at 208 nm.

Antibiotics: chloramphenicol from fish

MN Appl. No. 300770



Column type:
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. No. 730073

Sample pretreatment: homogenise fish sample in 0.1 mol/l acetate buffer pH 5.2, add ethyl acetate, homogenise again and centrifuge. Separate the ethyl acetate and repeat extraction. Combine ethyl acetate phases, evaporate to dryness and redissolve the residue in dichloromethane.

Sample application: slowly pour dichloromethane phase through the column

Column washing: dichloromethane

Elution: methanol; evaporate eluate, redissolve the residue in acetonitrile – water and degrease with *n*-hexane

Antibiotics: chloramphenicol residues in honey

MN Appl. No. 303730

S. Oepkemeier, H.-D. Winkeler; GIT Labor-Fachzeitschrift **46** (2002) 982 – 985

Column type:
CHROMABOND® XTR / 70 ml / 14.5 g
Cat. No. 730507

Sample pretreatment: 10 g honey are diluted with 15 ml water and placed in an ultrasonic bath for 10 min at 60 °C

Sample application: after cooling the sample is poured onto the XTR SPE column and absorbed for 60 min

Column washing: 40 ml hexane – ethyl acetate (95:5, v/v)

Elution: twice 40 ml hexane – ethyl acetate (50:50, v/v). The eluate is brought to dryness in a TurboVap 500 and reconstituted with 0.5 ml methanol – water (50:50, v/v). The sample solution is filtrated through a 0.45 µm PTFE filter (Cat. No. 729009).

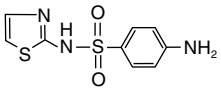
Further analysis: 1 µl is injected into a LC-MS system (see MN Appl. No. 119810 at *www.mn-net.com*)

Recovery rates: 85 – 105% (n = 10)

Immunochemical screening for antimicrobial drug residue in commercial honey

MN Appl. No. 302590

W. Heering et al., Analyst **123** (1998) 2759 – 2762



Column type:
CHROMABOND® C18 ec / 6 ml / 500 mg
Cat. No. 730014

Sample pretreatment: for sulfathiazole analysis, approximately 2.5 g of sample are weighed into a beaker, sodium acetate buffer (0.1 mol/l, pH 5.0) is added to give the fivefold weight, and mixed for 10 min with a magnetic stirrer

Column conditioning: 20 ml methanol, followed by 20 ml water

Sample application: 10 ml of the honey – acetate buffer mixture are slowly loaded on the column, followed by 2 ml of water; the column is dried for approximately 5 min under reduced pressure

Elution: 5 ml acetonitrile; evaporate the organic phase at 50 °C under reduced pressure with a rotary evaporator

Recovery rates:
(sulfathiazole from spiked honey samples determined by EIA)

Amount added [µg/kg]	Recovery [%]
200	105 ± 13.9
1000	100 ± 21.8

Detection of incurred dihydrostreptomycin residues in milk

MN Appl. No. 303700

G. Suhren, K. Knappstein; Analyst **123** (1998) 2797 – 2801

Compounds investigated: streptomycin (STR) and dihydrostreptomycin (DHS)

Column type:
CHROMABOND® C18 ec / 6 ml / 500 mg
Cat. No. 730014

Sample pretreatment: a 2 ml volume of oxalic acid (7.5% m/v oxalic acid dihydrate) is added to 10 g of milk (0.3 – 3.5% fat) in a polypropylene (PP) tube, thoroughly mixed (20 min in a shaker) and centrifuged (3 200 x g, 10 min). After fat removal, the supernatant is decanted into a PP tube, 2 ml of TCA (trichloroacetic acid) are added, mixed (20 min in a shaker) and centrifuged (3 200 x g, 10 min). The mixture is decanted into a PP tube, 0.80 ml HSA (0.2 M; 1.88 g sodium hexane-1-sulfonic acid dissolved in water and diluted to 50 ml) added, mixed and left at room temperature (20 °C for 15 min). After addition of 0.7 ml of NaOH (2 M) and mixing, the supernatant is centrifuged (3 200 x g, 10 min) and decanted into a PP tube.

Column conditioning: with 3 ml MeOH, 3 ml water and 3 ml HSA buffer (0.02 M; 1.88 g sodium hexane-1-sulfonic acid in water, 1 ml acetic acid, diluted with water to 500 ml, pH 3.3)

Sample application: slowly force or aspirate the sample through the column

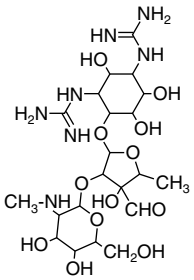
Column washing: twice with 3 ml of water

Elution: 2 ml eluent mixture (0.02 M HSA buffer – acetonitrile [9:11, v/v]). The eluate is concentrated in a vacuum concentrator to < 1 g; the mass is made up to 1 g with water.

Further analysis: 50 µl are injected into a LC system

Recovery rates:

Compound	Concentration [µg/kg]	Recovery [%]	LOD[a] [µg/kg]	LOQ[b] [µg/kg]
STR	∅	87	8	12
	10	96.1 ± 5.5		
	25	85.3 ± 10.6		
	50	92.2 ± 4.7		
	100	81.8 ± 12.8		
	200	77.6 ± 8.8		
DHS	∅	95	12	18
	10	106.8 ± 14.9		
	25	100.8 ± 10.2		
	50	95.4 ± 7.0		
	100	89.7 ± 8.6		
	200	81.6 ± 6.3		

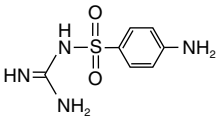


Streptomycin

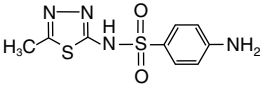
a) limit of detection, b) limit of quantitation

Sulfonamides

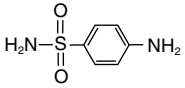
Sulfaguanidine



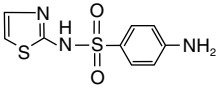
Sulfamethizole



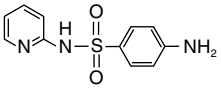
Sulfanilamide



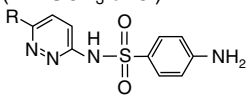
Sulfathiazole



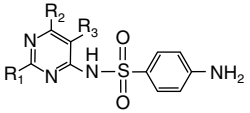
Sulfapyridine



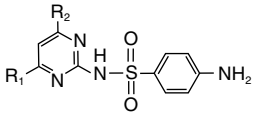
Sulfapyridazines
(R = OCH_3 or Cl)



Sulfadoxine R_1 = H, R_2 = R_3 = OCH_3
Sulfadimethoxine
R_1 = R_2 = OCH_3, R_3 = H



Sulfadiazine R_1=R_2=H
Sulfamerazine R_1 = H, R_2 = CH_3
Sulfadimidine R_1 = R_2 = CH_3



Sulfonamides in meat and kidney

MN Appl. No. 302710

B. Pacciarelli et al., Mitt. Gebiete Lebensm. Hyg. **82** (1991) 45 – 55

Compounds investigated: sulfaguanidine, sulfanilamide, sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfamethizole, sulfadimidine, sulfamethoxypyridazine, sulfachlorpyridazine, sulfadoxine, sulfadimethoxine

Column type:
CHROMABOND® SA (≡ SCX) / 3 ml / 500 mg
Cat. No. 730077

Sample pretreatment: homogenise 10 g sample and 60 ml dichloromethane – acetone (1:1, v/v) for 30 s with a Polytron. Centrifuge the homogenisate for 10 min at 2500 rpm. Filter the organic phase and wash the filter residue with a little dichloromethane – acetone. Add 5 ml glacial acetic acid to the filtered extract.

Column conditioning: apply 6 ml hexane and suck air until the column is dry (10 min). Then apply 6 ml dichloromethane – acetone – glacial acetic acid (10:10:1, v/v/v). Now the column must not run dry.

Sample application: 1/10 of the extract volume, flow rate about 2 ml/min; the column must not run dry

Column washing: 5 ml water, then 5 ml methanol; dry for 10 min under vacuum. Now suck NH_3 gas through the column until the acid is neutralised. To control the neutralisation process, press air through the column: a wet pH paper should indicate a neutral or basic pH value.

Elution: 3 ml methanol (1 – 2 ml/min); carefully concentrate the eluate on a rotation evaporator (40 °C/100 mbar), dissolve the residue in 0.5 ml of 5.5% acetonitrile in buffer (1.641 g sodium acetate in 1 l water, adjusted to pH 5 with glacial acetic acid) and centrifuge.

Further analysis: HPLC

Method for the quantitative determination of glucoside-glucose in wine must

MN Appl. No. 302650

S. Kraml, Forschungsanstalt Geisenheim, Germany

Compounds investigated: glucosidic bonded terpenes

Column type:
CHROMAFIX® C18 ec (M)
Cat. No. 731805

Sample application: adjust 10 – 20 ml clear filtered wine must to pH 2 with HCl

Column conditioning: 10 ml methanol, then 10 ml dist. water

Column washing: 2 x 25 ml dist. water

Elution: 1.5 ml ethanol (96%), then 3.0 ml dist. water; fill up to 5 ml with dist. water

Further analysis: enzymatic glucose determination

Anthocyanines, carbohydrates, organic acids from wine

MN Appl. No. 300160

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg and
CHROMABOND® SB (≡ SAX) / 3 ml / 500 mg
Cat. Nos. 730013 and 730079

Column conditioning: place C18 ec column on top of the SB column using the adaptor; condition with 2 column volumes methanol followed by 2 column volumes dist. water

Sample application: slowly force or aspirate 1 ml wine through the columns and collect the eluate

Column washing: 3 ml water, combine the eluates: carbohydrate fraction

Elution: separate columns, elute the C18 ec column with 2 ml 1% hydrochloric acid in methanol: anthocyanine fraction;
elute the SB column with 3 ml 1 mol/l hydrochloric acid: organic acids

Analysis of proanthocyanidins in malt with an ASPEC system

MN Appl. No. 303200

M. Papagiannopoulos et al., J. Chromatography A **956** (2002) 9 – 16

Column type:
CHROMABOND® PA / 6 ml / 1000 mg
Cat. No. 730127

Sample pretreatment: the crude malt extract is diluted with 24 ml water and mixed in liquid mode by aspirating and dispensing 10 ml of the diluted extract.

Column conditioning: 7 ml water

Sample application: the liquid is pushed through the column with air

Column washing: 8 ml water, then 1 ml DMF – water (85:15, v/v)

Elution: 2.5 ml of DMF – water (85:15, v/v)

Further analysis: HPLC

Recovery rates: 97% for five main malt proanthocyanidins

Anthocyan dyes from red wine

MN Appl. No. 300130

Column type:
CHROMABOND® C8 / 3 ml / 500 mg
Cat. No. 730023

Column conditioning: 1 column volume methanol, then 1 column volume dist. water

Sample application: slowly force or aspirate the wine sample through the column

Column washing: 1.5 ml dist. water

Elution: small volume of methanolic hydrochloric acid

Food additives: dyes from meat products

MN Appl. No. 300310

W. Arneth, E. Träger, Fleischwirtschaft **69** (1989) 621

Compounds investigated: fat- and water-soluble dyes

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg and
CHROMABOND® NH_2 / 1 ml / 100 mg
Cat. Nos. 730011 and 730031

Sample pretreatment: dry 2 x 10 – 20 g sample in thin slices under vacuum. Homogenise with 100 ml petroleum ether (40/60) and filter. Wash filter residue with petroleum ether, combine washing liquid with the filtrate and analyse for fat-soluble dyes as described below.

For determination of the **water-soluble dyes** proceed as follows:

Dry washed filter residue briefly at 60 °C. Add 60 ml of a 0.5% aqueous or methanolic sodium dodecylsulphate solution and homogenise. Stir 30 min at ambient temperature. When working with methanolic sodium dodecylsulphate solution, centrifuge, decant clear supernatant, evaporate to dryness at 30 °C in vacuum, and redissolve residue in 50 ml water. Precipitate sodium dodecylsulphate with 3 ml of a 5% calcium chloride solution. After 15 minutes filter through glass wool and glass fibre filter.

Conditioning of the NH_2 column: 1 column volume methanol, then 1 column volume dist. water

Sample application: pour the pretreated aqueous sample solution through the NH_2 column with about 1 drop/second applying pressure, at most until the adsorbent is completely coloured (if the column gets plugged, rinse with water and/or methanol)

Column washing: about 10 ml dist. water

Elution: 1 ml conc. ammonia solution – discard the first 3 – 5 colourless drops

For determination of the **fat-soluble dyes** proceed as follows:

Evaporate petroleum ether phase of the above filtration, shake the remaining fat with 10 ml methanol and cool. Decant methanol from the crystallised fat and filter.

Conditioning of the C18 ec column: 5 ml methanol

Sample application: force or aspirate the sample through the C18 ec column with 1 – 2 drops/second

Column washing: 2 – 3 ml methanol

Elution: 1 – 2 ml dichloromethane (without pressure or vacuum)

For further analysis we recommend thin layer chromatography on TLC ready-to-use plates SIL G-25, eluent: petroleum ether (60/80) – toluene – acetone – 96% acetic acid (80:10:4:5, v/v/v/v).

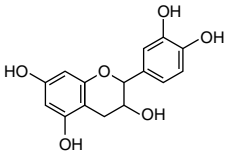
Flavonoids in strawberries

MN Appl. No. 303080

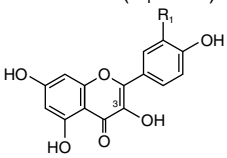
F. Breitfellner et al., Eur Food Res Technol **215** (2002) 28 – 31

Compounds investigated: flavonoids (+)-catechin, (–)-epicatechin, kaempferol-3-glucoside, quercetin-3-glucoside, quercetin-3-galactoside, ellagic acid and derivatives

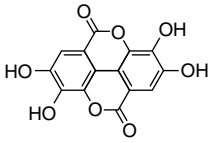
Catechin



Kaempferol (R_1 = H)
Quercetin (R_1 = OH)



Ellagic acid



Column type:
CHROMABOND® PA / 6 ml / 1000 mg
Cat. No. 730127

Sample pretreatment: fresh strawberries are washed with water and drained. Approximately 100 g of the whole fruits without caps are weighed into beakers, irradiated at room temperature (20 °C) in steps of 11 kGy up to 6 kGy; one portion was not irradiated, to serve as control. Irradiated and unirradiated samples are minced with a hand blender and subdivided into 5-g portions. The minced samples are homogenised under addition of 20 ml methanol and centrifuged (10 min, 3000 rpm). The residue is dissolved again in 20 ml methanol and centrifuged. The decanted solutions are combined, evaporated to dryness at 40 °C, and dissolved in 20 ml tridistilled water.

Column conditioning: 10 ml methanol – ammonia (98:2, v/v), followed by 10 ml of acetic acid – water (1:99, v/v) for neutralisation

Sample application: slowly aspirate the pretreated sample through the column

Column washing: sugars, organic acids and other water-soluble components are eluted with 20 ml water

Elution: 50 ml methanol (eluate 1); under these conditions ellagic acid remains adsorbed; it is eluted with 50 ml methanol – ammonia (98:2, v/v) (eluate 2). The eluates are concentrated under vacuum at 40 °C, transferred to 5 ml volumetric flasks, filled up with 0.5% (v/v) phosphoric acid and filtered through a 0.20 µm cellulose acetate membrane.

Further analysis: HPLC

Recovery rates:

(n=3 for each compound)

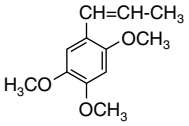
Compound	Recovery [%]
(+)-Catechin	98 ± 2
(–)-Epicatechin	98 ± 2
Quercetin-3-glucoside	88 ± 1
Kaempferol-3-glucoside	88 ± 1
Ellagic acid	82 ± 3

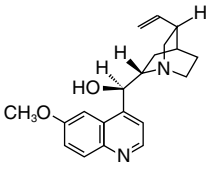
None of the strawberry samples contained quercetin or kaempferol as aglycone.

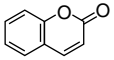
Flavour compounds from brandy

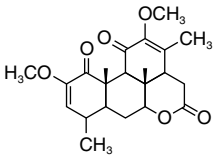
MN Appl. No. 300170

Compounds investigated: asarone, quinine, coumarin, quassin









Column type:
CHROMABOND® Phenyl / 6 ml / 1000 mg
Cat. No. 730412

Sample pretreatment: mix 10 ml sample with 90 ml water and 10 g sodium chloride and adjust to pH 7 with 0.1 mol/l sodium hydroxide solution

Column conditioning: 10 ml methanol, then 10 ml dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 2.5 ml water, then 2.5 ml pentane

Elution:

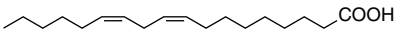
1) 2 x 2.5 ml pentane – diethyl ether (7:3, v/v): asarone, coumarin
2) 10 ml 1 mol/l basic methanol – diethyl ether (9:1, v/v): quinine
3) 5 ml chloroform: quassin

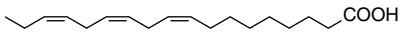
Influence of the acrospire of malted barley on the flavour stability and other quality parameters of beer

MN Appl. No. 302980

Private communication: A. Zürcher, M. Krottenthaler, W. Back, Lehrstuhl für Technologie der Brauerei 1, TU München, Germany

Compounds investigated: linoleic and linolenic acid hydroperoxides





Column type:
CHROMABOND® C18 ec / 3 ml / 200mg
Cat. No. 730012

Column conditioning: 12 ml methanol, then 12 ml water

Sample application: slowly aspirate 20 ml sample through the column

Column washing: 2 ml water

Elution: 8 ml tetrahydrofuran; evaporate to dryness and dissolve the residue in 2 ml acetonitrile

Further analysis: HPLC

Analysis of volatile components derived from raw and roasted earth-almond (*Cyperus esculentus* L.)

MN App. No. 302300

M. J. Cantalejo, R. Wild, J. Agric. Food Chem. **45** (1997) 1853 – 160

Compounds investigated: a total of 143 compounds is determined in the distillate; the main flavour compounds in raw earth-almonds are listed below.

Column type:
CHROMABOND® HR-P

Sample pretreatment: a mixture of 1 kg of earth-almonds and 1.7 l of water is stirred and distilled at 40 °C under a pressure of 5 x 10^3 Pa. From 1 kg of earth-almonds, 110 – 120 ml of extract are obtained.

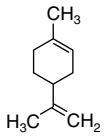
Sample application: the distillate is pressed through the column

Elution: *n*-pentane – dichloromethane (2:1, v/v); the eluate is concentrated to 100 – 200 µl of a residual colorless liquid on a water bath at 40 °C using a Vigreux column

Content of main flavor compounds in raw earth-almonds determined by GC/MS

Compound	Content in distillate [%]
n-Hexanol	21.6
Isoamyl alcohol	19.1
n-Amyl alcohol	6.6
Isobutyl alcohol	4.2
1-Heptanol	4.3
1-Octanol	3.5
1-Nonanol	3.2
Limonene	4.2
Aldehydes	6.4
Benzaldehyde	5.0

Limonene



Determination of 3-chloro-1,2-propanediol in liquid condiments

MN Appl. No. 303550

Private communication: Chinese government Lab „CIQ“

Column type:
CHROMABOND® XTR / 6 ml / 1000mg
Cat. No. 730487

Sample pretreatment: a 3-chloro-1.2-propanediol standard solution is obtained by mixing 0.1 g of 3-chloro-1,2-propanediol reference material and ethyl acetate in a 100 ml flask. The concentration of 3-chloro-1,2-propanediol is 1000 mg/l.

Sample application: 1 gram of sample is given on the XTR column and absorbed for 10 min

Column washing: 6 ml *n*-hexane

Elution: 8 ml ethyl acetate – ether (8:2, v/v). The eluate is collected in a 10 ml centrifuge tube with attached cap and then evaporated in a rotary evaporator or blown by nitrogen until nearly dry (not complete!) at 45 °C.

Further analysis: the sample, 50 ml 1-(heptafluorobutyryl) imidazole and *n*-hexane are mixed in a 1 ml flask and then shook for 1 min. After temperating the mixture at 70 °C for 30 min it is poured into 3 ml of distilled water and shaken for 1 min. Then the mixture is centrifuged at 3000 rpm for 3 min. The supernatant solvent is analysed by GC/ECD.

Recovery rates:

3-chloro-1,2-propanediol [mg/kg]	Recovery [%]
0.01	100.6
1.0	98.3

Extraction of alkylethoxylates

MN Appl. No. 303420

C. Asmussen, Dissertation (2000), Fakultät für Prozesswissenschaften, Institut für Lebensmittelchemie, Technische Universität Berlin, Germany

Column type:
CHROMABOND® HR-P / 6 ml / 200 mg
Cat. No. 730119

Sample pretreatment: 60 mg Brij 30 are added to 1 l water; 20 ml of the water sample are spiked with 10 µl internal standard (1 mg/ml stearyl alcohol)

Column conditioning: 2 column volumes methanol, then 2 column volumes water; the column must not run dry; use weak vacuum

Sample application: apply the 20 ml sample to the column with 5 ml/min

Column washing: 2 column volumes water, then dry the column under vacuum for 5 min, followed by 2 h under a nitrogen stream

Elution: 2 x 7.5 ml ethyl acetate; combine all fractions, evaporate to near dryness and dilute the residue in 1 ml methanol and transfer it to sample vials, dry the sample under a weak nitrogen stream.

Further analysis: HT-GC-AED after derivatisation with BSTFA

Recovery rates: average between 70 and 110% for C12 homologues, between 30 and 70% for C16 homologues

Fatty acid methyl esters from milk fat

MN Appl. No. 300260

F. Ulberth, E. Achs, J. Chromatography **504** (1990) 202 – 206

Column type:
CHROMABOND® SA (≡ SCX) / 3 ml / 500 mg
Cat. No. 730077

Sample pretreatment: esterify milk fat with methanolic KOH solution and dilute with *n*-hexane to a fatty acid methyl ester content of 2 mg/ml

Column conditioning: convert column to the NH_4^+ form by rinsing with 10 ml 1% (w/v) ammonium acetate solution, followed by 10 ml dist. water. Slowly percolate 2 ml of a 1% (w/v) silver nitrate solution into the column. Rinse with 10 ml methanol, 10 ml dichloromethane and 10 ml *n*-hexane.

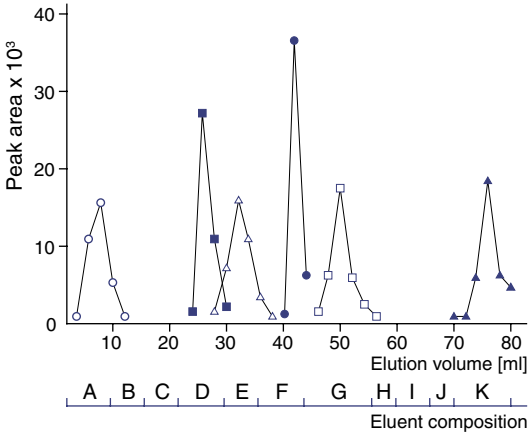
Sample application: force or aspirate 1 ml sample solution through the column

Column washing: 2 ml *n*-hexane

Elution: separation of the fatty acid methyl esters according to carbon number and number of double bonds (x:y)

Elute the conditioned column with 2 ml each of the sequence of eluents given below.

Further analysis: GC



(○ = 18:0, ■ = 18:1-trans, △ = 18:1-cis,
● = 18:2, □ = 18:3, ▲ = 20:4)

Composition of the eluents (v/v):

A) dichloromethane – *n*-hexane (1:99, v/v)
B) dichloromethane – *n*-hexane (5:95, v/v)
C) dichloromethane – *n*-hexane (10:90, v/v)
D) dichloromethane – *n*-hexane (50:50, v/v)
E) dichloromethane
F) methanol – *n*-hexane (3:97, v/v)
G) methanol – *n*-hexane (10:90, v/v)
H) methanol – *n*-hexane (50:50, v/v, lower phase)
I) methanol
J) acetonitrile – methanol (1:99, v/v)
K) acetonitrile – methanol (10:90, v/v)

Analysis of steryl esters in cocoa butter

MN Appl. No. 303360

W. Kamm, F. Dionisi, L.-B. Fay, C. Hirschenhuber, H.-G. Schmarr, K.-H. Engel, J. Chromatography A, **918** (2001) 341 – 349

Column type:
CHROMABOND® C18 ec / 6 ml / 500 mg
Cat. No. 730014

Sample pretreatment: 1 ml of a 4-dimethyl-aminopyridine solution (10 mg/ml in anhydrous pyridine) is added to a solution of 150 mg of the fatty acid chlorides (palmitoyl chloride, stearoyl chloride, oleoyl chloride, linoleolyl chloride) in 1 ml *n*-hexane. The mixture is vortexed until the solution becomes clear. About 200 mg of the sterol are added together with 30 ml of chloroform and the mixture is stirred for 2 h at room temperature in the absence of light. Upon stirring, the initially turbid mixture became clear, otherwise an additional ml of dichloromethane is added dropwise. The solution is washed three times with hydrochloric acid (0.1 mol/l) and the aqueous phase containing the excess of the *N*-acylpyridinium salt is discharged. To neutralise the excess of acid, the solution is washed with a sodium hydrogen carbonate solution. The organic phase is dried with sodium sulphate and the solvent evaporated using a rotary evaporator.

Column conditioning: about 4 ml methanol

Sample application: the solid residue is transferred from the flask onto the cartridge

Column washing: about 0.1 ml chloroform

Elution: 8 ml methanol and 8 ml of *n*-hexane – *tert.*-butyl methyl ether (9:1, v/v)
The steryl esters (purities >80%) are obtained after removal of the solvent of the latter fraction with a stream of nitrogen.

Further analysis: LC/GC

Food additives from orange juice

MN Appl. No. 300320

Compounds investigated: preservative sodium benzoate

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg and
CHROMABOND® OH (Diol) / 3 ml / 500 mg
Cat. Nos. 730013 and 730053

Sample pretreatment: adjust sample to pH 2.5 and filter

Conditioning of the C18 ec column: 1 column volume methanol, then 1 column volume 0.1 mol/l hydrochloric acid

Sample application: slowly force or aspirate 1 ml sample through the column

Column washing: 2 x 1 ml 0.1 mol/l hydrochloric acid; dry column under vacuum for 30 sec; aspirate 1 ml *n*-hexane through the column; dry column under vacuum for 1 min

Conditioning of the OH column: 1 column volume dichloromethane, then pour 2 ml dichloromethane into the column, do not apply vacuum

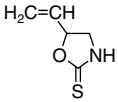
Sample transfer: place C18 ec column onto the OH column. Pour 2 x 1 ml dichloromethane through the columns; let each portion of dichloromethane remain in the C18 ec packing for 1 min, then aspirate solvent through the columns. Remove C18 ec column. Wash OH column with 1 ml dichloromethane and dry 1 min under vacuum.

Elution: leave 250 µl methanol in the column packing for 1 min, then elute benzoic acid with 2 x 500 µl methanol

Analysis of 5-vinyl-1,3-oxazolidine-2-thione in complex matrices at ppb level

MN Appl. No. 302480

N. Mabon et al., Talanta **49** (1999) 199 – 206



Column type:
CHROMABOND® C18, 2500 mg (special)

Sample pretreatment: 3.0 g of liver, kidney, lung, muscle, thyroid or 3 ml of plasma or milk are placed in a 16 x 100 mm glass tube with Teflon-coated screw caps. After preheating for 1 min in a 85 °C water bath, 4 ml of boiling phosphate buffer are added. Then, 500 µl of the aqueous solution of internal standard is added. The scattering of the blended matrices is achieved by vigorous manual shaking. The extract is kept in the water bath for 10 min and vortexed periodically, then cooled and centrifuged at 1000 x g for 10 min. The supernatant is transferred to a 15 x 100 mm test tube. The extraction is repeated twice but with 3 ml of phosphate buffer.

Column conditioning: 3 ml methanol, 2 ml acetonitrile, then 20 ml dist. water with special care in order to prevent air from entering

Sample application: about 10 ml of the sample are poured into the column reservoir and then percolated

Column washing: 2 ml dist. water to eliminate most of the polluting substances, then dry under aspiration for 1 min

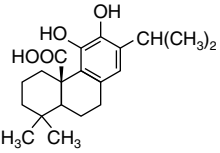
Elution: 2 ml methanol – acetonitrile (50:50, v/v); evaporate the eluate to dryness in a 10 ml conic flask using a rotating evaporator under vacuum (40 °C)

Further analysis: HPLC

Bioavailability of the antioxidative *Rosmarinus officinalis* compound carnosic acid in eggs

MN Appl. No. 302460

E. L. Krause, W. Ternes, Eur. Food Res. Technol. **210** (2000) 161 – 164



Column type:
CHROMABOND® HR-P / 3 ml / 500 mg
Cat. No. 730117

Sample pretreatment: fat and fat-soluble contents are extracted by the method of Twisselman. Forty grams of seasand are mixed with 4.00 g egg yolk. This mixture is homogenised with 30 g sodium sulphate and extracted for 5 h under reflux with 150 ml ethanol – cyclohexane (1:1, v/v).The remaining solvent is removed by heating at 50 °C under vacuum up to a volume of about 5 ml, followed by ventilation with nitrogen. The extract is dissolved in methanol and filtered through glass wool. The separation of fat from the sample is achieved in two steps. First it is filtered through a column containing 0.5 g Sephadex-LH-20 (dissolved in methanol) and eluted with methanol up to a sample volume of 30 ml. A part of the fat remains on the column without any loss of antioxidative compounds. Then the solution is frozen for 1.5 h at –18 °C in a deep freeze and for 15 min at – 21°C in a mixture of ice and salt (1:1) to separate the solution from remaining fat. The sample is centrifuged for 2 min at 1000 g at –18°C. The supernatant is separated and the solvent is concentrated at 60 °C under vacuum up to ~1.5 ml and validated with nitrogen. Partial precipitation of proteins is achieved by diluting the sample with 4 ml acetonitrile – water (6.5:3.5, v/v), filtering through a folded filter and washing with 1 ml acetonitrile – water (6.5:3.5, v/v). The filtrate is diluted with 4 ml of water.

Column conditioning: 4 ml of water

Sample application: slowly force or aspirate the pretreated sample through the column

Column washing: water

Elution: 5 ml acetonitrile – methanol (1:1, v/v), collected in a 5 ml volumetric flask; a sample of 1.5 ml of the eluate is concentrated under vacuum (50 °C) to a volume of about 250 µl and aerated with nitrogen

Further analysis: HPLC with electrochemical detection and diode array detection for confirmation of assignments

Metalorganic compounds in seafood

MN Appl. No. 301870

Compounds investigated: di- and tributyl tin

Column type:
CHROMABOND® Florisil® / 6 ml / 1000 mg
Cat. No. 730082

Sample pretreatment: homogenise 10 g sample with saturated NaCl solution, add 0.3 M HCl. Extract homogenate with *n*-hexane – diethyl ether (3:1, v/v)

Column conditioning: 5 ml acetone

Sample application: slowly force or aspirate the extract through the column

Elution: *n*-hexane – diethyl ether – acetic acid (75:25:1, v/v/v)

Quantification of hexavalent chromium in UHT milk and powdered milk

MN Appl. No. 302230 / 303460

J. Lameiras et al., Analyst **123** (1998) 2091 – 2095

Column type:
CHROMABOND® NH_2 / 3 ml / 500 mg
Cat. No. 730033

Sample pretreatment: UHT milk (40 ml) is mixed with 5 ml of 2% sodium acetate (pH 3.5). The sample is vortex mixed and centrifuged at 3000 rpm for 30 min. The supernatant is transferred to another tube, 0.001% alizarin (5 ml) are added and the mixture is agitated for 2 min. Thereafter, 40% sodium acetate are added up to pH 4.9. This solution is homogenised.

Powdered milk infant formulae are reconstituted with water and applied to the SPE column after precipitation of proteins

Column conditioning: two column volumes of 1 M nitric acid followed by two column volumes of distilled water

Sample application: pour the sample through the column at a flow rate of 4 ml/min, then dry the column under vacuum

Elution: two column volumes of 2 M HNO_3

Further analysis: both total chromium (directly in the milk sample) and hexavalent chromium after solid phase extraction are quantitated by atomic absorption spectrometry

Determination of acrylamide in food

MN Appl. No. 303580

J. Rosen, K. E. Hellenäs, B. Gutsche, R. Weißhaar, J. Buhlert; The Analyst **127** (2002) 871 – 879; Deutsche Lebensmittel-Rundschau **98** (2002) 437 – 443

Column type:
CHROMABOND® ABC18 / 6 ml / 500 mg
Cat. No. 730533

Sample pretreatment: a representative amount of sample (at least 50 g) is homogenised, e.g. by grinding or crushing in a suitable mill. About 5 g of homogenised sample are weighed in a folded filter and superficially defatted by slowly pouring 50 ml isohexane – butyl methyl ether (3:1, v/v) through the filter. The sample is spiked with about 2 – 5 µg of the internal standard (maximum 2 ml). After addition of 100 ml of bidistilled water the flask is ultrasonically heated at 40 °C for 10 min (shake now and then). The contents of the flask are mixed with 0.5 – 1 ml Carrez solution 1 and the with the same volume of Carrez solution 2 (mix well every time). The aqueous phase is filtered through a paper filter, about the first 10 ml are discarded, the next 10 – 20 ml are collected separately for further investigation. If necessary, a centrifugation step must precede the filtration.

Column conditioning: 10 ml methanol, then 10 ml bidist. water; aspirate air through the column for about 30 s

Sample application and elution: slowly force or aspirate 2 ml of the prepared sample through the column, discard the eluate; then force or aspirate another 5 ml* of the sample solution through the column, and collect the eluate

* The detection limit can be decreased to 40 µg/kg by using 25 ml instead of 5 ml sample solution.

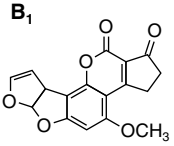
Further analysis: LC/MS/MS or GC/MS after extraction with 10 ml ethyl acetate, evaporation of the extract and filling up to 1 ml

Mycotoxins from cereal, nuts and peanut butter

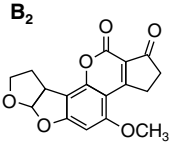
MN Appl. No. 300360

Compounds investigated: aflatoxins B_1, B_2, G_1, G_2

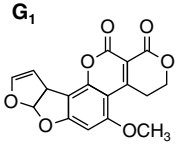
B_1



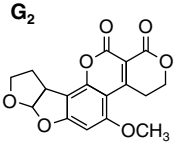
B_2



G_1



G_2



Column type:
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. No. 730073

Sample pretreatment: homogenise 50 g sample with 200 ml methanol – dist. water (85:15, v/v) for 30 min. Filter and mix. 40 ml of this filtrate with 40 ml aqueous sodium chloride solution (0.1 g/ml). Degrease the solution with 2 x 25 ml *n*-hexane, extract with 2 x 25 ml chloroform and concentrate the extract.

Column conditioning: 3 ml *n*-hexane, then 3 ml chloroform

Sample application: slowly pour the chloroform extract through the column

Column washing: 3 ml *n*-hexane, 3 ml diethyl ether, then 3 ml chloroform

Elution: 6 ml chloroform – acetone (9:1, v/v)

Mycotoxins from corn flour

MN Appl. No. 300370

Compounds investigated: aflatoxins B_1, B_2, G_1, G_2

Column type:
2 columns CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: homogenise 50 g corn flour with 100 ml methanol – water (8:2, v/v) (or acetonitrile – water, 9:1, v/v)

Conditioning of the 1st column: 1 column volume methanol, then 1 column volume methanol – water (8:2, v/v)

Sample application: slowly force or aspirate 3 ml homogenised unfiltered flour through the 1st column. Dilute 2 ml of the eluate to 5 ml with dist. water

Conditioning of the 2nd column: 1 column volume methanol, then 1 column volume dist. water

Sample application: slowly force or aspirate 5 ml of the diluted eluate from the 1st column through the 2nd column

Column washing: 1 column volume dist. water, then 1 ml *n*-hexane; dry column under vacuum for 5 min

Elution: 3 x 500 µl methylene chloride

Mycotoxins from liver

MN Appl. No. 300380

Compounds investigated: aflatoxins B_1 and M_1

Column type:
2 columns CHROMABOND® C18 ec / 6 ml / 500 mg
Cat. No. 730014

Sample pretreatment: homogenise 1 g liver with 2 ml saturated sodium chloride solution and 2.5 ml dist. water, add 10 ml methanol and shake 20 min; then centrifuge

Conditioning of the 1st column: 10 ml methanol, then 10 ml methanol – water (4:1, v/v)

Sample application: slowly force or aspirate the centrifuged solution through the column; dilute the eluate with twice its volume of dist. water

Conditioning of the 2nd column: 10 ml methanol, then 10 ml dist. water

Sample application: slowly force or aspirate the diluted eluate from the 1st column through the 2nd column

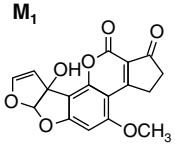
Column washing: 5 ml dist. water, dry column by aspirating air through it; then rinse column with 5 ml *n*-hexane, dry under vacuum for 3 – 4 min

Elution: elute B_1 with 3 ml *n*-hexane – dichloromethane (45:55, v/v); dry column 3 – 4 min under vacuum and elute M_1 with 3 ml dichloromethane – acetone (9:1, v/v)

Mycotoxins: aflatoxin M_1 from milk

MN Appl. No. 300430

M_1



Column type:
CHROMABOND® C18 ec / 6 ml / 500 mg
Cat. No. 730014

Sample pretreatment: dilute 20 ml milk with 30 ml dist. water

Column conditioning: 10 ml methanol, then 10 ml dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 10 ml dist. water, then 10 ml *n*-hexane;
dry column for 10 – 20 min at 50 °C or overnight at ambient temperature

Elution: 3 ml dichloromethane – acetone (4:1, v/v)

Mycotoxins: aflatoxins from maize

MN Appl. No. 300390

Column type:
CHROMABOND® Phenyl / 3 ml / 500 mg
Cat. No. 730084

Sample pretreatment: homogenise 50 g maize with 62.5 ml dist. water for 2 min, mix 100 g of the homogenate with 200 ml methanol and homogenise 3 min. Filter through filter paper MN 615 and mix 5 ml of the extract with 60 ml water – methanol – acetic acid (92.3:6.7:1, v/v/v)

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

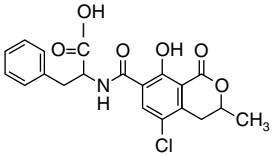
Sample application: force or aspirate the pretreated extract through the column with 10 ml/min

Column washing: 10 ml water; dry column under vacuum for 5 min

Elution: fill an empty CHROMABOND® column (6 ml) with 3 g anhydrous sodium sulphate, place it below the phenyl column using the adaptor, and elute aflatoxins with 7 ml chloroform (1 ml/min)

Mycotoxins: ochratoxin A from porcine serum

MN Appl. No. 300400



Column type:
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. No. 730073

Sample pretreatment: adjust 20 ml serum with 2 ml 2 M hydrochloric acid and 10 ml 0.4 M magnesium chloride to a pH value < 2. Add 10 ml chloroform. Shake 30 min and separate the emulsion by centrifugation.

Column conditioning: 2 column volumes toluene

Sample application: slowly force or aspirate 5 ml of the chloroform phase through the column

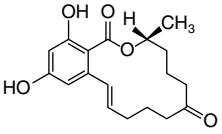
Column washing: 2 x 5 ml toluene

Elution: 2 x 10 ml toluene – acetic acid (9:1, v/v)

Mycotoxins from cereals and food

MN Appl. No. 300410

Compounds investigated: zearalenone, ochratoxin A



Zearalenone

Column type:
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. No. 730073

Sample pretreatment: mix 50 g ground sample with 250 ml chloroform and 25 ml 0.1 M phosphoric acid (for wheat and barley add 10 g celite kieselguhr); shake 45 min. For wheat and barley filter; for oats etc. centrifuge 10 min at 9 000 x g and then filter over 2 g celite. From this extract take 25 ml for wheat and barley, 15 ml for oats etc., evaporate almost to dryness and redissolve in 10 ml dichloromethane.

Column conditioning: place 2 g dry sodium sulphate onto the SiOH column (a combination column SiOH / Na_2SO_4 is available on request). Force or aspirate 5 ml *n*-hexane through the column, followed by 5 ml dichloromethane

Sample application: slowly force or aspirate the pretreated sample through the column

Column washing: 10 ml dichloromethane, then 10 ml *n*-hexane, finally 10 ml toluene

Elution: 8 ml toluene – acetone (95:5, v/v): zearalenone;
6 ml toluene – glacial acetic acid (90:10, v/v): ochratoxin A;
eluates can be concentrated in a stream of nitrogen

For further analyses we recommend HPLC using column NUCLEOSIL® 5 C_8, mobile phase methanol – 0.01 M phosphoric acid (58:42, v/v), 1 ml/min, fluorescence detection.

Mycotoxins from apple juice

MN Appl. No. 300420

T. Delgado et al., J. Chromatography **731** (1996) 109–114 and ibid **815** (1998) 93 – 97

Compounds investigated: alternariol and alternariol methyl ether

Step 1:

Column type:
CHROMABOND® C18 / 3 ml / 500 mg
Cat. No. 730003

Column conditioning: 6 ml methanol, then 6 ml dist. water

Sample application: slowly force or aspirate 10 ml apple juice through the column

Column washing: 2 ml dist. water, then 2 ml acetonitrile – dist. water (1:3, v/v)

Elution: 4 ml acetonitrile – acetic acid (99:1, v/v)

Evaporate the eluate and redissolve in 1.5 ml ethyl acetate (ultrasonic treatment)

Step 2:

Column type:
CHROMABOND® NH_2 / 3 ml / 500 mg
Cat. No. 730033

Column conditioning: 6 ml methylene chloride or ethyl acetate

Sample application: force or aspirate the redissolved eluate from step 1 through the column

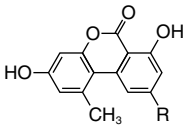
Column washing: 2 ml acetone, then 2 ml acetonitrile

Elution: 4 ml acetonitrile – formic acid (99:1, v/v)

Further analysis: HPLC

Recovery rates:

Compound	Recovery [%]	R
Alternariol	82.8	OH
Alternariol methyl ether	91.9	OCH_3



Pesticides from homogenised milk

MN Appl. No. 301460

Column type:
CHROMABOND® C18 ec / 3 ml / 200 mg
Cat. No. 730012

Sample pretreatment: mix 10 ml homogenised milk with 0.5% toluene and shake 10 min

Column conditioning: 10 ml acetonitrile, then 10 ml dist. water

Sample application: slowly force or aspirate the sample through the column

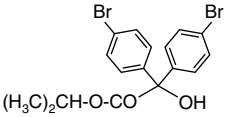
Column washing: 2 x 10 ml dist. water; dry column 30 min under vacuum

Elution: elute organochlorine pesticides with 2 ml *n*-hexane, triazines and weakly polar compounds with 2 ml methanol

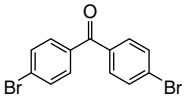
Pesticides: acaricides from honey

MN Appl. No. 301490

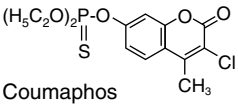
Compounds investigated: bromopropylate, coumaphos, 4,4-dibromobenzophenone, fluvalinate



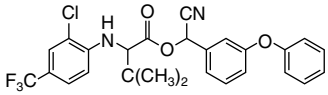
Bromopropylate



Dibromobenzophenone



Coumaphos



Fluvalinate

Column type:
CHROMABOND® C18 ec / 3 ml / 200 mg and
CHROMABOND® Florisil® / 3 ml / 500 mg
Cat. Nos. 730012 and 730081

Sample pretreatment: dissolve 5 g honey in 10 ml ethanol – water (1:1, v/v) and centrifuge

Column conditioning:
C18 ec: 1 column volume ethanol, then 1 column volume ethanol – water (1:1, v/v)
Florisil®: 1 column volume ethanol – water (1:1, v/v)

Sample application: place the Florisil® column onto the C18 ec column and slowly force or aspirate the sample through the column combination

Column washing: dist. water, then ethanol – water (1:1, v/v)

Elution: separate columns; elute acaricides from the C18 ec column with dichloromethane and ethyl acetate

Determination of pesticides in food samples (QuEChERS)

MN Appl. No. 303770

MN Chromatography Department - based on: Anastassiades et al., Journal of AOAC International **86** (2003) 412 – 431 (CVUA Stuttgart, Germany)

Column type:
CHROMABOND® Diamino (adsorbent)
Cat. No. 730653.20

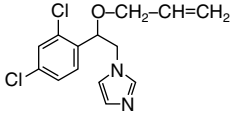
Sample pretreatment: 10 g sample (fruit or vegetables with low fat content) are homogenised with 10 ml acetonitrile. After adding the internal standard the sample is shaken with 4 g $MgSO_4$ and 1 g NaCl and centrifuged afterwards. 1 ml of the supernatant is spiked with 25 mg CHROMABOND® Diamino and 150 mg $MgSO_4$ and shaken again. After centrifugation the supernatant is injected into GC/MS.

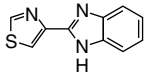
Further analysis: we recommend GC-MS

Pesticides: fungicides from citrus fruit

MN Appl. No. 301500

Compounds investigated: imazalil and thiabendazole





Column type:
CHROMABOND® OH (Diol) / 3 ml / 500 mg
Cat. No. 730053

Sample pretreatment: homogenise the peel and the white part of 100 – 200 g fruit 5 min with 100 ml dichloromethane, filter, again homogenise the residue with 100 ml CH_2Cl_2, filter; combine the filtrates and fill up to 200 ml

Column conditioning: 2 column volumes dichloromethane

Sample application: slowly pour 10 ml pretreated sample through the column

Column washing: 10 ml CH_2Cl_2; air dry column 5 min

Elution: 2 x 1 ml methanol – 0.1 M phosphoric acid (1:1, v/v)

Pesticides: imidazole from tomatoes

MN Appl. No. 301540

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg and
CHROMABOND® NH_2 / 3 ml / 500 mg
Cat. Nos. 730013 and 730033

Sample pretreatment: heat sample 2 h in 50 ml 2 mol/l hydrochloric acid, then filter

Column conditioning:

C18 ec: 2 column volumes MeOH, then 2 column volumes dist. H_2O

NH_2: 1 ml ethyl acetate, then 1 ml dichloromethane;
take care, that the column does not run dry

Sample application: slowly pour 40 ml sample through the C18 ec column

Column washing: 5 ml water; dry C18 ec column 5 min under vacuum, rinse with 1 ml dichloromethane, place C18 ec column onto NH_2 column

Elution: 1 ml ethyl acetate through both columns

Pesticides: atrazine from maize

MN Appl. No. 301630

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: homogenise 5 g maize with 10 ml acetonitrile and filter. Mix the filtrate with an equal volume of dist. water and evaporate to 10 ml at 60 °C under nitrogen.

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 ml dist. water

Elution: 2 ml methanol

Pesticides: atrazine from vegetable oil

MN Appl. No. 301620

Column type:
CHROMABOND® OH (Diol) / 3 ml / 500 mg
Cat. No. 730053

Sample pretreatment: mix 2 ml vegetable oil with 20 ml *n*-hexane

Column conditioning: 1 column volume *n*-hexane

Sample application: slowly force or aspirate the sample through the column

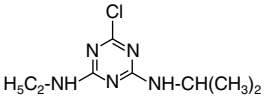
Column washing: 1 ml *n*-hexane

Elution: 1 ml methanol

Pesticides: atrazine from soy beans

MN Appl. No. 301610

Column type:
CHROMABOND® SA (≡ SCX) / 3 ml / 500 mg
Cat. No. 730077



Sample pretreatment: homogenise soy beans in acetonitrile, filter and add 15% acetic acid (1%)

Column conditioning: 2 column volumes acetonitrile, then 2 column volumes 1% acetic acid

Sample application: slowly force or aspirate the sample through the column

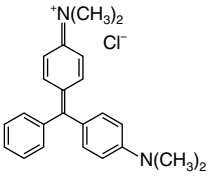
Column washing: 1 ml 1% acetic acid, then 1 ml acetonitrile, finally 1 ml 0.1 M dipotassium hydrogen phosphate

Elution: 1 – 2 ml acetonitrile – 0.1 M dipotassium hydrogen phosphate (1:1, v/v)

Pesticides: fungicide malachite green from fish

MN Appl. No. 301520

Column type:
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. No. 730073



Sample pretreatment: extract 25 g sample material with acetonitrile at pH 4. Mix with NaCl – dichloromethane and concentrate the organic phase. Redissolve the residue in 2 ml acetone – toluene (2:1, v/v).

Sample application: slowly force or aspirate the sample through the column; malachite green is seen in the column as small blue band

Column drying: under vacuum

Elution: pentanesulphonate solution

Xanthines: caffeine from decaffeinated cola

MN Appl. No. 300670

Column type:
CHROMABOND® Phenyl / 3 ml / 500 mg
Cat. No. 730084

Sample pretreatment: remove CO_2 from the beverage sample by shaking

Column conditioning: 1 column volume methanol, then 1 column volume dist. water

Sample application: pour 50 ml sample through the column with about 5 ml/min

Column washing: 2 column volumes 0.15 M aqueous ammonia solution – methanol (9:1, v/v), aspirate each portion of the washing liquid into the column packing and wait 30 sec; then dry the column under vacuum

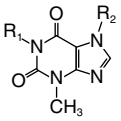
Elution: 2 x 1 ml acetonitrile – 1% aqueous acetic acid (3:7, v/v)

Xanthines from beverages

MN Appl. No. 300660

Compounds investigated: caffeine, theobromine, theophylline

Caffeine	$R_1 = CH_3$	$R_2 = CH_3$
Theobromine	$R_1 = H$	$R_2 = CH_3$
Theophylline	$R_1 = CH_3$	$R_2 = H$



Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

Sample pretreatment: mix beverage sample with internal standard β-hydroxyethyltheophylline

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate 1 ml sample through the column

Column washing: 1 column volume dist. water; dry column under vacuum for 10 min

Elution: 2 x 500 µl chloroform

Extraction of PAH and removal of chlorophyll, carotinoids and essential oils from vegetables

MN Appl. No. 301220

Column type:
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. No. 730073

Sample pretreatment: homogenise 25 g sample, mix with 2 ml internal standard (100 ng/ml phenanthrene-D_{10}, benzo[e]pyrene-D_{12} or perylene-D_{12} in methanol or cyclohexane) and reflux 4 h with 100 ml 2 mol/l methanolic potassium hydroxide solution in an amber glass flask. Transfer the still warm solution into an amber glass funnel, rinse the flask with 100 ml methanol – water (9:1, v/v) and extract twice with 150 ml each of cyclohexane. Clean cyclohexane extract by extraction with 100 ml methanol – water (1:1, v/v) and 100 ml water and dry over sodium sulphate; evaporate to about 2 ml in a rotation evaporator at 40 °C

Column conditioning: 5 ml cyclohexane

Sample application: slowly pour the sample through the column, collect eluate

Elution: 7 ml cyclohexane; combine eluates and concentrate in a stream of nitrogen

Benzo[a]pyrene from smoked meat products aromatised with artificial smoke aroma

MN Appl. No. 302720

K. Hartmann, Landesuntersuchungsamt Rheinland-Pfalz, Fachbereich Tiermedizin, Koblenz, Germany; Deutsche Lebensmitel-Rundschau **96** (2000) 136 – 166

Column type:
CHROMABOND® CN/SiOH / 6 ml / 500/1000 mg
Cat. No. 730135

Sample pretreatment: homogenise at least 100 g of meat with a suitable device. Weigh 20 g of the homogenised sample on a folded filter and insert it into an extraction thimble. Add 1 ml benzo[b]-chrysene standard solution and place the extraction thimble in a Soxhlet extractor. Fill the Soxhlet flask with 200 ml methanol and 25 ml KOH solution (50%). Extract the sample for 5 h on a sand or water bath. The extraction fluid must boil. Add 150 ml *n*-hexane to the cold extract and stir with a magnetic stirrer for 1 h. Add 100 ml dist. water and transfer all of the extract into an extraction funnel. Rinse the Soxhlet twice with 10 ml methanol each. After phase separtion collect the hexane phase and wash the aqueous phase twice with 100 ml *n*-hexane. The combined *n*-hexane phases are dried over sodium sulphate, evaporated on a rotation evaporator at 40 °C and redissolved in 1 ml cyclohexane.

Column conditioning: 5 ml dichloromethane, 5 ml cyclohexane

Sample application: slowly aspirate the pretreated sample through the column

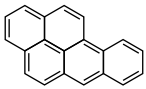
Column washing: 2 x 2 ml *n*-hexane, aspirate air through the column until it is dry

Elution: 5 x 5 ml acetonitrile; evaporate to dryness with a rotation evaporator at 40 °C; dissolve the residue in 1 ml acetonitrile; filter the solution through a 0.45 µm membrane filter (e.g. PET, Cat. No. 729023) or centrifuge with an ultra-centrifuge

Further analysis: HPLC (column NUCLEOSIL® 100-5 C_{18} PAH)

Recovery rates:
(determined by addition of 2 benzo[a]pyrene concentrations to the sample material, original contamination 0.013 µg/kg)

Concentration [µg/kg]	Recovery [µg/kg]	Recovery [%]
0.013 + 0.025	0.033	87
0.013 + 0.05	0.049	78



Rapid determination of heavy polycyclic aromatic hydrocarbons in edible fats and oils

MN Appl. No. 303060

R. Weißhaar, Eur. J. Lipid Sci. Technol. **104** (2002) 282 – 285

Column type:
CHROMABOND® HR-P / 6 ml / 500 mg
Cat. No. 730111

Sample pretreatment: in a small beaker, dissolve 2 – 2.5 g of sample in 5 ml isohexane – *t*-butyldimethylether, BME (95:5, v/v). Add 50 µl internal standard solution (benzo[b]chrysene, 1 ng/µl isooctane).

Column conditioning: 10 ml isohexane – BME (95:5, v/v)

Sample application: transfer the solution onto the column and rinse the beaker with additional 2 ml isohexane – *t*-butyldimethylether (95:5, v/v)

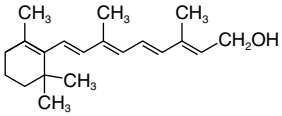
Column washing: 10 ml isohexane – BME (95:5, v/v), followed by a small volume of air (about 3 ml), then 10 ml isohexane – BME (80:20, v/v), followed by air as above, finally 10 ml isohexane – BME (50:50, v/v) followed by air as above; all eluates are discarded

Elution: 20 ml tetrahydrofuran; add 50 µl acetonitrile – diethylene glycol (2:1, v/v) and evaporate to dryness; dissolve the residue in 500 µl acetonitrile

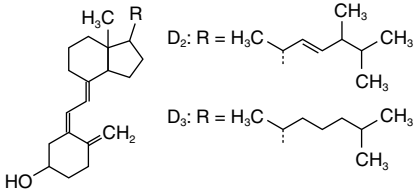
Further analysis: HPLC

Vitamins

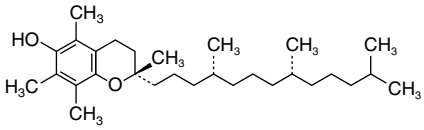
Vitamin A (retinol)



Vitamin D_2 (calciferol) **and D_3** (cholecalciferol)



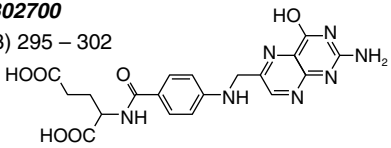
Vitamin E (α-tocopherol)



Vitamins: folic acid in food with HPLC

MN Appl. No. 302700

R. Gauch et al., Mitt. Gebiete Lebensm. Hyg. **84** (1993) 295 – 302



Column type:
CHROMABOND® C18 ec / 6 ml / 1000 mg
Cat. No. 730015

Sample pretreatment: add 80 mg Clara-Diastase, 80 mg trypsin and 100 ml phosphate buffer to 10 g of the homogenous food sample. Homogenise the sample for 3 min with a Polytron and hydrolise for 3 hours in a water bath at 40 °C. Shake gently every 30 min. Centrifugate the hydrolised sample for 20 min at 2000 rpm (15 °C) and filter the supernatant through a folded filter. Place 10 ml of the clear solution in a 50 ml beaker and add 0.8 g DEAE-Sephadex. Stir gently for 30 min. Rinse the contents of the beaker onto a filter column with 1/15 M phosphate buffer pH 7.0 (3.6 g KH_2PO_4 + 8.3 g K_2HPO_4 in 1 l dist. water). Place quartz wool on the surface of the ion exchanger and wash with 25 ml phosphate buffer (about 2 ml/min). Elute the folic acid fraction with 20 – 25 ml buffered sodium chloride solution (58 g NaCl in 1 liter 1/15 M phosphate buffer pH 7.0 as above, flow rate about 1 ml/min).

Column conditioning: 5 ml ethanol abs. and 5 ml 1/15 M phosphate buffer pH 7.0, using a vacuum manifold

Sample application: aspirate the complete eluate from the sample pretreatment through the column (about 970 mbar, 1 drop per second)

Column washing: 10 ml 1/15 M phosphate buffer pH 7.0, dry column for 1 min

Elution: 4 – 4.5 ml water, fill to 5 ml with dist. water

Further analysis: HPLC with column NUCLEOSIL® 120-5 C_{18} and UV or fluorescence detection

Recovery rates:

Food	Recovery [%]
Cacao drink	86
Cacao drink for babies and children	95
Corn flakes	94

Vitamins: folic acid from food

MN Appl. No. 300650

Column type:
CHROMABOND® SB (≡ SAX) / 3 ml / 500 mg
Cat. No. 730079

Sample pretreatment: homogenise 10 g food sample in 100 ml 0.01 M phosphate buffer pH 7.4 and filter

Column conditioning: 2 column volumes *n*-hexane, then 2 column volumes methanol, finally 2 column volumes dist. water

Sample application: force or aspirate 10 ml of the filtrate through the column

Column washing: 2 column volumes dist. water

Elution: 5 ml 10% sodium chloride in 0.1 M sodium acetate buffer

Vitamins A, D_2, D_3 and E from animal feed

MN Appl. No. 300570

Column type:
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. No. 730073

Sample pretreatment: homogenise 10 g sample with 20 ml *n*-hexane and filter

Column conditioning: 2 column volumes *n*-hexane

Sample application: slowly force or aspirate the sample through the column

Column washing: 1 ml *n*-hexane

Elution: 2 ml *n*-hexane – ethyl acetate (1:1, v/v)

Simultaneous quantification of vitamins A, D_3, and E in food samples

MN Appl. No. 303720

O. Heudi, M.-J. Trisconi, C.-J. Blake; J. Chromatography A **1022** (2004) 115 – 123

Column type:
CHROMABOND® XTR / 70 ml / 14.5 g
Cat. No. 730507

Sample pretreatment: the saponification procedure is similar to that described in the official CEN methods for Vitamins A, D_3 and E determination. Briefly, a well homogenised food sample (50 g of commercial powdered dietetic infant formulae and infant cereals) is dissolved in 100 ml of warm distilled water (40 °C) and thoroughly mixed to obtain a homogeneous slurry. A portion of the above mixture (30 g) is accurately weighed into a 250 ml Erlenmeyer flask and spiked with the internal standard solution containing Vitamin D_2 and DMT (5,7-dimethyltocol). For starch-containing products, about 0.2 g of takadiastase is added and the solution is incubated for 30 min at 45 °C. To the latter solutions, are added the following reagents under agitation: 7 g of potassium hydroxide, 50 ml of ethanol, 1 g of sodium sulphide and 1 g of sodium ascorbate. The solution is mixed under a nitrogen steam, then heated under reflux at 85 °C for 30 min. After cooling, the solution is quantitatively transferred into a 100 ml volumetric flask and 2 g of sodium 1-pentanesulfonate are added under agitation. The solution is made up to 100 ml volume with water.

Sample application: 20 ml of the saponified sample solution are poured onto the column and allowed to absorb for 15 min

Column washing: not performed

Elution: 100 ml of *n*-hexane (containing 5 mg BHT; 2,6-di-*tert*-butyl-*p*-cresol). The eluates are brought to dryness by a steam of nitrogen and the residue is dissolved in 4 ml of HPLC mobile phase.

Further analysis: If necessary the extract is diluted appropriately with the HPLC mobile phase before filtration through a 0.45 µm membrane filter. 40 µl are injected into a HPLC system (see MN Appl. No. 119820 at *www.mn-net.com*)

Recovery rates: (n = 6)

Vitamin A	102 ± 8%
Vitamin D_3	105 ± 7%
Vitamin E	96 ± 9%

Additives: preservatives from cosmetics

MN Appl. No. 300340

Compounds investigated: *p*-hydroxybenzoates

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: mix 1 g sample with 10 ml methanol, shake thoroughly and centrifuge. Dilute 100 µl supernatant to 2 ml with dist. water.

Column conditioning: 2 column volumes methanol, 2 column volumes water

Sample application: slowly force or aspirate 2 ml sample through the column

Column washing: 1 column volume dist. water

Elution: 2 x 500 µl methanol

For further analysis we recommend HPLC, e.g. with column NUCLEOSIL® 100-5 C_{18}.

Additives: preservatives from cosmetics

MN Appl. No. 300350

Compounds investigated: formaldehyde, especially in the presence of Dowicil 200

Column type:
CHROMABOND® SA (≡ SCX) / 3 ml / 500 mg
Cat. No. 730077

Sample pretreatment: dilute 1 g cosmetic sample with 100 ml tetrahydrofuran – water (9:1, v/v) and homogenise

Column conditioning: 3 column volumes tetrahydrofuran – water (9:1, v/v)

Sample application: slowly force or aspirate 1 ml sample through the column and collect the eluate

Elution: 1 ml tetrahydrofuran – water (9:1, v/v); combine the eluate with the eluate from the sample application

Derivatisation: mix 1 ml eluate with 0.4 ml 0.1% 2,4-dinitrophenylhydrazine and stir 1 min. Let stand 2 min at ambient temperature, then add 0.4 ml 0.1 M phosphate buffer of pH 6.8 and 0.7 ml 1 M sodium hydroxide solution.

Formaldehyde analysis with HPLC / biosensor coupling in cosmetics

MN Appl. No. 302180

J. Schultheiss, R. Galensa, Deutsche Lebensmittelrundschau **96** (2000) 98 – 103

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: dilute samples in 100 mM $Na_4P_2O_7$ x 10 H_2O buffer pH 8

Sample application: the whole sample is applied on the column. Formaldehyde shows no interactions with the sorbent and can be analysed in the eluate, while lipophilic matrix components are retained on the SPE column.

Further analysis: HPLC/biosensor coupling with formaldehyde dehydrogenase

Environmental samples and pollutants

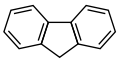
Envirionmental samples and pollutants

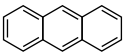
PAH

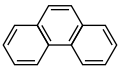
Naphthalene 

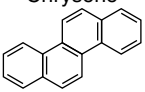
Acenaphthylene 

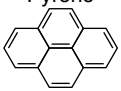
Acenaphthene 

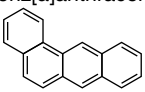
Fluorene 

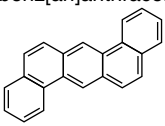
Anthracene 

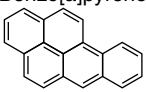
Phenanthrene 

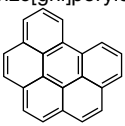
Chrysene 

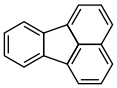
Pyrene 

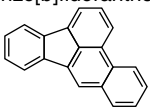
Benz[a]anthracene 

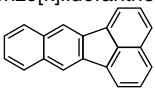
Dibenz[ah]anthracene 

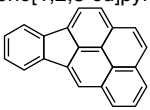
Benzo[a]pyrene 

Benzo[ghi]perylene 

Fluoranthene 

Benzo[b]fluoranthene 

Benzo[k]fluoranthene 

Indeno[1,2,3-cd]pyrene 

PAH from drinking water (EPA 550)

MN Appl. No. 302170

Methods for the determination of compounds in drinking water, supplement I, US EPA, office of R&D, Washington DC 20460, EPA/600/4-90/020

Column type:
CHROMABOND® C18 ec / 6 ml / 1000 mg
Cat. No. 730015

Sample pretreatment: add 100 mg $Na_2S_2O_3$ to 1000 ml water sample and adjust the pH value to pH 2 with 6 mol/l HCl

Column conditioning: 4 x 10 ml methlyene chloride, 4 x 10 ml methanol, then 4 x 10 ml ultra pure water

Sample application: aspirate or force the water sample through the column

Column washing: 10 ml ultra pure water, then dry the column for 10 min under vacuum

Elution: slowly aspirate 2 x 5 ml methylene chloride through the column; dry the combined fractions over Na_2SO_4, filter the suspension and wash with 2 ml methylene chloride; concentrate the sample to 1 ml under a nitrogen stream; for further analysis add 3 ml acetonitrile and concentrate to 0.5 ml with vacuum

Further analysis: HPLC, e. g. with NUCLEOSIL® 100-5 C_{18} PAH, according to MN Appl. No. 101680 at *www.mn-net.com*

For PAH analysis by GC we recommend our OPTIMA® fused silica capillary columns. Please see our application database at *www.mn-net.com*

PAH from water

MN Appl. No. 301230

Compounds investigated: benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene, fluoranthene, indeno[1,2,3-cd]pyrene

Column type:
CHROMABOND® PA / 6 ml / 500 mg
Cat. No. 730007

Column conditioning: 1 column volume dist. water

Sample application: force or aspirate 20 – 500 ml water sample through the column (0.04 ppm per PAH)

Column washing: some water; dry column 1 h in a stream of nitrogen

Elution: 3 ml tetrahydrofuran and 3 ml dichloromethane, evaporate eluate in a stream of nitrogen, redissolve in 100 µl tetrahydrofuran

Recovery rates: 85 ± 5%

PAH from water containing humic acids

MN Appl. No. 301260

Column type:
CHROMABOND® NH_2/C18, 6 ml, 500/1000 mg glass column
Cat. No. 730620 G

Sample pretreatment: add 25 ml 2-propanol to 500 ml water sample

Column conditioning: 10 ml methylene chloride, 10 ml methanol, then 10 ml dist. water – 2-propanol (9:1, v/v)

Sample application: aspirate 500 ml pretreated water sample through the column (about 5 ml/min)

Column washing: 2 ml dist. water – 2-propanol (9:1, v/v), then dry column (about 20 min, vacuum)

Elution: elute with 4 x 1 ml methylene chloride (let percolate first ml into the column packing without vacuum, then apply light vacuum), if necessary evaporate in a stream of nitrogen and fill up with a suitable solvent

Recovery rates:

Compound	Recovery [%]	Compound	Recovery [%]
Naphthalene	90	Benz[a]anthracene	88
Acenaphthylene	89	Chrysene	95
Acenaphthene	86	Benzo[b]fluoranthene	93
Fluorene	87	Benzo[k]fluoranthene	88
Phenanthrene	87	Benzo[a]pyrene	87
Anthracene	89	Dibenz[ah]anthracene	91
Fluoranthene	90	Benzo[ghi]perylene	90
Pyrene	93	Indeno[1,2,3-cd]pyrene	89

PAH from water

MN Appl. No. 301250

Column type:
CHROMABOND® C18 PAH / 6 ml / 2000 mg
Cat. No. 730166

Sample pretreatment: add 10 ml Methanol to 1000 ml water sample

Column conditioning: 1 column volume methanol, then 1 column volume dist. water

Sample application: aspirate 1000 ml water sample through the column (about 15 to 20 ml/min), then dry column (stream of nitrogen or 24 h in a desiccator over P_2O_5)

Elution: elute with 4 ml acetonitrile – benzene* (3:1, v/v) and evaporate or fill up to the volume required
* alternatively toluene can be used

Recovery rates:
(PAH from water containing 50 ng/l per component)

Compound	Recovery [%]	Compound	Recovery [%]
Naphthalene	87	Benz[a]anthracene	87
Acenaphthylene	89	Chrysene	95
Acenaphthene	90	Benzo[b]fluoranthene	91
Fluorene	82	Benzo[k]fluoranthene	89
Phenanthrene	85	Benzo[a]pyrene	90
Anthracene	90	Dibenz[ah]anthracene	97
Fluoranthene	89	Benzo[ghi]-perylene	91
Pyrene	89	Indeno[1,2,3-cd]pyrene	96

PAH from *n*-hexane

MN Appl. No. 301270

Column type:
CHROMABOND® HR-P / 3 ml / 500 mg
Cat. No. 730117

Column conditioning: 5 ml *n*-hexane

Sample application: aspirate 500 µl *n*-hexane solution through the column

Column washing: 5 ml *n*-hexane, then dry column thoroughly

Elution: 4 x 2.5 ml toluene

For further analysis we recommend TLC on Nano-SIL-PAH, Cat. No. 811051.

Recovery rates:
0.6 µg/ml per component

Compound	Recovery [%]	Compound	Recovery [%]
Benzo[ghi]perylene	94	Benzo[b]fluoranthene	96
Indeno[1,2,3-cd]pyrene	100	Benzo[k]fluoranthene	96
Benzo[a]pyrene	94	Fluoranthene	95

Recovery of PAH according to German Drinking Water Specifications

MN Appl. No. 302790

Private communication: Mr. Bromig, Staatl. Brautechnische Prüf- u. Versuchsanstalt, Freising-Weihenstephan, Germany

Column type:
CHROMABOND® *Easy* / 6 ml / 200 mg
Cat. No. 730755

Column conditioning: does not apply

Sample application: aspirate 500 ml drinking water through the column; then dry for 120 min under vacuum

Elution: 2 x 2 ml dichloromethane; evaporate to dryness at 20 °C, redissolve in 1 ml acetonitrile

Further analysis: HPLC with NUCLEOSIL® 100-5 C_{18} PAH, eluent acetonitrile – water (80:20, v/v), flow rate 0.75 ml/min

Recovery rates:
(unconditioned *Easy* columns)

Compound	Concentration [µg/l]	Recovery [%]
Fluoranthene	500	113
Benzo[b]fluoranthene	200	89
Benzo[k]fluoranthene	200	88
Benzo[a]pyrene	200	86
Benzo[ghi]perylene	200	89
Indeno[1,2,3-cd]pyrene	400	92

PAH from oil

MN Appl. No. 301290

Column type:
CHROMABOND® HR-P / 3 ml / 500 mg
Cat. No. 730117

Sample pretreatment: dissolve 1 g oil sample in 10 ml *n*-hexane

Column conditioning: 5 ml *n*-hexane

Sample application: aspirate oil / hexane solution through the column

Column washing: 5 ml *n*-hexane, then dry column thoroughly

Elution: 4 x 2.5 ml toluene

For further analysis we recommend TLC on Nano-SIL-PAH, Cat. No. 811 051.

Recovery rates:

Compound	Recovery [%]	Compound	Recovery [%]
Benzo[ghi]perylene	102	Benzo[b]fluoranthene	101
Indeno[1,2,3-cd]pyrene	99	Benzo[k]fluoranthene	99
Benzo[a]pyrene	100	Fluoranthene	89

PAH from *n*-hexane
MN Appl. No. 301280

Compounds investigated: 5 – 7 rings incl. benzo[a]pyrene

Column type:
CHROMABOND® Florisil® / 6 ml / 1000 mg
Cat. No. 730082

Sample pretreatment: concentrate sample to 1 ml under vacuum

Column conditioning: 1 column volume dichloromethane, then 2 column volumes *n*-hexane

Sample application: slowly force or aspirate the sample through the column

Column washing: 10 ml *n*-hexane

Elution: 8 ml *n*-hexane – dichloromethane (3:1, v/v)

PAH from crude oil
MN Appl. No. 301300

Column type:
CHROMABOND® Florisil® / 6 ml / 1000 mg
Cat. No. 730082

Column conditioning: 20 ml methanol, then 20 ml *n*-hexane

Sample application: slowly force or aspirate 200 µl oil through the column

Elution: 15 – 40 ml *n*-hexane

PAH elute according to the size of their aromatic system, therefore collect fractions

16 PAH according to EPA from soil or sludge
MN Appl. No. 302820

Column type:
CHROMABOND® *Easy* / 6 ml / 200 mg
Cat. No. 730755

Sample pretreatment: homogenise 5 g soil sample in 30 ml 2-propanol; filter solution and fill up to 250 ml with water

Column conditioning: 3 ml methanol, 3 ml water

Sample application: force or aspirate 250 ml sample solution through the column

Column washing: 2 x 3 ml water – methanol (95:5, v/v); dry column under vacuum

Elution: 2 x 1 ml dichloromethane (for GC analysis); for HPLC evaporate dichloromethane in a stream of nitrogen and dissolve in acetonitrile

Further analysis: HPLC with NUCLEOSIL® 100-5 C_{18} PAH or GC with OPTIMA® 5 MS, 0.25 µm film, 30 m x 0.25 mm ID (Cat. No. 726220.30)

For PAH analysis by HPLC we recommend columns NUCLEOSIL® 100-5 C_{18} PAH. Please see our application database at *www.mn-net.com*.

PAH from soil

MN Appl. No. 301310

Column type:
CHROMABOND® CN / SiOH, 6 ml, 500/1000 mg
Cat. No. 730135

Sample pretreatment: dry 30 g soil with sodium sulphate and reflux 4 hours with 250 ml petroleum ether in a Soxhlet extractor. For low PAH contents (colourless or weakly coloured extracts) concentrate extract to 1/10 of its volume in a rotation evaporator.

Column conditioning: 4 ml petroleum ether

Sample application: force or aspirate 20 ml of the extract through the column

Column washing: 2 ml petroleum ether

Elution: 2 x 2 ml acetonitrile – benzene (3:1, v/v), then evaporate or fill up to the volume required

For further analysis we recommend HPLC e.g. with a column 250 x 3 mm NUCLEOSIL® 100-5 C_{18} PAH.

Recovery rates:

Compound	Recovery [%]	Compound	Recovery [%]
Naphthalene	85	Benz[a]anthracene	84
Acenaphthylene	92	Chrysene	96
Acenaphthene	89	Benzo[b]fluoranthene	95
Fluorene	87	Benzo[k]fluoranthene	90
Phenanthrene	83	Benzo[a]pyrene	90
Anthracene	88	Dibenz[ah]anthracene	96
Fluoranthene	87	Benzo[ghi]perylene	87
Pyrene	90	Indeno[1,2,3-cd]pyrene	97

16 PAH according to EPA from water

MN Appl. No. 302830

Column type:
CHROMABOND® *Easy* / 6 ml / 200 mg
Cat. No. 730755

Sample: concentration of the standard 1 µg/l in drinking water

Column conditioning: 3 ml methanol, 3 ml water

Sample application: aspirate or force 500 ml drinking water through the column

Column washing: 3 ml water with 5% methanol; dry column under vacuum

Elution: 2 x 2 ml dichloromethane (for GC analysis)
for HPLC evaporate dichloromethane in a stream of nitrogen and dissolve in acetonitrile

Further analysis: HPLC with NUCLEOSIL® 100-5 C_{18} PAH or GC with OPTIMA® 5 MS, 0.25 µm film, 30 m x 0.25 mm ID (Cat. No. 726220.30)

PAH from soil and sludge

MN Appl. No. 301320

Column type:
CHROMAFIX® SA (M)
Cat. No. 731832

Sample pretreatment: air-dry soil or sludge sample, then grind (< 0.5 mm), homogenise and extract 5 g of the sample thus obtained with 10 ml acetonitrile in an ultrasonic bath for 1 h.

Column conditioning: 1 column volume methanol

Sample application and elution: force or aspirate 1 ml of the extract through the column, then rinse column with 2 ml methanol; collect the whole flow-through, then evaporate or fill up with methanol to the volume required

Further analysis: HPLC on a NUCLEOSIL® 100-5 C_{18} PAH, 150 x 4 mm ID

Recovery rates:
(PAH from soil, 150 ng/kg per component investigated)

Compound	Recovery [%]	Compound	Recovery [%]
Naphthalene	85	Benz[a]anthracene	85
Acenaphthylene	87	Chrysene	91
Acenaphthene	84	Benzo[b]fluoranthene	90
Fluorene	83	Benzo[k]-fluoranthene	87
Phenanthrene	89	Benzo[a]pyrene	91
Anthracene	88	Dibenz[ah]anthracene	92
Fluoranthene	90	Benzo[ghi]perylene	93
Pyrene	90	Indeno[1,2,3-cd]pyrene	95

PAH and PCB from soil

MN Appl. No. 301330

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: homogenise 5 g soil sample in 30 ml 2-propanol, filter and fill up the filtrate to 250 ml with dist. water

Column conditioning: 1 column volume 2-propanol, then 1 column volume dist. water – 2-propanol (85:15, v/v)

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 x 1 ml water – 2-propanol (85:15, v/v); dry column under vacuum for 5 min

Elution: 2 x 500 µl dichloromethane

PAH from water
MN Appl. No. 301240

Column type:
CHROMABOND® C18 ec / 6 ml / 500 mg
Cat. No. 730014

Column conditioning: 1 column volume methanol, then 1 column volume dist. water

Sample application: slowly force or aspirate 500 ml water sample through the column

Column washing: 2 ml dist. water

Elution: acetonitrile – water (8:2, v/v) or plain acetonitrile for elution of higher condensed PAH

PAH and PCB from water
MN Appl. No. 301340

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: mix 500 ml sample with 60 ml 2-propanol

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water – 2-propanol (85:15, v/v)

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 x 750 µl dist. water – 2-propanol (85:15, v/v)

Elution: 2 x 500 µl dichloromethane

Effects of heterocyclic PAH (N,S,O) on the biodegradation of typical tar oil PAH in a soil / compost mixture
MN Appl. No. 302210

S. Meyer, H. Steinhart, Chemosphere **40** (2000) 359 – 367

Compounds investigated: polycyclic aromatic hydrocarbons (PAH), PAH containing sulphur and oxygen (PASH and PAOH), PAH containing nitrogen (PANH)

Column type:
self-packed with 0.7 g CHROMABOND® SB and 2.0 g silica gel (inactivated with 10% water [w:w]) between three PTFE frits

Sample pretreatment: mix 20 g contaminated soil with 1 ml 1 M hydrochloric acid, dry with sodium sulphate and extract in a Soxhlet for 7.5 h with a mixture of dichloromethane (210 ml) and *n*-heptane (10 ml); concentrate the resulting extract to about 5 ml by rotary evaporation (40 °C, 600 mbar)

Column conditioning: *n*-hexane

Sample application: slowly force or aspirate the Soxhlet extract through the column

Elution:
Fraction 1 (PAH , PASH and PAOH): 3 ml *n*-hexane, 12 ml *n*-hexane – dichloromethane (85:15; v/v) and 2 ml dichloromethane
Fraction 2 (PANH and neutral metabolites): 1 ml dichloromethane, 6 ml methanol and 3 ml 0.05 mol/l hydrochloric acid in methanol

Further analysis: GC-FID or HPLC on NUCLEOSIL® 100-5 C_{18} PAH

Simultaneous determination of PAH, heteroPAH (N,S,O), and their degradation products in creosote-contaminated soils

MN Appl. No. 302420

S. Meyer et al., Anal. Chem. **71** (1999) 4023 – 4029

Compounds investigated: polycyclic aromatic hydrocarbons (PAH), PAH containing sulphur and oxygen (PASH and PAOH), PAH containing nitrogen (PANH)

1st step:

Column type:
self-packed by filling first 0.7 g of CHROMABOND® SB and then 2.0 g silica gel (dried 25 h at 180 °C, and deactivated with 10% water [w/w] before use) between three PTFE frits

Sample pretreatment: mix 20 g contaminated soil with 1 ml 1 M hydrochloric acid, dry by grinding with 20 g sodium sulfate and extract for 7.5 h with a mixture of 210 ml dichloromethane and 10 ml *n*-heptane using a Soxhlet apparatus. The dichloromethane is removed by concentrating the resulting extract to about 5 ml by rotary evaporation (40 °C, 600 mbar).

Column conditioning: 12 ml *n*-hexane

Sample application: transfer the soxhlet extract to the column

Elution:
Fraction 1 (PAH, PASH, and PAOH): 3 ml *n*-hexane, 12 ml *n*-hexane – dichloromethane (85:15, v/v), and 2 ml dichloromethane
Fraction 2 (PANH and neutral metabolites): 1 ml dichloromethane, 6 ml methanol, and 3 ml of 0.05 mol/l hydrochloric acid in methanol; this fraction is further separated in step 2
Fraction 3 (acidic metabolites): 6 ml of 0.05 mol/l hydrochloric acid in methanol

2nd step:

Column type:
CHROMABOND® SA / 3 ml / 500 mg
Cat. No. 730077

Column conditioning: 5 ml methanol

Sample application: fraction 2 (10 ml) was directly applied to the column

Elution:
Fraction 2a: neutral PANH and neutral metabolites are not retained; they elute immediately with the solvent of fraction 2 followed by 5 ml of methanol
Fraction 2b: basic PANH are eluted with 5 ml of 1 mol/l ammonia in methanol

Further analysis: GC-FID or HPLC (for GC, fractions are diluted, and internal standards are added as follows: 9-chloroanthracene and indeno[1,2,3-cd]fluoranthene for fraction 1, 2-chlorophenothiazin for fraction 2 a and 3, and indole for fraction 2b, respectively)

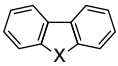
Recovery rates:

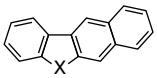
Compounds	Recovery [%]
PASH (GC-FID)	
Benzothiophene	55.3
Dibenzothiophene	92.2
Benzo[b]naphtho[2,3-d]thiophene	102.0
PAOH (GC-FID)	
Benzofuran	29.8
Dibenzofuran	89.8
Benzo[b]naphtho[2,3-d]furan	98.6
PANH, basic (HPLC-DAD)	
Quinoline	95.4
Isoquinoline	96.0
2-methylquinoline	100.8
4-methylquinoline	99.3
Acridine	98.2
Phenanthridine	98.9
Benzo[h]quinoline	96.3
Benz[a]acridine	91.4
Benz[c]acridine	91.8
Dibenz[a,c]acridine	91.7
PANH, neutral (HPLC-DAD)	
Indole	60.2
Carbazole	94.0
Dibenzo[a,i]carbazole	80.8
1-Cyanonaphthalene	97.6
9-Cyanophenanthrene	96.1
9-Cyanoanthracene	89.7
Metabolites, neutral (HPLC-DAD)	
2(1H)Quinoline	93.3
Coumarin	84.2
1-Indanone	75.8
Acenaphthene-1,2-dione	45.2
Dibenzothiophenesulfone	90.1
2-Naphthol	87.2
2-Hydroxycarbazole	55.3
Phenanthrene-9,10-dione	31.5
9-Fluorenol	93.7
9-Fluorenone	87.4
Anthracene-9,10-dione	96.4
Benz[de]anthracen-7-one	86.4
7,12-Benz[a]anthracenedione	89.8
Metabolites, acidic (HPLC-DAD)	
Salicylic acid	89.1
1-Naphthoic acid	98.1
9-Fluorenone-1-carboxylic acid	77.5
2-Naphthoic acid	102.9
3-Hydroxy-2-naphthoic acid	87.8
1-Hydroxy-2-naphthoic acid	86.4

Selected heterocycle structures

Benzofuran X = O
Benzothiophene X = S
Indole X = NH

Dibenzofuran X = O
Dibenzothiophene X = S
Carbazole X = NH

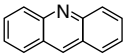


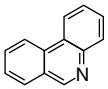


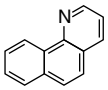
Benzo[b]naphtho[2,3-d]furan R = O
Benzo[b]naphtho[2,3-d]thiophene R = S

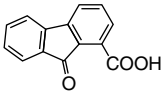
Quinoline 

Isoquinoline 

Acridine 

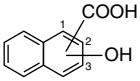
Phenanthridine 

Benzo[h]quinoline 



9-Fluorenone-
1-carboxylic acid

Naphthoic acids:



PCB from transformer oils

MN Appl. No. 301370

Column type:
CHROMABOND® C8 / 6 ml / 500 mg
Cat. No. 730024

Sample pretreatment: mix 0.5 g oil with 0.5 – 5 ml ethyl acetate (volume depends on type of oil) and dissolve the oil. Precipitate oil with 15 ml methanol and add 0.5 g sodium dodecylsulphate. Upon shaking the oil coagulates.

Column conditioning: slowly force or aspirate 5 ml *n*-hexane through the column, dry the column for 30 sec under vacuum, then force or aspirate 1 column volume methanol through the column; now the column must not run dry

Sample application: force or aspirate 250 µl sample solution through the column

Column washing: apply 1 ml acetonitrile – water (7:3, v/v) to the column, wait 30 sec; then vacuum dry column 10 min

Elution: 3 x 500 µl *n*-hexane

Automated sample preparation (BenchMate) for the determination of PCB from transformator oils

MN Appl. No. 302360

A. Joeris-Viethen, R. Weber, GIT Fachz. Lab. (1996) 1022 – 1027

Column type:
CHROMABOND® SA/SiOH / 3 ml / 500 mg
Cat. No. 730132

Sample pretreatment: dilute 200 mg oil in 10 ml *n*-hexane and spike with the internal standard PCB-209

Sample application: apply 500 µl sample directly on the column

Elution: 3 ml *n*-hexane; concentrate to 1 ml under dry nitrogen

Further analysis: GC/ECD

PCB from oil

MN Appl. No. 302680

Private communication: Institut für angewandte Chemie Gockel & Weischedel & Co GmbH, Stuttgart, Germany

Column type:
CHROMABOND® Florisil® / 1 ml / 100 mg
Cat. No. 730089

Column conditioning: 500 µl iso-octane

Sample application: apply 0.2 – 0.5 g oil sample (analytical balance) and allow to percolate into the packing

Elution: 10 ml *n*-hexane (gravity-controled flow, no vacuum); collect eluate in a headspace vial; apply warm stream of nitrogen until the eluate is concentrated to about 1 ml, add 10 ml acetonitrile and extract for 1 minute; use the acetonitrile phase.

Further analysis: GC/MS

DIN EN 12766-1 for determination of the content of up to 12 PCB congeners or defined unresolved groups of PCB in mineral oil products

MN Appl. No. 303490

Column type:
CHROMABOND® Kombi-Kit for extraction of PCB from oil, Cat. No. 730125 ($SiOH$-H_2SO_4/SA, 3 ml, 500/500 mg and SiOH / 3 ml / 500 mg), adaptor for coupling CHROMABOND® columns, Cat. No. 730100

Sample pretreatment: precisely (to 1 mg) weigh about 1.0 g of the homogenised, anhydrous oil sample in a 10-ml volumetric flask and mix with about 8 ml of the solvent (preferably heptane, but also hexane, cyclohexane, or 2,2,4-trimethylpentane, all with a low content of PCB); add 1 ml of the internal standard solution (2 mg/l of the congener 30 and 2 mg/l of the congener 209) and fill to the mark with the solvent.

Column conditioning: place the combined benzenesulfonic acid / sulphuric acid column on the 3 ml silica column with the aid of the adaptor. For purification of the stationary phase elute both columns three times with 2 ml each of the solvent and dry by applying a light vacuum

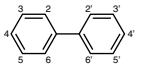
Sample application: apply 250 µl of the sample solution on the upper column and flush into the adsorbent with 0.5 ml solvent; distribute the sample evenly in the packing of the upper column, e.g. by applying a light vacuum

Elution: after at least 30 s elute the upper column twice with 1 ml solvent each; then remove the upper column; elute the silica column twice with about 0.5 ml solvent each into a 5-ml volumetric flask and fill to the mark with the solvent

Further analysis: GC/ECD

PCB from waste oil

MN Appl. No. 301390



Biphenyl

Column type:
CHROMABOND® SA/SiOH, 3 ml, 500/500 mg
Cat. No. 730132

Column conditioning: 1 ml *n*-hexane

Sample application: apply about 250 µl waste oil sample to the column and aspirate or force it into the adsorbent with 2 x 1 ml *n*-hexane

Elution: aspirate or force another 2 x 500 µl *n*-hexane through the column; collect all *n*-hexane fractions and if necessary adjust to a concentration suitable for subsequent analysis by either evaporating *n*-hexane in a stream of nitrogen or by dilution with *n*-hexane

Recovery rates:

Compound	Formula	Recovery [%]
PCB 28	2,4,4'-trichlorobiphenyl	97
PCB 52	2,2',5,5'-tetrachlorobiphenyl	96
PCB 101	2,2',4,5,5'-pentachlorobiphenyl	95
PCB 138	2,2',3,4,4',5'-hexachlorobiphenyl	90
PCB 153	2,2',4,4',5,5'-hexachlorobiphenyl	95
PCB 180	2,2',3,4,4',5,5'-heptachlorobiphenyl	96
PCB 209	2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl	100

PCB from sludge or soil

MN Appl. No. 301400

Compounds investigated: polychlorinated biphenyls (PCB); this procedure is also applicable for soil samples

Column type:
CHROMABOND® NAN / 6 ml / 700/2000/700 mg
Cat. No. 730149

Sample pretreatment: extract 2 g lyophilise sludge with 70 ml *n*-hexane, evaporate extract and finally fill to 10 ml with *n*-hexane

Column conditioning: 10 ml *n*-hexane

Sample application: aspirate 2 ml of the extract into the column

Elution: aspirate 40 ml *n*-hexane slowly through the column with weak vacuum, then evaporate and fill to 5 ml with *n*-hexane

Further analysis: GC, e.g. with capillary column OPTIMA® 5, 0.25 µm film, 50 m x 0.25 mm ID, Cat. No. 726056.50.

Recovery rates:

Compound	Recovery [%]	Compound	Recovery [%]
PCB 28	104	PCB 153	101
PCB 52	100	PCB 180	98
PCB 101	99	PCB 209	104
PCB 138	98		

PCB from soil, sludge or cement plaster

MN Appl. No. 302040

Private communication: E. Göldner, Institut für angewandte Chemie Gockel & Weischedel & Co. GmbH, Stuttgart, Germany

Column type:
CHROMABOND® NAN / 3 ml / 400/1400/400 mg
Cat. No. 730109

Sample pretreatment: ~20 g of a dry sample (soil, sludge or cement plaster) are extracted with 10 g sodium sulphate and 100 ml *n*-hexane for 8 hours in a soxhlet extractor, then the extract is concentrated to a volume of 1 – 2 ml

Column conditioning: 2 ml *n*-hexane

Sample application: slowly force or aspirate the extract of the sample pretreatment through the column

Elution: slowly aspirate 10 ml *n*-hexane through the column, then concentrate to 5 ml; extract 2 ml of the *n*-hexane-extract with 2 ml acetonitrile; after phase partition use ~1 ml of the acetonitrile phase for GC analysis with OPTIMA® δ-6 as described in our Appl. No. 250480

Recovery rates:

Compound	Extraction rate from *n*-hexane to acetonitrile [%]	Compound	Extraction rate from *n*-hexane to acetonitrile [%]
PCB 28	30.8	PCB 153	19.1
PCB 53	36.9	PCB 138	23.4
PCB 101	26.8	PCB 180	15.9

PCB in oil samples

MN Appl. No. 301380

Determination with reference to German Standard DIN 51 527

Column type:
CHROMABOND® SiOH-H_2SO_4/SA, 3 ml, 2 x 500 mg
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. Nos. 730085 and 730073 or Kombi-Kit PCB, Cat. No. 730125

Sample pretreatment: extract oil-contaminated solids with *n*-hexane. Homogenise other oil samples and dissolve 1.5 to 2.0 g in 50 ml *n*-hexane. Water which may cause turbidities can be removed with sodium sulphate.

Column conditioning: let 1 ml *n*-hexane flow through the CHROMABOND® SiOH-H_2SO_4/SA column

Sample application: Aspirate or force 500 µl sample through the CHROMABOND® SiOH-H_2SO_4/SA column. This phase offers better removal of interfering substances due to sulphonisation. Place CHROMABOND® SiOH-H_2SO_4/SA column on top of the SiOH column with the aid of an adaptor and after at least 30 seconds flush sample into the SiOH column with 2 x 1 ml *n*-hexane

Elution: elute SiOH column with 3 x 0.5 ml *n*-hexane; adjust to a suitable concentration by evaporation of *n*-hexane in a stream of nitrogen or by dilution with *n*-hexane

Further analysis: GC

Recovery rates:

Compound	Recovery [%]	Compound	Recovery [%]
PCB 28	99	PCB 153	99
PCB 52	95	PCB 180	96
PCB 101	99	PCB 209	101
PCB 138	94		

Pesticides and PAH from water

MN Appl. No. 301360

Column type:
CHROMABOND® C18 ec / 1 ml / 100 mg
Cat. No. 730011

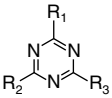
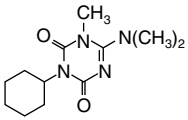
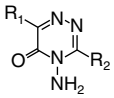
Column conditioning: 1 column volume methanol, then 1 column volume dist. water

Sample application: force or aspirate 50 – 100 ml water through the column, dry column under vacuum

Elution: pour 500 µl ethyl acetate into the column, let percolate without vacuum and collect the first 100 µl

For further analysis of PAH, PCB and pesticides we recommend gas chromatography with OPTIMA® fused silica capillary columns

Pesticides: triazines

	Compound	R_1	R_2	R_3
	Atrazine	Cl	NH – C_2H_5	NH – $CH(CH_3)_2$
	Cyanazine	Cl	NH – C_2H_5	NH – $C(CH_3)_2$ – CN
	Propazine	Cl	NH – $CH(CH_3)_2$	NH – $CH(CH_3)_2$
	Sebuthylazine	Cl	NH – C_2H_5	NH – $(CH_2)_3$ – CH_3
	Simazin	Cl	NH – C_2H_5	NH – C_2H_5
	Terbuthylazine	Cl	NH – C_2H_5	NH – $C(CH_3)_3$
	Atratone	O – CH_3	NH – C_2H_5	NH – $CH(CH_3)_2$
	Secbumeton	O – CH_3	NH – C_2H_5	NH – $CH(CH_3)$ – C_2H_5
	Terbumeton	O – CH_3	NH – C_2H_5	NH – $C(CH_3)_3$
	Ametryn	S – CH_3	NH – C_2H_5	NH – $CH(CH_3)_2$
	Desmetryn	S – CH_3	NH – CH_3	NH – $CH(CH_3)_2$
	Methoprotryn	S – CH_3	NH – $CH(CH_3)_2$	NH – $(CH_2)_3$ – OCH_3
	Prometryn	S – CH_3	NH – $CH(CH_3)_2$	NH – $CH(CH_3)_2$
	Simetryn	S – CH_3	NH – C_2H_5	NH – C_2H_5
	Terbutryn	S – CH_3	NH – C_2H_5	NH – $C(CH_3)_3$
	Hexazinone	–	–	–
	Metamitron	C_6H_5	CH_3	–
	Metribuzin	$C(CH_3)_3$	SCH_3	

Pesticides: triazines from water

MN Appl. No. 301590

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: pass 1 l water through a suitable glass fibre filter and acidify with 2 ml conc. hydrochloric acid

Column conditioning: 5 ml methanol, then 5 ml dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 2 ml acetonitrile – water (3:7, v/v); dry 20 min under vacuum

Elution: 20 ml acetonitrile; concentrate eluate on a rotation evaporator

Triazine herbicides from water

MN Appl. No. 303240

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108

Column conditioning: 1 column volume methanol , then 1 column volume dist. water; attention: the column must not run dry!

Sample application: aspirate 1000 ml water sample through the column, then dry column in a stream of nitrogen

Elution: 3 x 1 ml methanol – acetone (3:2, v/v), then evaporate to the volume required

Further analysis: GC

Recovery rates:

Compound	Recovery [%]	Compound	Recovery [%]
Atrazine	98	Desmetryn	93
Ametryn	94	Prometryn	95
Desisopropylatrazine	99	Simetryn	96
Desethylatrazine	98	Terbutryn	95
Desethylterbuthylazine	93	Atratone	94
Propazine	98	Secbumeton	98

Triazines

MN Appl. No. 303140

Column type: (6 ml / 2000 mg)
CHROMABOND® C18 ec, Cat. No. 730141
CHROMABOND® C18, Cat. No. 730130
CHROMABOND® C_6H_{11} ec, Cat. No. 730469
CHROMABOND® C18 Hydra, Cat. No. 730301
CHROMABOND® HR-P / 3 ml / 200 mg, Cat. No. 730108

Column conditioning: 10 ml methanol, then 10 ml water (pH 2)

Sample application: slowly force or aspirate 2000 mg sample through the column

Elution: 10 ml THF – methanol (1:1, v/v)

Recovery rates:

Phase	Recovery [%]			
	Atrazine	Desethylatrazine	Desisopropylatrazine	Prometryn
C18 ec	80	79	61	81
C18	81	81	104	84
C18 Hydra	88	87	37	88
C_6H_{11} ec	100	96	94	96
HR-P	104	92	87	97

Enrichment of triazines with cation exchangers

MN Appl. No. 302130

Compounds investigated: desisopropylatrazine, desethylatrazine, atrazine, prometryn, chlorpromazine

Column type:
CHROMABOND® SA, 6 ml, 500 mg, Cat. No. 730425
CHROMABOND® PSA, 6 ml, 500 mg, Cat. No. 730463
CHROMABOND® PCA, 6 ml, 500 mg, Cat. No. 730483
CHROMAFIX® PS-H$^+$ (M), Cat. No. 731861

Sample pretreatment: 200 µg desisopropylatrazine, 100 µg desethylatrazine, 100 µg atrazine, 100 µg prometryn, and 200 µg chlorpromazine are dissolved in 10 ml methanol, the solution is given in 200 ml dist. water

Column conditioning: 2 column volumes methanol, 2 column volumes acetic acid (1%)

Sample application: aspirate the sample with about 700 mbar through the column

Elution: 10 ml acetonitrile – 0.1 M K_2HPO_4 (1:1)

Further analysis: HPLC on NUCLEOSIL® 100-5 C_{18} (250 x 4 mm); eluents: A) water, B) methanol, gradient: 3 min 40% B, in 1 min to 60% B, in 3 min to 90% B, in 4 min to 100% B, flow rate: 1 ml/min; detection: UV, 280 nm

Recovery rates:

Compound	Recovery [%]			
	CHROMABOND®			CHROMAFIX®
	SA	PSA	PCA	PS-H$^+$
Desisopropylatrazine	51	54	3	49
Desethylatrazine	64	67	3	54
Atrazine	94	98	8	62
Prometryn	96	97	10	41
Chlorpromazine	71	99	15	35

Pesticides: herbicide metribuzin from leaf tissue

MN Appl. No. 301660

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg and
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. Nos. 730013 and 730073

Sample pretreatment: homogenise 2.5 g leaves with 20 ml methanol – water (8:2, v/v), filter and rinse the residue with 20 ml methanol – water (8:2, v/v) and 10 ml methanol. Evaporate under vacuum and redissolve in 25 ml chloroform. Extract with 20 ml 0.1 M potassium chloride. Extract aqueous phase twice with chloroform, evaporate chloroform phases and redissolve in 200 – 500 µl diethyl ether.

Sample application: slowly pour the pretreated sample through the column; blow diethyl ether from the column with nitrogen

Elution: elute metribuzin and nonpolar metabolites with 20 ml acetonitrile – water (1:1, v/v), concentrate under vacuum, redissolve in chloroform and apply to a CHROMABOND® SiOH column; elute with 20 ml chloroform

Pesticides: triazine herbicides from soil samples

MN Appl. No. 301640

Compounds investigated: atrazine, propazine, simazin

Column type:
CHROMABOND® SA (≡ SCX) / 3 ml / 500 mg
Cat. No. 730077

Sample pretreatment: suspend 100 g soil in 99 ml acetonitrile – water (9:1, v/v). Add 1 ml standard solution (0.2 mg/ml prometryn) and shake vigorously for 5 min. Filter through paper filter MN 616. Discard the first 5 ml, use the following 10 ml.

Column conditioning: flush with 1 column volume acetic acid (1%), then add 2 ml acetic acid (1%), place the reservoir onto the column with the adaptor

Sample application: pour 25 ml acetic acid (1%) into the reservoir, add 5 ml sample, stir and slowly aspirate the solution through the column; finally wash with 2 ml acetic acid (1%)

Column washing: 1 ml acetonitrile, then 1 column volume water, finally 1 ml 0.1 M dipotassium hydrogen phosphate; between the washing steps dry column briefly (about 15 sec) under vacuum

Elution: 2 ml acetonitrile – 0.1 M dipotassium hydrogen phosphate (1:1, v/v)

Pesticides from water

MN Appl. No. 302060

Compounds investigated: triazines and carboxylic amides

Column type:
CHROMABOND® C18 Hydra / 6 ml / 2000 mg
Cat. No. 730301

Sample pretreatment: adjust 1000 ml water to pH 7 – 8 with diluted NH_3 and add 100 µl of the internal standards (1 µg/l).

Column conditioning: 2 x 5 ml methanol, then 2 x 5 ml dist. water

Sample application: force or aspirate the sample through the column, then dry for 2 h with 2 bar N_2

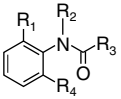
Elution: Slowly aspirate 10 ml methanol through the column. Evaporate the eluate to dryness in a tapered flask with a rotation evaporator at 30 °C and store in a refrigerator for ~ 15 min. Redissolve the residue in 200 µl cold, fresh *n*-hexane and transfer the solution to a conic HPLC vial (e. g. Cat. No. 702891). Store the solution in a refrigerator until chromatography.

Recovery rates: between 95 and 100%

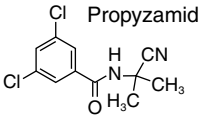
Further analysis: GC with OPTIMA® δ-3 or OPTIMA® δ-6 (e. g. application 250420) or HPLC in accordance with EN ISO 11369: 1997 on NUCLEOSIL® 120-3 C_{18} (application 110880)

Environmental samples and pollutants

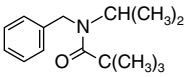
Pesticides: carboxylic acid amides

Structure	Compound	R_1	R_2	R_3	R_4
	Alachlor	C_2H_5	$CH_2 – OCH_3$	CH_2Cl	C_2H_5
	Benzanilide	H	H	C_6H_5	H
	Metalaxyl	CH_3	$CH(CH_3) – CO – OCH_3$	$CH_2 – OCH_3$	CH_3
	Metazachlor	CH_3	$CH_2 –$ 	CH_2Cl	CH_3
	Metolachlor	CH_3	$CH(CH_3) – CH_2 – OCH_3$	CH_2Cl	C_2H_5

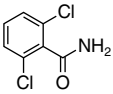
Propyzamid



Tebutam



2,6-Dichlorobenzamide



Pesticides: herbicides from water

MN Appl. No. 301570

Compounds investigated: alachlor, atrazine, metolachlor

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: filter sample, if necessary

Column conditioning: 2 ml isooctane – ethyl acetate (9:1, v/v), then 6 ml methanol, finally 6 ml dist. water, all with about 3 ml/min

Sample application: force or aspirate the sample through the column with about 6 ml/min

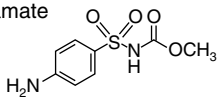
Column washing: 6 ml water; then dry column thoroughly under vacuum

Elution: 2 ml isooctane – ethyl acetate (9:1, v/v)

Pesticides: herbicide asulam from water

MN Appl. No. 301650

Compounds investigated: asulam = methyl-4-aminophenylsulfonyl carbamate



Column type:
CHROMABOND® SA (≡ SCX) / 3 ml / 500 mg
Cat. No. 730077

Sample pretreatment: adjust 100 ml water sample to pH 3 with oxalic acid; if Ca^{2+} is present, it will be precipitated and has to be decanted or filtered (store sample in an amber glass bottle)

Column conditioning: 2 x 5 ml methanol, then 5 ml dist. water and 2 ml 0.6% oxalic acid

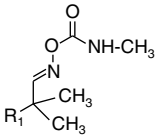
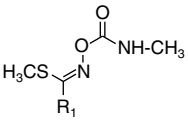
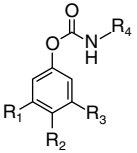
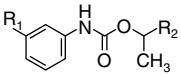
Sample application: slowly force or aspirate 100 ml adjusted water sample through the column

Column washing: 3 ml dist. water

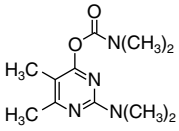
Elution: 3 x 1 ml acetonitrile – ammonia (25%) – aqueous barium chloride solution (10%) – water (7:27:1:33, v/v/v/v); precipitate Ba^{2+} by addition of 500 µl H_2SO_4

For further pesticide analysis we recommend gas chromatography with OPTIMA® fused silica capillary columns.

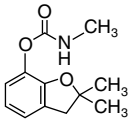
Pesticides: carbamates

Structure	Compound	R_1	R_2	R_3	R_4
	Aldicarb	SCH_3			
	Aldicarb sulfoxide	$SO - CH_3$			
	Aldicarb sulfone	$SO_2 - CH_3$			
	Methomyl	CH_3			
	Oxamyl	$CO - N(CH_3)_2$			
	Methiocarb	CH_3	SCH_3	CH_3	CH_3
	Promecarb	CH_3	H	$CH(CH_3)_2$	CH_3
	Karbutilate	$NH - CO - N(CH_3)_2$	H	H	$C(CH_3)_3$
	Chlorpropham	Cl	CH_3		
	Propham	H	CH_3		
	Carbetamide	H	$CO - NH - C_2H_5$		

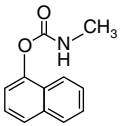
Pirimicarb



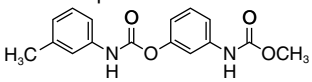
Carbofuran



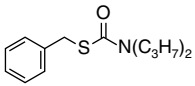
Carbaryl



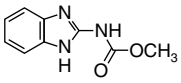
Phenmedipham



Prosulfocarb



Carbendazim



Pesticides: aldicarb from water

MN Appl. No. 301480

Column type:
CHROMABOND® C18 ec / 3 ml / 200 mg
Cat. No. 730012

Column conditioning: 1 column volume methanol, 2 column volumes water

Sample application: slowly force or aspirate 20 ml water sample through the column; dry column 1 min in a stream of nitrogen

Column washing: does not apply

Elution: 400 µl acetonitrile, then 1100 µl 0.01 M HCl

Pesticides: recovery of carbamates

MN Appl. No. 303130

Column type: (6 ml / 2000 mg)
CHROMABOND® C18 ec, Cat. No. 730141
CHROMABOND® C18, Cat. No. 730130
CHROMABOND® C_6H_{11} ec, Cat. No. 730469
CHROMABOND® C18 Hydra, Cat. No. 730301
CHROMABOND® HR-P / 3 ml / 200 mg, Cat. No. 730108

Column conditioning: 10 ml methanol, then 10 ml water

Sample application: slowly force or aspirate 2000 mg sample through the column

Elution: 10 ml THF – methanol (1:1, v/v)

Recovery rates:

Phase	Recovery [%] promecarb	carbofuran	aldicarb
C18 ec	75	91	90
C18	93	97	91
C18 Hydra	96	87	74
C_6H_{11} ec	86	98	95
HR-P	87	85	99

Recovery of pesticides

MN Appl. No. 303220

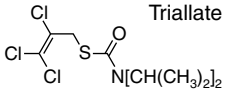
Private communication: Mr. Kühn, GUB, Waldshut Tiengen, Germany

Column type:
CHROMABOND® *Easy* / 3 ml / 200 mg
Cat. No. 730754

Column conditioning: 1 ml water, 3 ml methanol, 1 ml water

Sample application: aspirate the sample through the column

Elution: 3 x 1 ml acetone



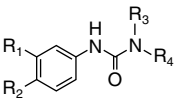
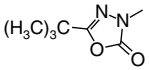
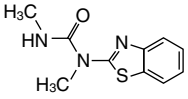
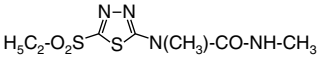
Triallate

Further analysis: HPLC with NUCLEOSIL® 120-5 C_{18}

Recovery rates:

Compound	Recovery [%]	Compound	Recovery [%]
Desisopropylatrazine	90.3	Metalaxyl	95.5
2,6-Dichlorobenzamide	93.1	Isoproturon	93.5
Desethylatrazine	92.7	Diuron	94.4
Hexazinone	69.3	Metazachlor	97.0
Terbacil	65.1	Propazine	94.6
Simazin	81.4	Terbuthylazine	93.2
Cyanazine	92.8	Linuron	95.7
Desethylterbuthylazine	90.6	Metolachlor	97.3
Methabenzthiazuron	93.7	Triallate	61.4
Chlortoluron	91.4	Standard	63.7
Atrazine	92.4		

Pesticides: phenylurea derivatives

Structure	Compound	R_1	R_2	R_3	R_4
	Chloroxuron	H	O – C_6H_4 – Cl	CH_3	CH_3
	Chlortoluron	Cl	CH_3	CH_3	CH_3
	Diflubenzuron	H	Cl	H	
	Dimefuron	Cl		CH_3	CH_3
	Diuron	Cl	Cl	CH_3	CH_3
	Isoproturon	H	$CH(CH_3)_2$	CH_3	CH_3
	Linuron	Cl	Cl	CH_3	OCH_3
	Metobromuron	H	Br	CH_3	OCH_3
	Metoxuron	Cl	OCH_3	CH_3	CH_3
	Monolinuron	H	Cl	CH_3	OCH_3
	Monuron	H	Cl	CH_3	CH_3
	Pencycuron	H	H	cyclopentyl	CH_2 – *p*-C_2H_4Cl
	Methabenzthiazuron				Ethidimuron

Determination of pesticide fluxes in beech forests

MN Appl. No. 303440

Private communication: A. Bernhardt,et al., Institute of Ecology and Environmetal Chemistry, University of Lüneburg, Germany

Compounds investigated: desisopropylatrazine, metamitron, desethylatrazine, bentazone, 2,4-D, bromoxynil, chlortoluron, atrazine, metazachlor, isoproturon, phenmedipham, ethofumesat, terbuthylazine, prosulfocarb, pendimethalin

Column type:
CHROMABOND® C18 / 1 ml / 100 mg
Cat. No. 730001

Sample collection: beech trees are furnished with a rin made of tin foil to collect stem-flow water; for method evaluation stem-flow water and normal water samples are spiked with the pesticides

Sample application: 500 ml of the water sample are passed through the column

Elution: 1 ml methanol

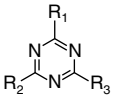
Further analysis: RP-HPLC

Pesticides from water

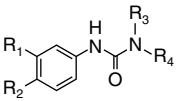
MN Appl. No. 301550

Compounds investigated:

T = triazine derivatives

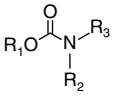


U = phenylurea derivatives



CA = carboxylic acid amides

C = carbamates



S = others

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108

Column conditioning: 1 column volume methanol, then 1 column volume dist. water

Sample application: aspirate 1000 ml water sample through the column, then dry column in a stream of nitrogen

Elution: elute with 3 x 1 ml methanol – acetone (3:2, v/v) and then evaporate or dilute to the volume required

Further analysis: GC with OPTIMA® fused silica capillary columns

* Applicability of this procedure as given in the table below:

	applicable
	limited applicability
	not applicable

If you are interested in GC procedures for pesticide analyses, please see our application database at *www.mn-net.com.*

Recovery rates:

Compound	Type	Recovery [%]
Aldicarb	C	95
Aldicarb sulfoxide	C	97
Aldicarb sulfone	C	96
Ametryn	T	87
Atratone	T	95
Atrazine	T	98
Benzanilide	CA	89
Bifenox	CA	98
Bromacil	S	88
Carbaryl	C	98
Carbetamide	CA	70
Carbofuran	C	17
Chloridazon	S	87
Chloroxuron	U	96
Chlorpropham	C	81
Chlortoluron	U	97
Crimidine	T	91
Cyanazine	T	96
Desethylatrazine	T	97
Desethyldesisopropylatrazine	T	15
Desethylsimazin	T	97
Desethylterbuthylazine	T	90
Desisopropylatrazine	T	98
Desmetryn	T	88
Dimefuron	U	99
Diuron	U	96
Ethidimuron	U	83
Ethofumesat	S	95
Fluazifop-butyl	S	0
Flurochloridon	S	70
Fluroxypyr-methyl heptyl ester	S	20
Haloxyfop ethoxyethyl ester	S	0
Hexazinone	T	96
3-Hydroxycarbofuran	C	95

Compound	Type	Recovery [%]
Isoproturon	U	99
Karbutilate	C	94
Linuron	U	95
Methabenzthiazuron	U	94
Metalaxyl	CA	47
Metamitron	T	85
Metazachlor	CA	94
Methiocarb	C	98
Methomyl	C	95
Methoprotryn	T	90
Metobromuron	U	100
Metolachlor	CA	92
Metoxuron	U	96
Metribuzin	T	72
Monolinuron	U	95
Oxamyl	C	99
Pencycuron	U	99
Pendimethalin	S	73
Pirimicarb	C	82
Promecarb	C	22
Prometryn	T	87
Propazine	T	90
Propham	C	94
Propyzamid	CA	86
Prosulfocarb	C	64
Sebuthylazine	T	97
Secbumeton	T	98
Simazin	T	97
Simetryn	T	88
Tebutam	CA	80
Terbumeton	T	80
Terbuthylazine	T	94
Terbutryn	T	88

Triazines and pesticides from water

MN Appl. No. 303021

Private communication: Mr. Reif, Erftverband, Zentrallabor, Bergheim, Germany

Column type:
CHROMABOND® *Easy* / 3 ml / 60 mg
Cat. No. 730753

Sample pretreatment: spike water with the compounds listed below to a concentration of 1000 pg/µl or 1 ppm

Column conditioning: 2 ml acetone, 2 ml water

Sample application: aspirate the spiked water sample through the column

Column washing: 10 ml water

Elution: acetone or acetone – ethyl acetate (see table below)

Further analysis: GC

Recovery rates:

Compound	Recovery [%] for elution with acetone		acetone – ethyl acetate		Signal [nm]
	c.	n.c.	c.	n.c.	
Desisopropylatrazine	48	50	50.5	55.5	214
Desethylatrazine	92.5	80.5	69	77	214
Desethylterbuthylazine	88	87.5	82	83	214
Metazachlor	87.5	85.5	82	85.5	214
Metolachlor	82	85	72	75	214
Simazin	90.5	85	81	82.5	220
Cyanazine	89	86	81.5	82	220
Methabenzthiazuron	92.5	89.5	96	97	220
Atrazine	89.5	89	80.5	85	220
Sebuthylazine	83.5	89	77.5	82.5	220
Propazine	83.5	92.5	75.5	80	220
Terbuthylazine	83.5	88.5	75.5	78.5	220
Prometryn	81.5	89	67.5	72	220
Terbutryn	81.5	87	65.5	70.5	220
Metoxuron	96	94	89.5	91.5	245
Hexazinone	90	89.5	85.5	89.5	245
Monuron	93.5	94	89	89.5	245
Chlortoluron	91	93.5	86.5	89.5	245
Monolinuron	83	87	73	71.5	245
Diuron	89.5	94.5	85.5	90.5	245
Isoproturon	89	91.5	84.5	87	245
Metobromuron	87	91	79	82.5	245
Linuron	86.5	93	79.5	86	245
Chloroxuron	84	93.5	80	87	245
Chloridazon	93	96.5	89.5	92	280
Metamitron	77	74	74.5	74	300
Metribuzin	83	77.5	70	72	300

c. = conditioned; n.c. = not conditioned

Pesticides from water

MN Appl. No. 301560

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108

Column conditioning: 1 column volume methanol, then 1 column volume dist. water

Sample application: aspirate 1 l water sample through the column, then dry column in a stream of nitrogen

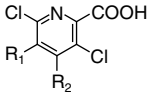
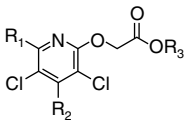
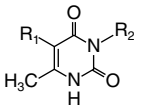
Elution: elute with 3 x 1 ml acetonitrile – methanol (1:1, v/v) and then evaporate or dilute to the volume required

For further analysis we recommend GC, e.g. with capillary column OPTIMA® 5, 0.25 µm film, 50 m x 0.25 mm ID, Cat. No. 726056.50.

Recovery rates:
(100 ng/l per component)

Compound	Recovery [%]	Compound	Recovery [%]
Linuron	97	Propham	94
Monolinuron	99	Atrazine	96
Diuron	100	Simazin	97
Isoproturon	99	Cyanazine	99
Metobromuron	96	Sebuthylazine	96
Chlortoluron	97	Terbuthylazine	94
Methabenzthiazuron	98	Desisopropylatrazine	97
Metoxuron	96	Desethylatrazine	96
Metazachlor	98	Hexazinone	96
Metolachlor	97		

Miscellaneous pesticides containing nitrogen heterocycles

Structure	Compound	R_1	R_2	R_3
	**Pyridinecarboxylic acid derivatives:**			
	Clopyralid	H	H	–
	Picloram	Cl	NH_2	–
	**Pyridyloxyacetic acid derivatives:**			
	Fluroxypyr-MHE	F	NH_2	$CH(CH_3) – (CH_2)_5 – CH_3$
	Triclopyr	Cl	H	H
	**Uracil derivatives:**			
	Bromacil	Br	$CH(CH_3) – C_2H_5$	–
	Terbacil	Cl	$C(CH_3)_3$	–

Pesticides from water

MN Appl. No. 302880

Private communication: Mr. Schmidt-Leistner, Bayr. Landesamt f. Wasserwirtschaft, Germany

Column type:
CHROMABOND® *Easy* / 3 ml / 200 mg
Cat. No. 730754

Sample pretreatment: spike 1000 ml water with 100 ng each of the internal standards

Column conditioning: 3 ml methanol, then 3 ml ultra-pure water

Sample application: slowly force or aspirate the sample through the column, then dry the column with vacuum

Elution: 3 ml methanol – acetonitrile (1:1, v/v)

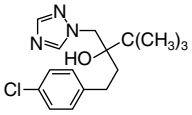
Further analysis: GC

Recovery rates:

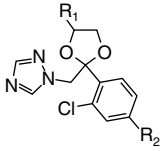
Compound	Recovery [%]	Compound	Recovery [%]
Desethylsimazin	89	Imidacloprid	97
2,6-Dichlorobenzamide	82	Desethylsebuthylazine	89
Metamitron	72	Metribuzin	82
Chloridazon	89	1-(3,4-Dichloro-phenyl)urea	91
Desethylatrazine	83		
Metoxuron	94	1-(4-Isopropylphenyl)-3-methylurea	93
Carbetamide	87		
Bromacil	70	Metalaxyl	73
Simazin	79	Referenzpeak	88
Cyanazine	92	Diuron	94
Desethylterbuthylazine	96	Pirimicarb	86
1-(4-Isopropylphenyl)urea	80	Dimefuron	97
Methabenzthiazuron	86	Triadimenol	90
Chlortoluron	90	Linuron	86
Desmetryn	78	Ethofumesat	85
Atrazine	77	Flurochloridon	90
Monolinuron	57	Prosulfocarb	57
Reference peak	74	Pendimethalin	50
Isoproturon	81	Carbendazim	89
Metobromuron	75	Hexazinone	84
Metazachlor	73	1-(3,4-Dichlorophenyl)-3-methylurea	88
Sebuthylazine	81		
Propazine	71	Benzanilide	83
Terbuthylazine	75	Epoxiconazol	77
Terbutryn	77	Azoxystrobin	80
Diflubenzuron	84	Tebuconazol	88
Metolachlor	64	Propiconazol	80
Fluroxipyr-MHE	43	Aclonifen	67
Ethidimuron	95	Difenoconazol	63

Miscellaneous pesticides containing nitrogen heterocycles

Tebuconazol



Propiconazol
(R_1 = C_3H_7, R_2 = Cl)
Difenoconazol
(R_1 = CH_3, R_2 = O – C_6H_4Cl)



Pesticides from water

MN Appl. No. 303250

Private communication: Bayerisches Landesamt für Wasserwirtschaft, Germany

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108

Sample pretreatment: spike 1 l drinking water with PSM

Column conditioning: 1 column volume methanol, then 1 column volume dist. water

Sample application: aspirate 1000 ml water through the column; dry column in a stream of nitrogen

Elution: 3 x 1 ml methanol – acetone (3:2, v/v) and then evaporate to the needed volume

Further analysis: HPLC/UV

Recovery rates:

Compound	Concentration [ng/l]	Recovery [%]
Desethylsimazin	100	86
Metamitron	200	73
Bromacil	300	103
Simazin	200	77
Cyanazine	200	85
Desethylterbuthylazine	200	77
Methabenzthiazuron	100	70
Chlortoluron	100	83
Atrazine	200	72
Monolinuron	200	56
Metobromuron	100	71
Metazachlor	200	75
Sebuthylazine	100	76
Propazine	100	69
Terbuthylazine	200	67
Terbutryn	200	68
Metolachlor	400	59
Desmetryn	200	71
Diflubenzuron	200	74

Recovery of selected pesticides from water

MN Appl. No. 302920

Private communication: St. Geilen, Bergisches Wasser- und Umweltlabor der BTV-GmbH, Wuppertal, Germany

Column type:
CHROMABOND® HR-P / 6 ml / 500 mg
Cat. No. 730111

Column conditioning: 2 ml acetone

Sample application: 1 l water spiked with 0.2 µg bromacil

Column washing: 3 ml dist. water, then suck to dryness for 30 min

Elution: apply 2 ml acetone and elute with further 2 ml acetone

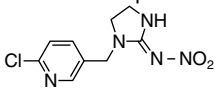
Recovery rates:

Compound	Recovery [%]		Compound	Recovery [%]	
	lot 1	lot 2		lot 1	lot 2
Dichlobenil	91	88	Desisopropylatrazine	94	99
Carbofuran	102	97	Desethylatrazine	97	92
Simazin	91	91	Trifluralin	89	93
Atrazine	95	92	Desethylterbuthylazine	89	93
Propazine	98	95	Sebuthylazine	82	88
Terbuthylazine	90	94	Desmetryn	90	101
γ-HCH	90	80	Vinclozolin	93	99
Alachlor	96	93	Prometryn	89	95
Bromacil	101	0*	Cyanazine	97	93
Metolachlor	88	86	Pendimethalin	95	93
Metazachlor	98	101	Quintozen	93	99
Endosulfan-α	87	90	Heptachlor	75	90
Endosulfan-β	89	96	Dieldrin	82	101
Propham	92	95	Endrin	77	92

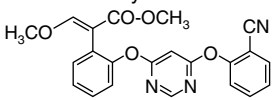
* To ensure the recovery of bromacil the sample pH must be adjusted to pH = 2 before application on the column!

Miscellaneous pesticides containing nitrogen heterocycles

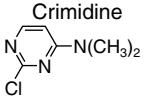
Imidacloprid



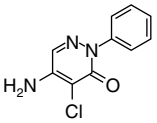
Azoxystrobin



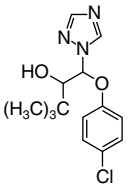
Crimidine



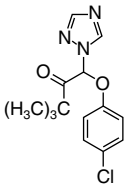
Chloridazon



Triadimenol



Triadimefon



Pesticides from water

MN Appl. No. 303150

Column type:
CHROMABOND® C18 Hydra / 6 ml / 2000 mg
Cat. No. 730301

Sample pretreatment: spike 1 l drinking water with each compound as listed below

Column conditioning: 3 ml acetone, then 3 ml water

Sample application: aspirate 1 l water sample through the column

Column washing: 3 ml dist. water, dry column for 5 min in a stream of nitrogen

Elution: let 2 ml acetone percolate into the adsorbent, then elute with 2 ml acetone

Recovery rates:

Compound	Recovery [%]	Compound	Recovery [%]
Alachlor	100	Metazachlor	91
Atrazine	99	Metolachlor	96
Carbofuran	88	Propazine	96
Chlorfenvinphos	97	Terbuthylazine	95
Dichlobenil	100	Azinphos-ethyl	62
Endosulfan-α	92	Endosulfan-β	65
γ-HCH	99		

Pesticides

MN Appl. No. 302760

Column type:
CHROMABOND® *Easy* / 3 ml / 500 mg
Cat. No. 730759

Column conditioning: 5 ml methanol, 5 ml water

Sample application: slowly force or aspirate 200 ml water sample through the column

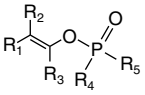
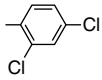
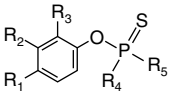
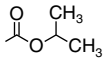
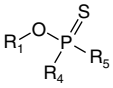
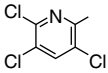
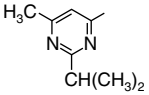
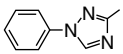
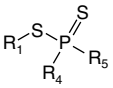
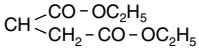
Column washing: 10 ml water

Elution: 2 x 5 ml MeOH – THF (1:1, v/v)

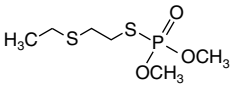
Recovery rates:

Compound	Concentration [µg/l]	Recovery [%]	
		conditioned	unconditioned
Hexazinone	100	85	85
Terbuthylazine	100	89	88
Prometryn	150	93	88
Desethylatrazine	100	82	86
Aldicarb	200	92	100
Carbofuran	150	91	96
Promecarb	100	87	96
Fluazifop-butyl	100	87	88

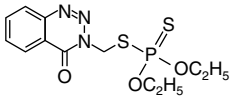
Organophosphorus pesticides

Structure	Compound	R_1	R_2	R_3	R_4	R_5
	Chlorfenvinphos	Cl	H		OC_2H_5	OC_2H_5
	Dichlorphos	Cl	Cl	H	OCH_3	OCH_3
	Mevinphos	$CO-OCH_3$	H	CH_3	OCH_3	OCH_3
	Fenitrothion	NO_2	CH_3	H	OCH_3	OCH_3
	Fenthion	SCH_3	CH_3	H	OCH_3	OCH_3
	Isofenphos	H	H		OC_2H_5	
	Parathion	NO_2	H	H	OC_2H_5	OC_2H_5
	Parathion-methyl	NO_2	H	H	OCH_3	OCH_3
	Chlorpyrifos				OC_2H_5	OC_2H_5
	Diazinon				OC_2H_5	OC_2H_5
	Triazophos				OC_2H_5	OC_2H_5
	Carbophenothion	$CH_2-S-C_6H_4Cl$			OC_2H_5	OC_2H_5
	Dimethoate	$CH_2-CO-NH-CH_3$			OCH_3	OCH_3
	Fonofos	C_6H_5			C_2H_5	OC_2H_5
	Malathion				OCH_3	OCH_3

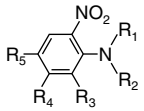
Demeton-S-methyl



Azinphos-ethyl



Miscellaneous pesticides: nitroaniline derivatives

Structure	Compound	R_1	R_2	R_3	R_4	R_5
	Pendimethalin	H	$CH(C_2H_5)_2$	NO_2	CH_3	CH_3
	Trifluralin	C_3H_7	C_3H_7	NO_2	H	CF_3
	Aclonifen	H	H	Cl	$O-C_6H_5$	H

Pesticides from water

MN Appl. No. 301470

Compounds investigated: carbaryl, chlorpyrifos, iprodione, isofenphos, triadimefon

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: mix 1 l water sample with 1 ml acetone

Column conditioning: 3 ml methanol, then 3 ml dist. water

Sample application: slowly force or aspirate the sample through the column

Column drying: dry the column thoroughly under vacuum

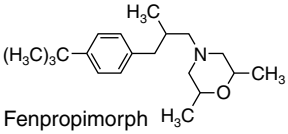
Elution: 5 ml methylene chloride

If you wish to further fractionate the pesticides, you can first elute chlorpyrifos and isofenphos with 5 ml *n*-hexane, then elute the other compounds with 5 ml methylene chloride.

Pesticides: organophosphorus pesticides from water

MN Appl. No. 301580

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108



Fenpropimorph

Sample pretreatment: adjust 1000 ml water sample to pH 6 with 1 M phosphate buffer

Column conditioning: 2 x 2 ml ethyl acetate, 3 x 2 ml methanol, then 3 x 2 ml dist. water

Sample application: aspirate adjusted water sample through the column

Column washing: 2 ml dist. water, then dry column for 30 min under vacuum

Elution: 2 x 1 ml ethyl acetate – *n*-hexane (50:50, v/v)

Further analysis: GC

Recovery rates:
150 ng/l per component

Compound	Recovery [%]	Compound	Recovery [%]
Azinphos-methyl	90	Fenpropimorph	90
Carbophenothion	89	Fenthion	94
Chlorfenvinphos	88	Fonofos	93
Demeton-*S*-methyl	91	Malathion	89
Diazinon	95	Methyl parathion	91
Dichlorphos	90	Mevinphos	96
Dimethoate	92	Parathion	93
Fenitrothion	95	Triazophos	96

For further pesticide analysis we recommend gas chromatography with OPTIMA® fused silica capillary columns.

Pesticides (PBSM-8A + triazines) from water

MN Appl. No. 302960

Private communication: E. Göldner et al., Institut für angewandte Chemie Gockel und Weischedel GmbH, Stuttgart, Germany

Compounds investigated: isoproturon, dichlobenil, desisopropylatrazine, desethylatrazine, trifluralin, 2,6-dichlorobenzamide, desethylterbuthylazine, dimethoate, simazin, atrazine, propazine, terbuthylazine, diazinon, sebuthylazine, metalaxyl, fenitrothion, malathion, parathion-ethyl, metolachlor, metazachlor, pendimethalin

Column type:
CHROMABOND® HR-P / 3 ml / 100 mg
Cat. No. 730342.1

Column conditioning: 2 x 1.5 ml acetone, then one column volume of dist. water

Sample application: slowly aspirate one to two liters of water sample through the column, then aspirate to dryness for about 10 min (adsorbent colour will change to orange). Elute directly or store sealed column in a refrigerator.

Elution: apply 2 ml acetone and allow to elute by gravity flow into a 2-ml volumetric flask; rinse with acetone, until the volumetric flask is filled to the mark

Further analysis: GC/MS

Pesticides: insecticides from river water and suspended sediments

MN Appl. No. 303430

R. Schulz et al., Water SA **27** (2001) 65 – 70

Compounds investigated: endosulfan-α, endosulfan-β, chlorpyrifos, azinphos-methyl

Column type:
CHROMABOND® C18 / 6 ml / 500 mg
Cat. No. 730004

Column conditioning: 6 ml methanol, then 6 ml water

Water samples:

Sample application: aspirate 500 – 900 ml river water through the column; air-dry for 30 min and keep column at –18 °C until analysed

Suspended sediment samples:

Sample pretreatment: samples are placed in 250-ml PP bottles and centrifuged; the supernatant water is discarded and 50 ml methanol are added; the bottles are shaken until the contents are well mixed, placed in an ultrasonic bath for 30 min and then centrifuged; the supernatant methanol is filtered through glass filter paper into 500 ml measuring cylinders; another 50 ml of methanol are added to the sediment and again mixed well, placed in the ultrasonic bath and centrifuged. The two methanol extracts from each sample are pooled and made up to 350 ml with pure water.

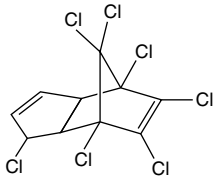
Sample application: aspirate a 50-ml aliquot of the extract through the column

Elution: 2 ml *n*-hexane, then 2 ml dichloromethane; the eluates are evaporated in a stream of nitrogen and then dissolved in 1 ml *n*-hexane (0.5 ml for water samples)

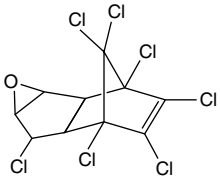
Further analysis: GC with electron-capture and nitrogen-phosphorus detector

Organochlorine pesticides

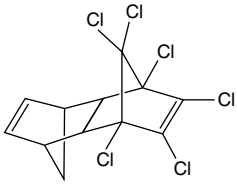
Heptachlor



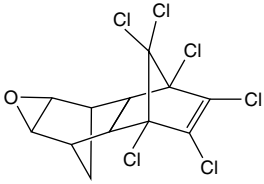
Heptachlor epoxide



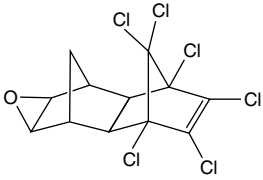
Aldrin



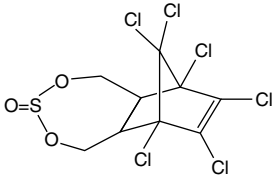
Dieldrin



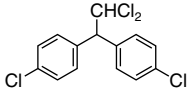
Endrin



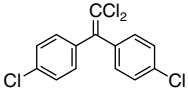
Endosulfan



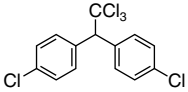
p,p'-DDD



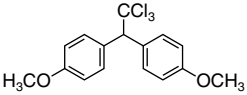
p,p'-DDE



p,p'-DDT



p,p'-Methoxychlor



Pesticides from drinking water

MN Appl. No. 301690

Compounds investigated: organochlorine pesticides aldrin, *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT, endosulfan, α-BHC, β-BHC, δ-BHC, heptachlor, heptachlor epoxide, methoxychlor

Column type:
CHROMABOND® C8 / 6 ml / 500 mg
Cat. No. 730024

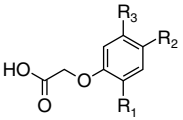
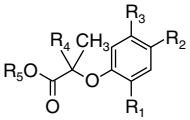
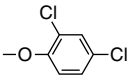
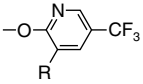
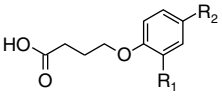
Column conditioning: 2 x 3 ml methanol, then 2 ml dist. water

Sample application: slowly pour 100 ml drinking water through the column

Column washing: 1 ml dist. water; dry column 5 min under vacuum

Elution: 2 x 500 µl *n*-hexane – ether (50:50, v/v)

Pesticides: phenoxycarboxylic acids

Compound	R_1	R_2	R_3	R_4	R_5
 **Phenoxyacetic acid derivatives**					
2,4-D	Cl	Cl	H	–	–
2,4,5-T	Cl	Cl	Cl	–	–
MCPA	CH_3	Cl	H	–	–
 **Phenoxypropionic acid derivatives**					
2,4-DP = Dichlorprop	Cl	Cl	H	H	H
2,4,5-TP = Fenoprop	Cl	Cl	Cl	H	H
MCCP = Mecoprop	CH_3	Cl	H	H	H
Clofibric acid	H	Cl	H	CH_3	H
Diclofop	H		H	H	H
Fluazifop-butyl	H	 R = H	H	H	C_4H_9
Haloxyfop ethoxyethyl ester	H	R = Cl	H	H	$(CH_2)_2 - OC_2H_5$
 **Phenoxybutyric acid derivatives**					
2,4-DB	Cl	Cl	–	–	–
MCPB	CH_3	Cl	–	–	–

Pesticides from drinking water

MN Appl. No. 301740

Compounds investigated: phenoxycarboxylic acid herbicides 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, 2-(2,4,5-trichlorophenoxy)-propionic acid

Column type:
CHROMABOND® C18 ec / 6 ml / 500 mg
Cat. No. 730014

Sample pretreatment: adjust 250 ml sample to pH 2 with about 0.2 ml conc. HCl

Column conditioning: 2 column volumes methanol, then 2 column volumes acidified water (0.2 ml conc. HCl per 250 ml water)

Sample application: slowly force or aspirate 250 ml sample through the column

Column washing: 1 ml acidified water (as above); dry column 10 min under vacuum

Elution: 2 x 500 µl methanol or acetone

Pesticides: phenoxycarboxylic acids from water

MN Appl. No. 302860

Private communication: Mr. Maes, Stadtwerke Wiesbaden, Germany

Column type:
CHROMABOND® *Easy* / 3 ml / 200 mg
Cat. No. 730754

Sample pretreatment: 1000 ml water are spiked with 100 ng of each internal standard; the pH value is adjusted to pH 2.0

Column conditioning: 2 x 2 ml acetone, then 2 x 2 ml ultra-pure water

Sample application: slowly force or aspirate the whole sample of 1000 ml water through the column, dry the column under vacuum

Elution: 2 x 2 ml methanol – acetone (1:1, v/v); alternatively 2 x 2 ml ethyl acetate – acetone (1:1, v/v) can be used

Further analysis: GC

Recovery rates:

Compound	Recovery [%]	Compound	Recovery [%]
Clopyralid	77	Bentazone	100
Dicamba	99	Picloram	103
MCPP	96	Fluazifop	109
MCPA	92	Haloxyfop	102
2,4-DP	94	MCPB	95
2,4-D	96	Fenoprop	97
Triclopyr	97	Bromacil	107
2,4,5-T	96	Clofibric acid	94
2,4-DB	108	Fluroxypyr	101

Pesticides from water

MN Appl. No. 301730

Compounds investigated: chlorophenoxycarboxylic acids

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: adjust 500 ml water to pH 2 with hydrochloric acid and add 5 ml methanol

Column conditioning: 1 column volume *n*-hexane, 1 column volume methanol, 1 column volume water (pH 2, acidified with HCl)

Sample application: slowly aspirate the sample through the column with vacuum; dry 15 min under vacuum

Column washing: aspirate 1 ml *n*-hexane into the column packing, wait 2 min, aspirate solvent through the column and wash with 1 ml *n*-hexane (chlorohydrocarbons); then dry column under vacuum

Elution: aspirate 1 ml methanol into the column packing, wait 2 min, then wash with 1 ml methanol

Acid herbicides from soil samples

MN Appl. No. 303000

J. L. Luque-Garcia et al., Chromatographia **55** (2002) 117 – 122

Compounds investigated: bentazone, 2,4-dichlorophenoxyacetic acid (2,4-D), triclopyr, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4,5-trichlorophenoxypropionic acid (2,4,5-Tp)

Column type:
CHROMABOND® C18 Hydra / 3 ml / 1000 mg
Cat. No. 730298

Sample pretreatment: spike 500 g of each soil type (clayey, slimy, limy and sandy) with the acid herbicides to a final total concentration of 25 µg/g (5 µg/g of each herbicide); dissolve the sample in 50 ml water using ten microwave irradiation periods of 2.5 min each interrupted by 1-min resting periods; filter the extract and merge the sample solution with an acetic acid/sodium acetate buffer at pH 4.5 in order to protonate the acid herbicides

Column conditioning: methanol, then water

Sample application: after the microwave leaching step described above the solution is passed through the column

Elution: methanol

Further analysis: HPLC

The complete sample preparation and HPLC procedure is automated.

Pesticides from drinking water

MN Appl. No. 302800

Private communication: Mr. Lahr, Stadtwerke Mainz, Germany

Column type:
CHROMABOND® *Easy* / 6 ml / 200 mg
Cat. No. 730755

Sample pretreatment: add 100 ng/l of the standard

Column conditioning: 2 ml methanol, then 2 ml water

Sample application: aspirate 1 l water through the column, then dry under vacuum

Elution: 2 x 2 ml MeOH – THF (1:1, v/v) or acetone; evaporate to dryness at 20 °C

Further analysis: GC/MS

Recovery rates:

Compound	Recovery [%] blind value without SPE (MeOH – THF)	standard solution (MeOH – THF)	standard solution (acetone)
Mecoprop	114	105	96
Dicamba	117	91	98
MCPA	123	94	101
Dichlorprop	117	99	97
2,4-D	133	109	90
Bentazone	134	95	96

Comparison of different phases for extraction of pesticides from water

MN Appl. No. 302140

Column type:
CHROMABOND® C18 ec / 6 ml / 2000 mg, Cat. No. 730141
CHROMABOND® C18 / 6 ml / 2000 mg, Cat. No. 730130
CHROMABOND® C_6H_{11} ec / 6 ml / 2000 mg, Cat. No. 730469
CHROMABOND C18 Hydra / 6 ml / 2000 mg, Cat. No. 730301
CHROMABOND HR-P / 3 ml / 200 mg. Cat. No. 730108

Column conditioning: 10 ml methanol, then 10 ml dist. water

Sample application: aspirate the sample through the column

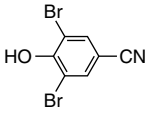
Elution: 10 ml THF – methanol (1:1, v/v)

Recovery rates:

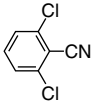
Compound	Recovery [%]				
	C18 ec	C18	C_6H_{11} ec	C18 Hydra	HR-P
Desisopropylatrazine	61	104	37	94	87
Desethylatrazine	79	81	87	96	92
Atrazine	80	81	88	100	104
Prometryn	81	84	88	96	97
Aldicarb	75	93	96	86	87
Carbofuran	91	97	87	98	85
Promecarb	90	91	74	95	99
4,4'-DDT	85	87	98	90	62
Fluazifop-butyl	87	83	84	98	67
Dichlorprop	77	81	82	98	78
Diclofop	68	63	71	96	81
2,4-DB	72	79	80	95	68

Miscellaneous pesticides

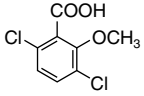
Bromoxynil



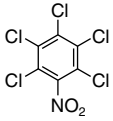
Dichlobenil



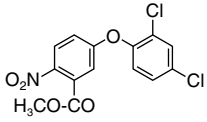
Dicamba



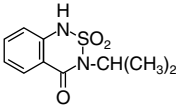
Quintozen



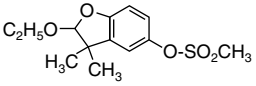
Bifenox



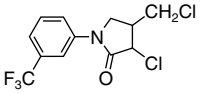
Bentazone



Ethofumesat



Flurochloridon



Pesticides from water, soil and lean tissues

MN Appl. No. 301710

Compounds investigated: organochlorine pesticides

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: adjust water samples to pH 7; soil or lean tissues: homogenise 10 g sample with 50 ml methanol, filter and fill 25 ml of the filtrate with water up to 2000 ml; adjust to pH 7

Column conditioning: 2 column volumes methanol, 2 column volumes water

Sample application: slowly pour sample or parts thereof through the column

Column washing: 2 x 500 µl water; dry column about 10 min under vacuum

Elution: 2 x 500 µl *n*-hexane – dichloromethane (7:3, v/v)

Pesticides: organochlorine insecticides from water

MN Appl. No. 301700

Compounds investigated: aldrin, *p,p'*-DDE, *o,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, dieldrin, endosulfan I, endosulfan II, endrin, heptachlor, heptachlor epoxide, lindane, *p,p'*-methoxychlor

Column type:
CHROMABOND® C18 ec / 6 ml / 500 mg
Cat. No. 730014

Sample pretreatment: filter sample, if necessary

Column conditioning: 2 column volumes ethyl acetate, then 1 column volume methanol, finally 1 column volume dist. water

Sample application: slowly force or aspirate the sample through the column

Column washing: 1 column volume dist. water; dry column 15 min thoroughly under vacuum

Elution: 2 x 500 µl ethyl acetate; concentrate eluate to about 250 µl in a stream of nitrogen at 40 °C; if necessary, perform a second purification:

Column type:
CHROMABOND® SiOH / 6 ml / 1000 mg
Cat. No. 730075

Column conditioning: 200 µl water, then 10 ml *n*-hexane

Sample application: mix concentrated C18 ec eluate with 1 ml *n*-hexane and force or aspirate it through the column; collect the eluate

Elution: 10 ml *n*-hexane; combine eluates: 1st fraction; elute a 2nd fraction with benzene – hexane (6:4, v/v); analyse both fractions separately

Phenoxycarboxylic acids from water

MN Appl. No. 303110

Column type: (6 ml / 2000 mg)
CHROMABOND® C18 ec, Cat. No. 730141
CHROMABOND® C18, Cat. No. 730130
CHROMABOND® C_6H_{11} ec, Cat. No. 730469
CHROMABOND® C18 Hydra, Cat. No. 730301
CHROMABOND® HR-P / 3 ml / 200 mg, Cat. No. 730108

Column conditioning: 10 ml methanol, then 10 ml water (pH 2)

Sample application: slowly force or aspirate 2.00 mg sample through the column

Elution: 10 ml THF – methanol (1:1, v/v)

Recovery rates:

Phase	Recovery [%]		
	dichlorprop	diclofop	2,4-DB
C18 ec	77	68	72
C18	81	63	79
C18 Hydra	98	96	95
C_6H_{11} ec	82	71	80
HR-P	78	81	68

Recovery of halogenated pesticides

MN Appl. No. 303120

Column type: (6 ml / 2000 mg)
CHROMABOND® C18 ec, Cat. No. 730141
CHROMABOND® C18, Cat. No. 730130
CHROMABOND® C_6H_{11} ec, Cat. No. 730469
CHROMABOND® C18 Hydra, Cat. No. 730301
CHROMABOND® HR-P / 3 ml / 200 mg, Cat. No. 730108

Column conditioning: 10 ml methanol, then 10 ml water

Sample application: slowly force or aspirate 2000 mg sample through the column

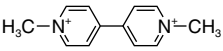
Elution: 10 ml THF – methanol (1:1, v/v)

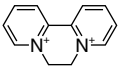
Recovery rates:

Phase	Recovery [%]	
	4,4'-DDT	fluazifop-butyl
C18 ec	85	87
C18	87	83
C18 Hydra	98	84
C_6H_{11} ec	90	98
HR-P	62	67

Pesticides: herbicides paraquat and diquat from water

MN Appl. No. 301670





Column type:
CHROMABOND® CN / 3 ml / 500 mg
Cat. No. 730063

Sample pretreatment: adjust water sample to pH 7.5 with diluted ammonia

Column conditioning: 3 ml methanol, then 5 ml dist. water

Sample application: slowly force or aspirate 100 ml adjusted water sample through the column

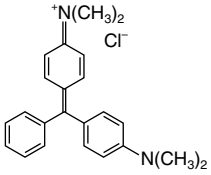
Column washing: 3 ml dist. water

Elution: elute analytes with 3 x 1 ml 1.5 mol/l HCl, neutralise with 750 µl ammonia (25%) and if necessary fill to the volume required

For further analysis we recommend HPLC.

Pesticides: fungicide malachite green from water

MN Appl. No. 301510



Column type:
CHROMABOND® OH (Diol) / 3 ml / 500 mg
Cat. No. 730053

Column conditioning: 1 column volume dist. water

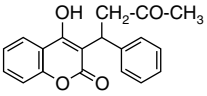
Sample application: slowly force or aspirate 40 ml sample solution (200 µg/l) through the column

Column washing: 1 column volume dist. water

Elution: 2 x 500 µl methanol with 2% acetic acid

Pesticides: rodenticide warfarin from water

MN Appl. No. 301750



Column type:
CHROMABOND® C18 ec / 3 ml / 200 mg
Cat. No. 730012

Sample pretreatment: acidify 1 l water with acetic acid to pH 4.3

Column conditioning: 5 ml acetonitrile, then 5 ml water pH 4.3

Sample application: slowly force or aspirate the sample through the column

Column washing: 20 ml acetonitrile – water pH 4.3 (1:4, v/v)

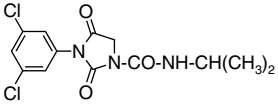
Elution: 1 ml acetonitrile – phosphate buffer pH 7.4 (1:1, v/v)

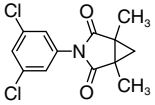
Pesticides: fungicides from drinking water

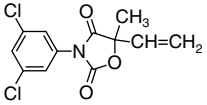
MN Appl. No. 301530

I. Wassmuth-Wagner, H. York, J. Planar Chromatogr. **2** (1989) 297

Compounds investigated: iprodione, procymidone, vinclozolin etc.







Column type:
CHROMABOND® C18 / 3 ml / 500 mg
Cat. No. 730003

Column conditioning: 3 ml *n*-hexane, 3 ml dichloromethane, 3 ml methanol, then 10 ml dist. water

Sample application: force or aspirate 1 l drinking water through the column with 5 – 8 ml/min

Column washing: dry column 30 min under vacuum; then aspirate 2 x 1 ml *n*-hexane into the column packing and dry again under vacuum for 15 min

Elution: apply 2 x 1 ml dichloromethane to the column and let them percolate into the packing; then elute slowly

Removal of humic compounds from surface water and sewage plant efflux for further pesticide analysis

MN Appl. No. 303170

Private communication: Mr. Schmidt-Leistner, Bayrisches Landesamt für Wasserwirtschaft, München, Germany

Step 1:

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108

Sample application: aspirate 250 – 500 ml water through the PS/DVB column

Elution: acetonitrile – methanol (1:1, v/v); evaporate and dissolve in 5 ml ethyl acetate

Step 2:

Column type:
CHROMABOND® SiOH / 3 ml / 500 mg
Cat. No. 730073

Sample application: apply eluate from step 1 to the silica column

Column washing: 2 ml ethyl acetate

Elution: acetonitrile – methanol (1:1, v/v)

Further analysis: HPLC

For further pesticide analysis we recommend gas chromatography with OPTIMA® fused silica capillary columns.

Aromatic pollutants and pesticides from water

MN Appl. No. 303160

Column type:
CHROMABOND® C18 Hydra / 6 ml / 2000 mg
Cat. No. 730301

Sample pretreatment: spike 1 l drinking water with each compound as listed below

Column conditioning: 3 ml acetone, then 3 ml water

Sample application: aspirate 1 l water sample through the column

Column washing: 3 ml dist. water, dry column for 5 min in a stream of nitrogen

Elution: let 2 ml acetone percolate into the adsorbent, then elute with 2 ml acetone

Recovery rates:

Compound	Recovery [%]	Compound	Recovery [%]
Phenol	105	Carbofuran	95
2,4-Dinitrophenol	90	Promecarb	90
Pentachlorophenol	85	4,4'-DDT	85
Atrazine	100	Fluazifop-butyl	95
Desethylatrazine	95	Dichlorprop	95
Desisopropylatrazine	90	Diclofop	93
Prometryn	95	2,4-DB	93
Aldicarb	85		

Hydrocarbons from water acc. to DIN H-53 / EN ISO 9377-2:2000

MN Appl. No. 302090

Column type:
CHROMABOND® Na_2SO_4/Florisil® / 6 ml / 2000/2000 mg glass column
Cat. No. 730249 G

Internal standard solution: dissolve 20 mg *n*-tetracontane ($C_{40}H_{82}$) in petroleum ether, add 20 ml *n*-decane ($C_{10}H_{22}$) and fill up to 1 liter with petroleum ether. For preparation of the extraction solution dilute standard solution 1:10 with petroleum ether.

Sample pretreatment: adjust 900 ml water (10 °C) with HCl (12 mol/l) to pH 2 and add 80 g $MgSO_4$. Add 50 ml of the extraction solution, close the bottle and stir the suspension intensely for 30 min. Add enough dist. water to separate the organic from the aqueous phase.

Column conditioning: 5 ml petroleum ether

Sample application: slowly aspirate or force the sample through the column

Elution: wash with 10 ml petroleum ether. Evaporate the combined solution from sample application and elution to 1 ml at about 75 °C. If necessary, fill up to 1 ml again. (If the hydrocarbon content is high, evaporation to 1 ml may not be necessary.)

Recovery rates: must be > 80% for *n*-tetracontane

Different hydrocarbon fractions from diesel fuel

MN Appl. No. 301770

Column type:
CHROMABOND® SiOH / 6 ml / 2 g glass column
Cat. No. 730107 G

Sample pretreatment: dissolve 1 g diesel fuel in 10 ml *n*-pentane, filter

Column conditioning: heat column for 1 h at 140 °C to increase the activity and reproducibility. After cooling to about 30 °C force or aspirate 5 ml *n*-pentane through the column

Sample application: slowly pour 200 µl sample solution through the column

Elution: elute with the following solvents (1 ml/min), collect 1-ml fractions, concentrate in a stream of nitrogen:

2 ml *n*-pentane:	dead volume
4 ml *n*-pentane:	aliphatics
8.5 ml CH_2Cl_2 – *n*-pentane (5:95, v/v:	monoaromatics
5.0 ml CH_2Cl_2 – *n*-pentane (10:90, v/v):	diaromatics
5.0 ml CH_2Cl_2 – *n*-pentane (60:40, v/v:	polyaromatics
5.0 ml acetic acid – methanol (25:75, v/v):	polar compounds

Further analysis: GC

Nitroaromatics from water

MN Appl. No. 302870

Private communication: Mr. Steinbach, Universität Marburg, Germany

Column type:
CHROMABOND® *Easy* / 3 ml / 200 mg
Cat. No. 730754

Sample pretreatment: 500 ml water are spiked with 20 ng of the internal standards

Column conditioning: 2 x 2 ml acetone, then 2 x 2 ml ultra-pure water

Sample application: slowly aspirate the water sample through the column, then dry the column by sucking air through it

Elution: 2 x 2 ml MeOH – THF (1:1, v/v)

Further analysis: HPLC with NUCLEOSIL® 120-3 C_{18}

Recovery rates:

Compound	Recovery [%]	Compound	Recovery [%]
Octogen	95	2-Amino-4,6-dinitrotoluene	106
Hexogen	83	2,6-Dinitrotoluene	106
1,3,5-Trinitrobenzene	96	2,4-Dinitrotoluene	101
1,3-Dinitrobenzene	132	2-Nitrotoluene	101
2,4,6-Trinitrotoluene	85	4-Nitrotoluene	101
4-Amino-2,6-dinitrotoluene	101	3-Nitrotoluene	102

Naphthols and nitroaromatics from water

MN Appl. No. 301780

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108

Sample pretreatment: adjust pH of water sample to 2 with dilute HCl

Column conditioning: 2 x 3 ml methanol, then 3 ml dist. water

Sample application: aspirate 1000 ml water sample (pH 2) through the column

Elution: 3 x 1 ml acetonitrile – methanol (1:1, v/v)

Further analysis: HPLC, e.g. with column 250 x 4 mm NUCLEOSIL® 100-5 C_{18}.

Recovery rates:
100 µg/l per component

Compound	Recovery [%]	Compound	Recovery [%]
2-Naphthol	98	2-Nitrotoluene	96
1-Naphthol	95	4-Nitrotoluene	92
2,4-Dinitrotoluene	98	3-Nitrotoluene	95

Aromatic hydrocarbons: explosives from water

MN Appl. No. 301790

Compounds investigated: 1,3-dinitrobenzene, 2,6-dinitrotoluene, 2,4-dinitrotoluene, nitrobenzene, RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine), tetryl (*N*-methyl-*N*,2,4,6-tetranitroaniline), 1,3,5-trinitrobenzene, 2,4,6-trinitrotoluene

Column type:
CHROMABOND® C18 ec / 6 ml / 500 mg
Cat. No. 730014

Sample pretreatment: adjust 500 ml sample to pH 6 and dissolve 150 g sodium chloride in this sample; filter

Column conditioning: 2 column volumes methanol, 2 column volumes water

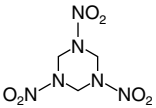
Sample application: slowly force or aspirate the sample through the column

Column washing: 1 ml dist. water; dry column under vacuum for 5 min

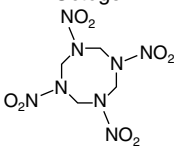
Elution: 2 x 1 ml methanol

Nitroaromatics

Hexogen (= RDX)



Octogen



Aromatic hydrocarbons from water

MN Appl. No. 301800

Compounds investigated: halogenated anilines

Column type:
CHROMABOND® C18 ec / 6 ml / 3 g (special)

Sample pretreatment: adjust 1 l water sample to pH 9 with sodium hydroxide, filter through a glass fibre filter and mix with 350 g sodium chloride

Column conditioning: for cleaning first rinse the column with 8 ml acetone, then pass nitrogen (80 – 90 ml/sec) through the column for 30 min, and finally pour 8 ml methanol, then 8 ml water through the column

Sample application: force or aspirate the sample through the column in about 3 h

Column drying: pass nitrogen (80 – 90 ml/sec) through the column for 30 min

Elution: 6 ml ethyl acetate; concentrate eluate in a nitrogen stream

Enrichment of explosives from water samples

MN Appl. No. 302910

Private communication: T. Bausinger, Geographisches Institut, Universität Mainz, Germany

Column type:
CHROMABOND® LV-HR-P / 15 ml / 200 mg
Cat. No. 732108

Column conditioning: 5 ml methanol, 3 ml acetonitrile and then 10 ml water without vacuum; the column must not run dry

Sample application: aspirate 400 ml of the sample through the column with 10 – 15 ml/min

Column washing: water, then dry using vacuum for 40 – 60 min

Elution: apply 1ml methanol – acetonitrile (50:50, v/v) on the column; the adsorbent should become wet; but do not suck the solvent through the column; wait for 5 min, then elute with 1.5 ml of the same solvent under vacuum; fill the eluate up to 4 ml with water

Further analysis: RP-HPLC

Recovery rates:

(test mixture containing 5 µg/l of each compound in water)

Compound	Recovery [%]	Compound	Recovery [%]
Octogen	114 ± 6	4-Amino-2,6-dinitrotoluene	107 ± 1
2,4-Diamino-6-nitrotoluene	63 ± 25	2-Amino-4,6-dinitrotoluene	107 ± 2
Hexogen	99 ± 14	2,6-Dinitrotoluene	98 ± 2
1,3,5-Trinitrobenzene	105 ± 2	2,4-Dinitrotoluene	97 ± 1
2-Amino-6-nitrotoluene	102 ± 1	2-Nitrotoluene	103 ± 2
2-Amino-4-nitrotoluene	101 ± 1	4-Nitrotoluene	87 ± 1
1,3-Dinitrobenzene	106 ± 1	3-Nitrotoluene	92 ± 6
2,4,6-Trinitrotoluene	103 ± 1		

Enrichment of explosives from water samples

MN Appl. No. 303010

Private communication: T. Bausinger, Geographisches Institut, Universität Mainz, Germany

Column type:
CHROMABOND® LV-*Easy* / 15 ml / 30 mg
Cat. No. 732470

Column conditioning: under normal pressure apply 5 ml methanol – acetone (50:50, v/v) on the column and then slowly aspirate 10 ml water through the column; the column must not run dry

Sample application: aspirate 400 ml of the sample through the column with 10 – 15 ml/min

Column washing: water, then dry using vacuum for 40 – 60 min

Elution: apply 1 ml methanol – acetone (50:50, v/v) on the column; the adsorbent should become wet; but do not suck the solvent through the column; wait for 5 min, then elute with 1.5 ml of the same solvent under vaccum; fill the eluate up to 4 ml with water

Further analysis: RP-HPLC

Recovery rates:

(test mixture containing 5 µg/l of each compound in water)

Compound	Recovery [%]	Compound	Recovery [%]
Octogen	91 ± 3	4-Amino-2,6-dinitrotoluene	107 ± 1
2,4-Diamino-6-nitrotoluene	94 ± 15	2-Amino-4,6-dinitrotoluene	105 ± 2
Hexogen	97 ± 4	2,6-Dinitrotoluene	95 ± 1
1,3,5-Trinitrobenzene	84 ± 4	2,4-Dinitrotoluene	94 ± 0
2-Amino-6-nitrotoluene	103 ± 2	2-Nitrotoluene	91 ± 3
2-Amino-4-nitrotoluene	100 ± 2	4-Nitrotoluene	88 ± 2
1,3-Dinitrobenzene	104 ± 2	3-Nitrotoluene	87 ± 4
2,4,6-Trinitrotoluene	98 ± 1		

Aromatic hydrocarbons from water

MN Appl. No. 301760

Compounds investigated: monochloronitrobenzene

Column type:
CHROMABOND® C18 ec / 6 ml / 500 mg
Cat. No. 730014

Column conditioning: 2 column volumes methanol, then 1 column volume dist. water

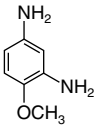
Sample application: pour sample through the column with about 5 – 6 ml/min

Column washing: 1 column volume dist. water

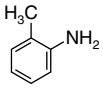
Elution: 4 x 500 µl *n*-hexane

Aromatic amines

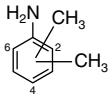
2,4-Diaminoanisole



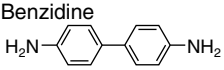
o-Toluidine



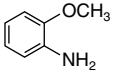
Xylidine isomers



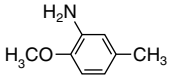
Benzidine



o-Anisidine



p-Cresidine



Aromatic amines from water

MN Appl. No. 302450

T. C. Schmidt et al., Chromatographia **48** (1998) 436 – 442

Compounds investigated: 2-aminotoluene, 3-aminotoluene, 4-aminotoluene, 2,3-diaminotoluene, 2,4-diaminotoluene, 2,6-diaminotoluene, 3,4-diaminotoluene, 2-amino-3-nitrotoluene, 2-amino-4-nitrotoluene, 2-amino-5-nitrotoluene, 2-amino-6-nitrotoluene, 4-amino-2-nitrotoluene, 2-amino-4,6-dinitrotoluene, 4-amino-4,6-dinitrotoluene, 2,4-diamino-6-nitrotoluene, 2,6-diamino-4-nitrotoluene, aniline, 1,2-phenylenediamine, 1,3-phenylenediamine, 1,4-phenylenediamine, 2-nitroaniline, 3-nitroaniline, 4-nitroaniline, 2,4-dinitroaniline, 2,5-dinitroaniline, 2,6-dinitroaniline, 3,5-dinitroaniline, *o*-anisidine, *m*-anisidine, *p*-anisidine, 2,3-dimethylaniline, 2,4-dimethylaniline, 2,5-dimethylaniline, 2,6-dimethylaniline, 3,4-dimethylaniline, 3,5-dimethylaniline, *N,N*-dimethylaniline, diphenylamine, 1-naphthylamine, 2-naphthylamine, benzidine, 2-aminobiphenyl, 4-aminobiphenyl, 4-isopropylaniline, 2,6-diethylaniline, 2-ethyl-6-methylaniline, 4-chloro-*N*-methylaniline, 2-chloroaniline, 3-chloroaniline, 4-chloroaniline, 3,4-dichloroaniline, 4-chloro-2-methylaniline, 3-chloro-4-methylaniline, 3-chloro-4-methoxyaniline, 3-chloro-4-fluoroaniline, 4-bromoaniline

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108

Sample pretreatment: immediately before measurements, samples are adjusted to about pH 9 with a concentrated NaOH solution (c=10 M in water); if necessary, samples are filtered through 0.45 µm cellulose nitrate membrane filters

Column conditioning: twice with 1 ml methanol, and twice with 1 ml acetonitrile, then twice with 1 ml dist. water, adjusted to pH 9

Sample application: depending on the matrix and the expected concentration range, 0.1 – 1 liter sample are passed through the column with a peristaltic pump, set to 10 ± 0.2 ml/min

Column washing: dist. water, then dry under vacuum for 1 min

Elution: 3 times with 1 ml methanol – acetonitrile (1:1, v/v); for subsequent derivatisation transfer eluates to 24 ml borosilicate glass vials and reduce volume under a gentle stream of nitrogen at 40 °C to < 0.5 ml

Further analysis: GC

Recovery rates: for most analytes 80 – 120%

Determination of azo dyes / aromatic amines in coloured textile materials

MN Appl. No. 302100

acc. to § 35 of the German law for food and consumer goods / LMBG

Column type:
CHROMABOND® XTR / 70 ml / 14.5 g,
for max. 20 ml aqueous solution
Cat. No. 730507

Sample pretreatment: weigh about 1 g of a cut-up textile sample (coloured textiles about 0.1 g) in a 100 ml threaded vial. Degrease leather samples before processing: cover sample with technically pure *n*-hexane and put the vial in an ultrasonic bath for 20 min. After decanting the *n*-hexane rinse with a small amount of *n*-hexane and dry sample by gently blowing in air or N_2. Add 250 µl IS (1.2 mg/ml tetramethylbenzidine in methanol – ethyl acetate 1:1, v/v), 17.0 ml citrate buffer (pH 6) (25.05 g citric acid and 12.64 g NaOH, filled up with deion. H_2O to 2 l) and heat 30 min to 70 °C. Then add 3 ml of a freshly prepared soution of 0.2 g/ml sodium dithionite in water and heat for exactly 30 min to 70 °C while shaking occasionally.

Sample application: cool the solution immediately (put vial in water – stopping of reductive cleavage). After 5 –10 min pour liquid onto the column (squeeze textile remains).

Elution: allow solution to be soaked up by the adsorbent for 15 min. Then elute four times with 20 ml each of diethyl ether or diethyl ether – ethanol (90:10, v/v) (see recovery rates), using the first 40 ml to rinse the sample remains. Evaporate the eluate to 3 ml with a rotation evaporator and transfer the solution to a 10 ml measuring flask with the help of a Pasteur pipette and by rinsing with methanol. Fill up to the mark with methanol, shake and fill about 1 ml into a vial.

Further analysis: HPLC on NUCLEOSIL® 100-5 C_{18} HD (MN Appl. No. 110500) or GC on OPTIMA® δ-3 (MN Appl. No. 210820), see our application database at *www.mn-net.com*

Recovery rates:

Compound	Recovery [%]	
	Et_2O	Et_2O – EtOH (9:1, v/v)
Tetramethylbenzidine (IS)	112	66
o-Toluidine	90	124
2,4- and 2,6-Xylidine	85	120
o-Anisidine	91	127
p-Chloroaniline	85	131
p-Cresidine	88	116
2,4,5-Trimethylaniline	85	48
4-Chloro-*o*-toluidine	85	124
2,4-Toluenediamine	17	30
2,4-Diaminoanisole	2	12
2-Naphtylamine	80	98
4-Aminobiphenyl	89	99
4,4'-Oxydianiline	90	81
4,4'-Diaminodiphenylmethane	97	76
Benzidine	90	66
4,4'-Diamino-3,3'-dimethylphenylmethane	86	80
3,3'-Dimethylbenzidine	85	80
4,4'-Thiodianiline	81	84
3,3'-Dimethoxybenzidine	91	71
4,4'-Methylene-bis-(2-chloroaniline)	89	102
3,3'-Dichlorobenzidine	85	92

Aromatic amines from water samples

MN Appl. No. 301810

Private communication: M. Leß, T.C. Schmidt, Section of chemistry, University of Marburg, Germany 1997

Compounds investigated: aromatic amines

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108

Sample pretreatment: adjust pH to 9 with 10 mol/l NaOH

Column conditioning: 2 ml each of methanol, acetonitrile and 10^{-5} mol/l sodium hydroxide solution

Sample application: aspirate sample through the column with about 10 ml/min

Column washing: 2 ml dist. water, then dry 5 min under vacuum

Elution: 3 x 1 ml methanol/acetonitrile (1:1, v/v)

Recovery rates:
(matrix A: drinking water, matrix B: polluted ground water)

Compound	Recovery [%] A	Recovery [%] B
Aniline	80 ± 6	83 ± 6
4-Aminotoluene	80 ± 2	111 ± 7
4-Chloro-*N*-methylaniline		107 ± 3
3,5-Dimethylaniline	99 ± 5	
2,6-Dimethylaniline	111 ± 5	
3,4-Dimethylaniline	66 ± 2	107 ± 6
3,4-Dichloroaniline	100 ± 3	89 ± 5
1,3-Phenylenediamine	35 ± 0.3	23 ± 1
3-Nitroaniline	89 ± 5	85 ± 2
4-Nitroaniline	97 ± 6	100 ± 3
3-Chloro-4-methoxyaniline	106 ± 5	91 ± 3
2-Amino-6-nitrotoluene	88 ± 8	92 ± 2
4-Amino-2-nitrotoluene	84 ± 4	60 ± 1
2,4-Diaminotoluene	52 ± 2	48 ± 1
2,6-Diaminotoluene	80 ± 6	104 ± 3
2-Amino-3-nitrotoluene	80 ± 7	
2-Amino-5-nitrotoluene	90 ± 5	90 ± 2
2-Amino-4-nitrotoluene	91 ± 5	91 ± 2
2-Naphthylamine	82 ± 4	87 ± 3
1-Naphthylamine	79 ± 4	100 ± 4
2,6-Dinitroaniline	60 ± 5	75 ± 3
2-Aminobiphenyl	80 ± 5	84 ± 2
3,5-Dinitroaniline	83 ± 7	97 ± 4
4-Amino-2,6-dinitrotoluene	89 ± 6	73 ± 1
4-Aminobiphenyl	94 ± 6	95 ± 1
2-Amino-4,6-dinitrotoluene	81 ± 6	86 ± 3
2,4-Diamino-6-nitrotoluene	95 ± 5	104 ± 1
2,6-Diamino-4-nitrotoluene	109 ± 6	
Benzidine	69 ± 4	58 ± 2

Compound	Recovery [%] A
3-Chloroaniline	71 ± 3
p-Anisidine	67 ± 7
3-Chloro-4-methylaniline	94 ± 3
2-Nitroaniline	76 ± 1
4-Chloroaniline	85 ± 4
2-Chloroaniline	71 ± 3
2,4-Dimethylaniline	107 ± 5
m-Anisidine	92 ± 10
4-Bromoaniline	93 ± 6

Aromatic hydrocarbons: phenols in water

MN Appl. No. 302930

Private communication: D. Jahr, Landesuntersuchungsamt für das Gesundheitswesen Südbayern, Oberschleißheim, Germany

Column type:
CHROMABOND® C18 / 6 ml / 1000 mg
Cat. No. 730005

Sample pretreatment: add the internal standard $^{13}C_6$-2,4,6-trichlorophenol and 20 ml acetanhydride to 5 l water sample; stir for 15 min, then add 100 ml methanol; filter the sample

Column conditioning: 6 ml methanol, then 6 ml water

Sample application: 1 liter per hour, then dry with nitrogen

Elution: 4 ml acetone

Further analysis: GC/MS, from 0.5 ml eluate use 2 µl

Chlorophenols from soil and sludge

MN Appl. No. 301820

M. Syrhe, G. Hanschmann and R. Heber, GIT **38** (1994) 1232 – 1236

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108

Sample pretreatment: add 200 – 300 ml water to 20 – 30 g fresh sludge (or soil), add 36 g NaCl per 100 g water, and sulphuric acid to pH 1. Subject mixture to a steam distillation. Adjust pH of the distillate to pH 2 with dilute HCl.

Column conditioning: 2 x 3 ml tetrahydrofuran, then 3 ml water

Sample application: force or aspirate adjusted distillate through the column

Elution: 3 x 1 ml tetrahydrofuran

For subsequent GC analysis we recommend derivatisation with TMSH.

Aromatic hydrocarbons: phenols from water

MN Appl. No. 301840

Compounds investigated: chlorophenols after acetylation

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: dissolve 10 g sodium hydrogen carbonate in 250 ml water sample and mix with 1 ml acetic acid anhydride. Shake the sample, until the formation of CO_2 has subsided

Column conditioning: 1 column volume methanol, then 1 column volume water

Sample application: slowly pour 100 – 250 ml sample through the column

Column drying: 3 – 4 min under vacuum

Elution: 3 x 300 µl benzene (leave on the column for about 1 min)

Comparison of different phases for phenol analysis

MN Appl. No. 302150

Column type:
CHROMABOND® C18 ec, 6 ml, 2000 mg, Cat. No. 730141
CHROMABOND® C18, 6 ml, 2000 mg, Cat. No. 730130
CHROMABOND® C_6H_{11} ec, 6 ml, 2000 mg, Cat. No. 730469
CHROMABOND® C18 Hydra, 6 ml, 2000 mg, Cat. No. 730301

Column conditioning: 10 ml acetone, 10 ml methanol, and 10 ml dist. water (pH 2)

Sample application: aspirate the sample through the column.

Elution: 10 ml methanol

Recovery rates:

Compound	Recovery [%]			
	C18 ec	C18	C_6H_{11} ec	C18 Hydra
Phenol	92	96	93	101
2,4-Dinitrophenol	81	86	93	91
Pentachlorophenol	96	92	93	88

Aromatic hydrocarbons: phenols from water

MN Appl. No. 301830

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108

Sample pretreatment: adjust water sample to pH 2 with diluted HCl

Column conditioning: 2 x 3 ml THF, then 3 ml dist. water

Sample application: aspirate 1000 ml water sample (pH 2) through the column

Elution: elute with 3 x 1 ml THF and then evaporate to 1 ml in a stream of nitrogen

Further analysis: HPLC.

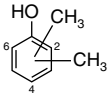
Recovery rates:
50 µg/l per phenol

Compound	Recovery [%]	Compound	Recovery [%]
Phenol	97	2,4-Dimethylphenol	95
4-Nitrophenol	92	4-Chloro-3-methylphenol	92
2,4-Dinitrophenol	98	2,4-Dichlorophenol	89
2-Nitrophenol	93	2,4,6-Trichlorophenol	95
2-Chlorophenol	90	Pentachlorophenol	90
2-Methyl-4,6-dinitrophenol	93		

Phenols in aqueous samples

MN Appl. No. 302370

Cresol isomers: 

Xylenol isomers: 

R. Ciupe et al., GIT Fachz. Lab (1996) 764 – 766

Column type:
CHROMABOND® HR-P
Cat. No. 730615

Sample pretreatment: water samples with two different concentration ranges (0.1 and 0.003 mg/kg) are prepared without pH adjustment or desalting with methanol as auxillary solvent

Column conditioning: 4 ml methanol, then 4 ml water; the column must not run dry

Sample application: aspirate 50 ml (0.1 mg/kg) or 500 ml (0.003 mg/kg) of the prepared samples through the column; dry the column by sucking air for 1 min

Elution: 4 ml methanol; concentrate eluate to 1 or 0.5 ml by heating to 70 °C.

Further analysis: GC

Recovery rates:

Compound	Recovery [%]		Compound	Recovery [%]	
	0.1 mg/l	0.003 mg/l		0.1 mg/l	0.003 mg/l
Phenol	99.02 ± 1.0	98.8 ± 0.9	2,4+2,5-Xylenol	99.66 ± 0.7	98.6 ± 0.8
o-Cresol	99.08 ± 0.7	97.9 ± 0.8	3,4-Xylenol	98.94 ± 0.6	97.4 ± 0.7
m + *p*-Cresol	99.24 ± 0.7	97.5 ± 0.8	2,3-Xylenol	99.06 ± 0.4	98.9 ± 0.6
2,6-Xylenol	97.81 ± 1.2	99.2 ± 0.7	3,5-Xylenol	99.38 ± 0.3	98.5 ± 0.7

Aromatic hydrocarbons: phenols from water

MN Appl. No. 301850

Column type:
CHROMABOND® Phenyl / 3 ml / 50 mg
Cat. No. 730084

Sample pretreatment:
Adjust 100 – 500 ml water to pH 2 with 1 mol/l nitric acid, add 30 g sodium chloride per 100 ml sample

Column conditioning: 5 ml acetonitrile, then 5 ml dist. H_2O

Sample application: slowly force or aspirate the sample through the column

Column washing: 10 ml 0.01 mol/l nitric acid

Elution: 2 x 2.5 ml acetonitrile

Aromatic hydrocarbons: phenols from water

MN Appl. No. 301860

Column type:
CHROMABOND® C18 / 3 ml / 500 mg
Cat. No. 730003

Sample pretreatment: mix 300 ml water sample with 60 g sodium chloride and adjust to pH 2 with 1 mol/l hydrochloric acid

Column conditioning: 2 column volumes methanol, then 2 column volumes 0.01 mol/l hydrochloric acid

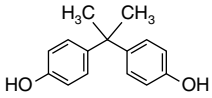
Sample application: slowly force or aspirate the sample through the column

Column washing: 2 x 500 µl 0.01 mol/l HCl; dry column 10 min under vacuum

Elution: 3 x 500 µl methanol

Determination of bisphenol A in water

MN Appl. No. 303211/303212



Column type:
CHROMABOND® HR-P / 3 ml / 200 mg, Cat. No. 730108
or CHROMABOND® *Easy* / 3 ml / 200 mg, Cat. No. 730754

Sample pretreatment: 250 ml water sample are spiked with internal standard

Column conditioning: 3 ml methanol, 3 ml dist. water

Sample application: 250 ml sample are aspirated through the column with 15 ml/min

Column washing: dist. water pH 2.0

Elution: 5 ml acetone – glacial acetic acid (99:1, v/v)

Further analysis: GC

Metals: trace elements from water

MN Appl. No. 301880

Compounds investigated: Bi, Cd, Co, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Ti

Column type:
CHROMABOND® Phenyl / 3 ml / 500 mg
Cat. No. 730084

Sample pretreatment: adjust up to 500 ml water to pH 8 – 9 and add 1 ml 0.1% aqueous sodium diethyl dithiocarbamate solution

Column conditioning: 1 column volume methanol, 1 column volume water

Sample application: force or aspirate sample through the column with 3 – 4 ml/min

Column washing: 2 ml dist. water; dry column under vacuum for 3 – 4 min

Elution: 2 column volumes methanol

Metals from water
MN Appl. No. 301900

Compounds investigated: Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: mix 250 ml water sample with 0.1 ml 5% 8-hydroxyquinoline solution in 0.5 M HCl; adjust pH value to 8.5 with aqueous ammonia solution

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water, which was adjusted to pH 8.5 with ammonia

Sample application:
pour sample through the column with 3 – 4 ml/min

Column washing: 2 x 500 µl water, which was adjusted to pH 8.5 with ammonia; dry column under vacuum for 3 – 4 min

Elution: 2 x 750 µl methanol

Metals: trace elements from water
MN Appl. No. 301910

Compounds investigated: Al, Be, Cu, Cr(VI), Mo(VI), V(V)

Column type:
CHROMABOND® NH_2 / 3 ml / 500 mg
Cat. No. 730033

Sample pretreatment: mix 100 ml water sample with 5 ml 0.001% alizarinsulphonic acid solution and adjust to pH 5.5 with acetic acid or sodium acetate

Column conditioning: 2 column volumes 1 mol/l nitric acid, then 2 column volumes dist. water

Sample application: force or aspirate sample through the column with 3 – 4 ml/min

Column washing: 2 ml dist. water; dry column under vacuum for 4 min

Elution: 2 column volumes 2 mol/l nitric acid

Transition metal traces from water
MN Appl. No. 301890

Column type:
CHROMABOND® Phenyl / 3 ml / 500 mg
Cat. No. 730084

Sample pretreatment: mix 100 ml water sample with 10 ml ammonium citrate buffer pH 6 – 7 (6.25 g citric acid, 0.63 g EDTA and 10 ml aqueous ammonia solution in 100 ml water) and 1 ml 0.1% sodium diethyl dithiocarbamate solution

Column conditioning: 2 column volumes methanol, then 2 column volumes dist. water

Sample application: slowly force or aspirate 100 ml sample through the column

Column washing: 2 ml water, which was adjusted to pH 6 – 7 with ammonium citrate buffer

Elution: 3 x 1 ml methanol

AOX determination in waters containing high salt loads / organic pollutants (DIN 38409 – H 22)

MN Appl. No. 302080

Column type:
CHROMABOND® AOX / 6 ml / 500 mg
Cat. No. 730111.AOX

Column conditioning: 5 ml methanol, 10 ml dist. water. Do not let the column run dry!

Sample application: force or aspirate 100 ml original or diluted sample (pH 1) through the column (3 – 5 ml/min), don't let the column run dry. Discard the flow-through.

Column washing: 50 ml nitrate rinsing solution (dissolve 17 g $NaNO_3$ in 100 ml dist. water, add 1.4 ml HNO_3 10 M, fill up to 1000 ml; take 50 ml and fill to 1000 ml with dist. water). Discard the flow-through.

Elution: slowly aspirate 1 x 1 ml , then 1 x 4 ml methanol and 1 x 10 ml dist. water through the column. Collect eluates in a 100 ml volumetric flask and fill to 100 ml with dist. water.

Determination of haloacetic acids in aqueous environments

MN Appl. No. 302990

R. Loos, D. Barcelo, J. Chromatography A, **938** (2001) 45 – 55

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108

Sample pretreatment: adjust pH of water sample to 1.8 using sulphuric acid

Column conditioning: 5 ml methanol, then 3 ml water (acidified to pH 2.5 with sulphuric acid) at a flow rate of 1 ml/min

Sample application: aspirate the water sample (usually 50 ml) through the column at a flow rate of 5 ml/min, do not allow the column to run dry

Column washing: 1 ml water (pH 2.5)

Elution: 4 ml of a mixture of 0.5 ml water + 3.5 ml methanol – acetone (1:1, v/v); evaporate under a gentle stream of nitrogen until only the water is left

Further analysis: LC/MS

Recovery rates:
(from 50 ml spiked groundwater at pH 1.8)

Compound	Concentration [µg/l]	Recovery [%]
Monochloroacetic acid	300	27 ± 2
Monobromoacetic acid	200	57± 3
Dichloroacetic acid	300	54 ± 3
Bromochloroacetic acid	200	53 ± 4
Dibromoacetic acid	100	45 ± 3
Trichloroacetic acid	100	74 ± 3
Bromodichloroacetic acid	200	39 ± 3
Dibromochloroacetic acid	500	22 ± 2
Tribromoacetic acid	1000	25 ± 1

Polar carboxylic acids from aqueous extracts of inorganic multi-component incineration residues

MN Appl. No. 302670

H. Hirschlag et al., Fresenius J Anal Chem **263** (1998) 274 – 280

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg, glass column
Cat. No. 730108 G

Sample pretreatment: slags from a municipial waste incineration plant are dried for 2 h at 105 °C, ground (< 200 µgm) and subjected to annealing in air at 550 °C for a week in order to oxidise all organic constituents. By means of X-ray diffraction, it is ensured that annealing has not caused any mineralogical conversions. These slags are then doped with 1 ml aqueous acid mixture. Acid concentration in the slags is 100 – 200 µg/kg. For doping, acid droplets are added with a calibrated pipette. Subsequently, the mixture is homogenised thoroughly in the mortar. Prior to extraction, the doped samples are stored for one day at room temperature. Samples that have been stored for a longer period of time (up to 3 months) yield the same recovery rates. Samples are extracted with a hot extraction system (see original literature).

Column conditioning: 3 x 1 ml methanol, followed by 3 x 1 ml water (pH 1, NaCl-saturated)

Sample application: 5 ml of the sample (about 1 mg/l per acid, < pH 1, NaCl-saturated) are loaded slowly on the column. After the addition of the sample, the residual water is removed from the columns in a centrifuge at about 1000 rpm for 10 min and the columns are then dried in a stream of nitrogen for 1 min.

Elution: a Na_2SO_4 column is connected to the outlet of the SPE column by column adapters, followed by slow elution with 4 x 1 ml MTBE (methyl *tert.*-butyl ether); the eluate is collected in pre-calibrated 5 ml V-shaped glasses; the collected eluates are reduced to ~1 ml in a nitrogen flow and subjected to derivatisation.

Further analysis: GC/MS

Recovery rates:
(derivatisation and solid phase enrichment, ME = methyl ester, DME = dimethyl ester)

Compound	Recovery [%]	Compound	Recovery [%]
Butanoic acid, ME	51 ± 5	Benzoic acid, ME	72 ± 9
Lactic acid, ME	42 ± 6	Salicylic acid, ME	80 ± 5
Propanedioic acid, DME	65 ± 7	Hexanedioic acid, DME	67 ± 9
Butanedioic acid, DME	84 ± 5	Octanedioic acid, DME	52 ± 11

Removal of halides from aqueous samples: trace analysis of nitrate besides an excess of Cl^- or Br^-

MN Appl. No. 301930 / 302750

Column type:
CHROMAFIX® PS-Ag^+ (M)
Cat. No. 731865

Column conditioning: 1 ml dist. water

Sample application and elution: apply 4 x 1 ml sample fractions to the cartridge, discard 1st ml, collect 2nd, 3rd and 4th ml separately

Further analysis: HPLC with column 250 x 4 mm NUCLEOSIL® Anion II; eluent 2 mM potassium hydrogen phthalate pH 6; 2 ml/min; detection: indirect UV, 280 nm

Sample preparation for the determination of bromate from water according to EN ISO 15061

MN Appl. No. 303270

Column type:
CHROMAFIX® PS-Ag^+ (S) Cat. No. 731866
CHROMAFIX® PS-Ba^{2+} (S) Cat. No. 731871
CHROMAFIX® PS-H^+ (S) Cat. No. 731867

Column conditioning: ultra-pure water

Sample application: using a flow rate of 1 – 1.5 ml/min aspirate 15 ml water sample through the cation exchanger in the Ba^{2+} form to remove sulphate. Reject the first 2 ml of the eluate.

Aspirate 10 ml of the sulphate-free sample through the cation exchanger in the Ag^+ form to remove halides from the sample; reject the first 2 ml of the eluate.

Aspirate about 8 ml of the sulphate- and halogenide-free sample through the cation exchanger in the H^+ form to remove metals and carbonates, reject the first 2 ml of the eluate.

Alternatively it is possible to connect all three cation exchange cartridges in series. In this case reject the first 3 ml of the eluate from the last cartridge.

Flush the remaining eluate ((about 5 ml) with an inert gas (e.g. nitrogen or helium) to remove carbon dioxide.

Further analysis: ion chromatography

Fluoride from aqueous samples

MN Appl. No. 302550

Column type:
CHROMABOND® NO_2 / 3 ml / 500 mg
Cat. No. 730143

Sample pretreatment: ^{18}F is prepared from 5 ml 2-fluoroaniline by photon bombardment (25 MeV). The organic phase is extracted with 4 ml dist. water. The aqueous phase is washed with 1 ml *n*-hexane.

Column conditioning: 1.5 ml HCl (0.5 M), then dist. water until the eluate shows a pH value of 5 – 6

Sample application: slowly force or aspirate 3.8 ml of the aqueous sample through the column

Column washing: 20 ml dist. water

Elution: 20 ml K_2CO_3 (50 mM)

Carbonyl compounds from air

MN Appl. No. 302900

Column type:
CHROMAFIX® DNPH (M)
Cat. No. 731855

Sample application: air flow: 2 l/min

Elution: 3 x 1 ml acetonitrile with 1 ml/min

Further analysis: HPLC on NUCLEOSIL® HD, MN Appl. No. 110490 (see our application database at *www.mn-net.com*)

Acetaldehyde from mineral water stored in PET bottles after derivatisation with 2,4-dinitrophenyl hydrazine

MN Appl. No. 302950

E. Göldner et al., Institut für angewandte Chemie Gockel und Weischschedel GmbH, Stuttgart, Germany

Column type:
self-packed CHROMABOND® HR-P / 8 ml / 100 mg
glass column with PTFE frits

Sample pretreatment: derivatisation: add 3 ml citrate buffer to 100 ml of the aqueous sample, adjust the pH value to 3.0 ± 0.1 using a pH electrode (with HCl suprapur or 1 mol/l NaOH), then add 1 ml 2,4-dinitrophenyl hydrazine (DNPH) in acetonitrile and wait for 5 min

Column conditioning: 9 ml acetonitrile, then ~20 ml citrate buffer; in both steps the column must not run dry!

Sample application: aspirate 100 ml of the derivatisised sample through the column, then dry column by aspirating dry air for 10 min

Elution: apply 5 ml acetonitrile and slowly aspirate into a 5 ml volumetric flask; fill up to 5 ml with acetonitrile

Further analysis: GC/MS

Determination of furanic compounds in insulating oil by HPLC/MS

MN Appl. No. 302220

O. Kóréh et al., Rapid Communications in Mass Spectrometry **12** (1998) 1515 – 1519

Column type:
CHROMABOND® SiOH / 6 ml / 1000 mg
Cat. No. 730075

Sample pretreatment: a standard solution containing the six marker furanic compounds (5 µg/g each) is prepared in methanol – water (40:60, w/w). Fresh insulating oil is spiked with these furan derivates. 10 cm^3 of oil sample are dissolved in 10 ml HPLC-grade *n*-pentane.

Column conditioning: 2 ml *n*-pentane at atmospheric pressure

Sample application: the 20 ml sample is passed through the column under vacuum at a flow rate of 2.5 ml/min

Column washing: 20 ml *n*-pentane; dry column by suction, maintaining the vacuum for 5 min

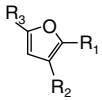
Elution: HPLC-grade methanol – distilled water (40:60, w/w), first 2 ml are collected in sample vials of 3.74 ml volume and filtered with CHROMAFIL® AO-45/25 (pore size 0.45 µm, Cat. No. 729013)

Further analysis: qualitative and quantitative determination of the extracted compounds using HPLC/UV and LC/MS

Recovery rates:

Compound	Recovery [%]	Compound	Recovery [%]
3-Furoic acid methyl ester	69 ± 3	2-Furfuryl alcohol	90 ± 4
2-Acetylfuran	62 ± 3	2-Furaldehyde	70 ± 3
5-Hydroxymethyl-2-furaldehyde	82 ± 4	5-Methyl-2-furaldehyde	60 ± 3

Furanic compounds

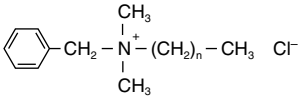
Structure	Compound	R_1	R_2	R_3
	3-Furoic acid methyl ester	H	$CO - OCH_3$	H
	2-Acetylfuran	$CO - CH_3$	H	H
	5-Hydroxymethyl-2-furaldehyde	CHO	H	CH_2OH
	2-Furfuryl alcohol	CH_2OH	H	H
	2-Furaldehyde	CHO	H	H
	5-Methyl-2-furaldehyde	CHO	H	CH_3

Benzalkonium chloride from waste water

MN Appl. No. 301940

K. Kümmerer et al., J. Chromatography **774** (1997) 281 – 286

Compounds investigated: 0.5 – 2.0 mg/l benzalkonium chloride (n = 11 – 13)



Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Column conditioning: force or aspirate 1 column volume methanol, then 1 column volume dist. water through the column

Sample application: force or aspirate 10 ml of the waste water sample through the column

Column washing: force or aspirate 1 column volume dist. water, then 1 column volume ethyl acetate through the column

Elution: 3 x 1 ml methanol – ethyl acetate (1:1, v/v) with 1% calcium chloride

Recovery rates: 93 – 95%

Benzalkonium chloride from aqueous solutions

MN Appl. No. 301960

Column type:
CHROMABOND® CN / 6 ml / 1000 mg
Cat. No. 730065

Column conditioning: 1 column volume acetonitrile, dry column 30 sec under vacuum, then 2 column volumes dist. water; now the column must not run dry

Sample application: force or aspirate 50 ml sample (containing about 0.005% benzalkonium chloride) through the column in about 10 min. For more concentrated solutions reduce sample volume.

Column washing: 1 column volume dist. water, then 2 ml 1.5 mol/l hydrochloric acid; dry column under vacuum for 30 sec

Elution: 2 x 1 ml methanol – 1.5 mol/l hydrochloric acid (4:1, v/v); leave each portion on the column for about 30 sec

Solid-phase extraction of polar hydrophilic aromatic sulfonates

MN Appl. No. 302740

R. Loos et al., J. Chromatography A, **890** (2000) 225 – 237

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108

Sample pretreatment: sulfonate standard stock solutions of 1000 mg/l are prepared by dissolving 50 mg of each compound in 50 ml ultra-pure water. The working standard solutions are prepared by further diluting the stock standard solution with water. The standard mixtures are produced from these single-compound solutions. The standard mixtures are further diluted for capillary electrophoresis and LC analysis, calibrations and preparation of fortified SPE samples, all solutions are stored at 4 °C in the dark.

Column conditioning: 7 ml methanol, then 3 ml water (acidified to pH 2.5 with sulphuric acid) at a flow rate of 1 ml/min; do not allow the adsorbent to run dry

Sample application: different volumes of spiked water samples are passed through the cartridge at a flow rate of 5 ml/min; after passing the water samples, the cartridges are not dried

Elution: compounds are eluted into glass vials, first with 1 ml water containing 5 mM TEA – acetic acid and then with 6 ml of methanol – acetone (1:1, v/v); evaporate the methanol – acetone solvent under a gentle stream of nitrogen until the 1 ml water is left. If necessary, (if some water has been evaporated) the vials are filled up with water (containing 5 mM TEA – 5 mM acetic acid) to a final volume of approximately 1 ml (the overall enrichment factor by the extraction of 150 ml water is 150).

Further analysis: LC/UV/DAD/MS

Recovery rates:
(extraction of 150 ml spiked ground water at 50 µg/l)

Compound	Recovery [%]
1-Amino-5-naphthalenesulfonate	21
Benzenesulfonate	4
1-Amino-4-naphthalenesulfonate	37
1-Hydroxy-6-amino-3-naphthalenesulfonate	25
3-Nitrobenzenesulfonate	113
1-Amino-6-naphthalenesulfonate	54
4-Methylbenzenesulfonate	63
1-Hydroxy-4-naphthalenesulfonate	85
4-Chlorobenzenesulfonate	110
2-Amino-1-naphthalenesulfonate	85
1-Amino-7-naphthalenesulfonate	67
4-Chloro-3-nitrobenzenesulfonate	94
1-Naphthalenesulfonate	92
2-Naphthalenesulfonate	85
Diphenylamine-4-sulfonate	66

Removal of oxidisable organic compounds from water

MN Appl. No. 300030

Compounds investigated: oxidisable organic compounds in water with COD > 100 mg/l

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Sample pretreatment: if necessary, filter sample with CHROMAFIL® PET-45/25 (Cat. No. 729020)

Column conditioning: 2 column volumes methanol, then 2 column volumes methanol – water (1:1, v/v)

Sample application: slowly force or aspirate the sample through the column and collect the eluate

Determination of 1,2-ethanediol in motor oil according to German standard DIN 51375-2

MN Appl. No. 302730

Column type:
CHROMABOND® OH (Diol) / 3 ml / 500 mg
Cat. No. 730053

Sample pretreatment: warm oil sample to 60 °C and shake vigorously, homogenise in an ultrasonic bath for 5 min; then weigh 2.5 g of the sample and 1.6 g of standard solution (100 mg 1,3-propanediol and 75 g isobutyl methyl ketone) into a 10 ml measuring flask; fill up to 10 ml with cyclohexane

Column conditioning: does not apply

Sample application: aspirate 2 ml of the sample solution through the column (vacuum 0.1 kPa)

Column washing: 3 x 2 ml cyclohexane, dry column with air for 5 min

Elution: 2 x 1 ml ethanol, each elution volume has to be in contact with the adsorbent for 30 s; then elute with 2 x 1 ml ethanol directly.

Further analysis: GC

o-Nitrophenylhydrazones from cyclohexane

MN Appl. No. 300050

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg
Cat. No. 730013

Column conditioning: 3 ml methanol, then 2 ml cyclohexane

Sample application: slowly force or aspirate the *o*-nitrophenylhydrazone-containing cyclohexane phase through the column

Column drying: 20 min under vacuum

Elution: 2 x 500 µl acetonitrile

Plasticisers (phthalates and adipates) from drinking water (EPA 506)

MN Appl. No. 302160

Methods for the determination of compounds in drinking water, supplement I, US EPA, office of R&D, Washington DC 20460, EPA/600/4-90/020

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg glass column
Cat. No. 730013 G

Sample pretreatment: add 5 ml methanol to 1000 ml water sample

Column conditioning: 2 x 10 ml methylene chloride, 2 x 10 ml methanol, then 10 ml ultra pure water

Sample application: aspirate or force up to 1000 ml water sample through the column

Column washing: 10 ml ultra pure water

Elution: slowly aspirate 10 ml methylene chloride through the column; concentrate the sample under a nitrogen stream to about 0.5 ml and dry over Na_2SO_4

Further analysis: GC according to EPA 606 on an OPTIMA® 1 or OPTIMA® 5 column, see MN Appl. Nos. 201210 and 201220 at *www.mn-net.com*

Plasticisers (phthalates) from water

MN Appl. No. 301970

Column type:
CHROMABOND® C18 ec / 3 ml / 500 mg glass column
Cat. No. 730013 G

Column conditioning: 2 column volumes methanol, 2 column volumes water

Sample application: force or aspirate up to 1000 ml water sample through the column

Column washing: 1 column volume water; dry column 5 min under vacuum

Elution: 1 ml ethyl acetate

Anionic surfactants from water

MN Appl. No. 301980

Compounds investigated: aryl sulfonates R–C_6H_4–SO_3H,
R = C_7H_{15} to $C_{13}H_{27}$, about 100 µg/l

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108

Sample pretreatment: adjust pH value to 6.5 and add 0.1 g tetrabutylammonium bromide to 500 ml water sample

Column conditioning: 2 x 3 ml methanol, then 5 ml dist. water and 3 ml 0.5 mM aqueous tetrabutylammonium bromide solution

Sample application: force or aspirate 500 ml of the pretreated water sample through the column

Column washing: 3 ml dist. water; then dry column under vacuum

Elution: elute analytes with 3 x 0.5 ml methanol or methylene chloride – acetone (7:3, v/v), evaporate to dryness under vacuum and redissolve in dist. water

For further analysis we recommend HPLC.

Separation of different classes of surfactants

MN Appl. No. 302620

Private communication: N. Buschbaum, A. Kruse, Westfälische Wilhelms-Universität, Münster; R. Schulz, Th. Goldschmidt AG, Organic Analytical Laboratories, Essen, Germany

Compounds investigated: anionic surfactants (an): sodium laurylsulfate; cationic surfactants (cat): cetylpyridinium chloride monohydrate; non-ionic surfactans (nio): Triton X100; amphoteric surfactants (am): Tego Betain L7 (cocamidopropyl betaine)

Step 1:

Column type:
self-packed CHROMABOND® SiOH, 500 mg (lower part) and Al_2O_3, 500 mg (upper part)

Sample pretreatment: the above compounds are chosen as model compounds for the different classes of surfactants; the separation can be performed for an amount of 2.5 mg of each compound

Sample application: slowly force or aspirate the model mixture in water through the column

Elution:
1st fraction: non-ionic surfactants: chloroform – methanol (9:1, v/v)
2nd fraction: anionic, amphotere and cationic surfactants: methanol – 2 mol/l HCl (5:1, v/v)

Step 2:

Column type:
CHROMABOND® SiOH / 6 ml / 1000 mg
Cat. No. 730075

Sample application: the second fraction of step one is slowly forced or aspirated through the SiOH column

Elution:
3rd fraction: anionic surfactants: 100% methanol
4th fraction: amphoteric surfactants: MeOH – 2 mol/l NH_3 (3:1, v/v)
5th fraction: cationic surfactants: MeOH – 2 mol/l HCl (5:1, v/v)

Epichlorhydrin from water

MN Appl. No. 303290

Column type:
CHROMABOND® HR-P / 3 ml / 200 mg
Cat. No. 730108

Sample pretreatment: mix 100 ml of the sample with the internal standard

Column conditioning: 5 ml diisopropylether, let the column run empty and apply 5 ml methanol, let the column again run empty, but not dry; finally apply 5 ml water and make sure that the surface of the water is above the column packing

Sample application: aspirate the whole sample through the column with a constant flow rate of 1 – 3 ml/min; do not apply more than 100 ml sample; after extraction remove the main portion of water by applying a stream of nitrogen (1 – 2 ml/min) for 5 min; ensure that 250 – 350 mg water remain in the adsorbent!

Elution: 2 ml diisopropyl ether

Further analysis: GC

Thiocyanate from water

MN Appl. No. 301990

Column type:
CHROMABOND® SB (≡ SAX) / 3 ml / 500 mg
Cat. No. 730079

Sample pretreatment: adjust pH value to 7

Column conditioning: 1 column volume dist. water

Sample application: force or aspirate 20 ml sample through the column in about 4 – 5 min

Column washing: 2 ml dist. water

Elution: 2 x 1 ml 6 mol/l hydrochloric acid

Antibiotics from surface water

MN Appl. No. 303260

Column type:
CHROMABOND® *Easy* / 3 ml / 200 mg
Cat. No. 730754

Sample pretreatment: filter a mixed surface water sample through 0.45 µm glass fibre filters and fill into 250 ml flasks; spike with 0.2 ml of the standard solution (concentration range 7 – 15 µg), adjust to pH 3.4 with 900 µl glacial acetic acid

Column conditioning: 4 ml methanol, 6 ml demineralised water

Sample application: aspirate the sample through the column with about 5 ml/min

Column washing: rinse the flask with 3 ml dist. water and apply the water on the column; then dry column in a stream of nitrogen

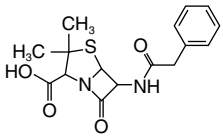
Elution: 6 ml methanol; the eluate is evaporated and the residue dissolved in 1 ml methanol.

Recovery rates:

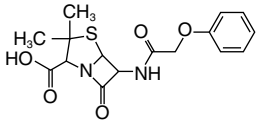
Compound	Recovery [%]		
	A	B	C
Chlorotetracycline	52	48	44
Doxycycline	43	41	45
Oxytetracycline	60	60	69
Sulfadiazine	58	61	52
Sulfadimidine	68	70	61
Penicillin G	62	64	54
Penicillin V	60	68	54
Ciprofloxacin	17	13	23

A = conditioned column, B and C = unconditioned column

Penicillin G



Penicillin V



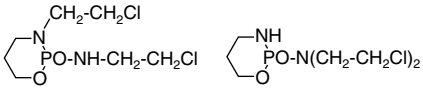
for other structures see index from page 239

Antineoplastic agents from sewage water

MN Appl. No. 300920

T. Steger-Hartmann, K. Kümmerer, J. Schecker; J. Chromatography **726** (1996) 179 – 184

Compounds investigated: ifosfamide, cyclophosphamide



Step 1:

Column type:
CHROMABOND® C18 / 6 ml / 500 mg
Cat. No. 730004

Sample pretreatment: 500 ml effluent from a laboratory-scale sewage treatment plant were filtered through filter paper and then through glass fibre filtres

Column conditioning: 3 ml *n*-hexane, 3 ml methanol, then 10 ml deionised water with 3 ml/min each

Sample application: force or aspirate the filtered sample through the column with 3 ml/min

Column washing: 10 ml deionised water with 3 ml/min; then evacuate the column, dry it under a stream of nitrogen and finally wash with 500 µl *n*-hexane

Elution: 2 ml methanol – acetone (95:5, v/v); evaporate the eluate in a stream of nitrogen and redissolve the residue in 200 µl ethyl acetate

Step 2:

Column type:
CHROMABOND® SiOH / 1 ml / 100 mg
Cat. No. 730071

Column conditioning: 1 ml *n*-hexane

Sample application: slowly force or aspirate the final solution from step 1 through the column

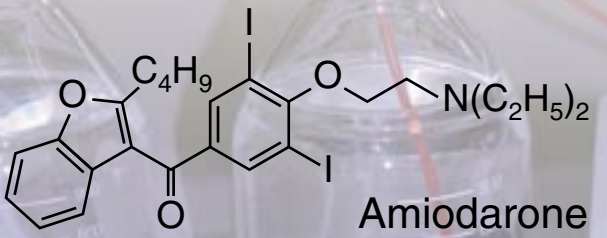
Column washing: ethyl acetate

Elution: 1 ml acetone – *n*-hexane (9:1, v/v), then 1 ml acetone

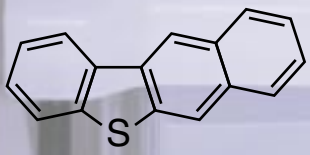
Dry eluate in a stream of nitrogen, wash the residue twice with hexane and redissolve the remaining residue, which is insoluble in hexane, in di-isopropyl ether. Evaporate the ether fraction to dryness and redissolve in 100 µl ethyl acetate. These last purification steps allow further discrimination of interfering sewage water components.

Further analysis: part of the final ethyl acetate fraction is derivatised with trifluoroacetic anhydride at 80 °C for 20 min. Both the derivatised and underivatised samples are further analysed on an OPTIMA® 5 column with 0.25 µm film, 25 m x 0.25 mm ID (Cat. No. 726056.25).

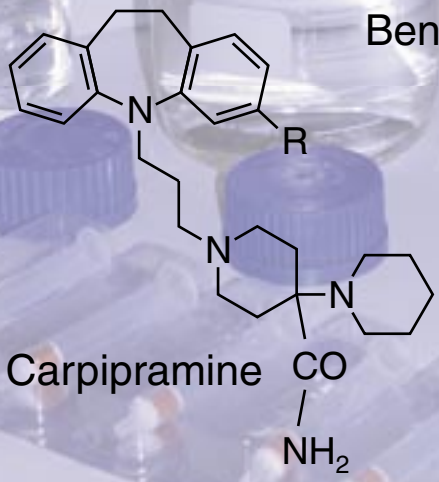
Substance index
Structure index



Amiodarone



Benzonaphthothiophene



Carpipramine

Substance index

Substance index

Substance	Sample matrix	SPE phase	Page
Basic drugs	serum	C18	104
	urine	Drug	103
Bentazone	beech stem-flow water	C18	161
	soil	C18 Hydra	176
	water	Easy	175, 176
Benz[a]acridine	soil	SB/SiOH + SA	148
Benz[c]acridine	soil	SB/SiOH + SA	148
Benzaldehyde	earth-almond distillate	HR-P	119
Benzalkonium chloride	plasma	C18 ec	48
	water	C18 ec	199
		CN	199
Benzanilide	water	Easy	166
		HR-P	163
Benz[a]anthracene	soil	CN/SiOH	145
	soil, sludge	SA	146
	water	C18 PAH	142
		NH_2/C18	141
7,12-Benz[a]anthracenedione	soil	SB/SiOH + SA	148
Benz[de]anthracen-7-one	soil	SB/SiOH + SA	148
Benzenesulfonate	water	HR-P	200
Benzidine	textile materials	XTR	188
	water	HR-P	187, 189
Benzocaine	serum	C18 ec	72
Benzodiazepines	hair	C18 ec	92
	serum	C18 ec	93
	urine	C18 ec	93
Benzo[b]fluoranthene	*n*-hexane	HR-P	142
	oil	HR-P	143
	soil	CN/SiOH	145
	soil, sludge	SA	146
	water	C18 PAH	142
		Easy	143
		NH_2/C18	141
		PA	141
Benzo[k]fluoranthene	*n*-hexane	HR-P	142
	oil	HR-P	143
	soil	CN/SiOH	145
	soil, sludge	SA	146
	water	C18 PAH	142
		Easy	143
		NH_2/C18	141
		PA	141
Benzofuran	soil	SB/SiOH + SA	148
Benzoic acid methyl ester	incineration residues	HR-P	196
Benzo[b]naphtho[2,3-d]furan	soil	SB/SiOH + SA	148
Benzo[b]naphtho[2,3-d]-thiophene	soil	SB/SiOH + SA	148

Substance index

Substance index

Substance	Sample matrix	SPE phase	Page
Chrysene	soil	CN/SiOH	145
	soil, sludge	SA	146
	water	C18 PAH	142
		NH_2/C18	141
Cimetidine	plasma	C18 ec	83
Cinoxacin	recovery	Easy	90
		Easy, HR-P, C18 ec	89
Ciprofloxacin	blood, surface water	Tetracycline	89
	crude extracts	C18 ec	88
	recovery	Easy	90
		Easy, HR-P, C18 ec	89
	surface water	Easy	204
Clocapramine	urine, plasma, blood	C18 ec	91
Clofibric acid	water	Easy	175
Clopyralid	water	Easy	175
Cobalt	water	C18 ec	194
		Phenyl	193
Cocaine	hair	C18	97, 100
		C18 ec	99
	urine, blood	C18 ec	101
Cocamidopropyl betaine	water	SiOH + Al_2O_3	203
Codeine	blood	C18 ec	97
	blood, serum	Drug	95
	hair	C18 ec	99
	liquid-liquid extraction	XTR	104
	plasma, blood	C18 ec	96
	serum	Drug	101, 102
	serum, urine, hair	Drug	102
Codeine + glucuronide	urine	C18 ec	98
Copper	water	C18 ec	194
		NH_2	194
		Phenyl	193
Cotinine	plasma	C18 ec	43
Coumaphos	honey	C18 ec + Florisil®	130
Coumarin	brandy	Phenyl	118
	soil	SB/SiOH + SA	148
p-Cresidine	textile materials	XTR	188
Cresol isomers	water	HR-P	192
Crimidine	water	HR-P	163
Crosslinks, pyridinium	urine	Crosslinks	50
Cyanazine	recovery	Easy	160
	water	Easy	164, 166
		HR-P	163, 165, 167, 168
9-Cyanoanthracene	soil	SB/SiOH + SA	148
1-Cyanonaphthalene	soil	SB/SiOH + SA	148
Cyanopeptolins	cyanobacteria	C18	50
9-Cyanophenanthrene	soil	SB/SiOH + SA	148

Substance index

Substance	Sample matrix	SPE phase	Page
Desethylsimazin	water	Easy	166
		HR-P	163, 167
Desethylterbuthylazine	recovery	Easy	160
	water	Easy	164, 166
		HR-P	155, 163, 167, 168, 172
Desipramine	urine, plasma, blood	C18 ec	91
Desisopropylatrazine	beech stem-flow water	C18	161
	recovery	C18 ec, C18, C_6H_{11} ec, C18 Hydra, HR-P	155
		Easy	160
		SA, PSA, PCA, PS-H^+	156
	water	C18 ec, C18, C_6H_{11} ec, C18 Hydra, HR-P	177
		C18 Hydra	182
		Easy	164
		HR-P	155, 163, 165, 168, 172
Desmetryn	water	Easy	166
		HR-P	155, 163, 167, 168
Despropyldisopyramide	serum	C18 ec	77
2,4-Diaminoanisole	textile materials	XTR	188
4,4'-Diamino-3,3'-dimethyl-phenylmethane	textile materials	XTR	188
4,4'-Diaminodiphenylmethane	textile materials	XTR	188
2,4-Diamino-6-nitrotoluene	water	Easy	186
		HR-P	185
Diaminonitrotoluene isomers	water	HR-P	187, 189
Diaminopyridine	urine	C18 ec	51
Diaminotoluene isomers	water	HR-P	187, 189
Diaromatic hydrocarbons	diesel fuel	SiOH	183
Diazepam	hair	C18 ec	92
	serum	C18 ec	93
	urine	C18 ec	73
Diazinon	water	HR-P	171, 172
Dibenz[a,c]acridine	soil	SB/SiOH + SA	148
Dibenz[ah]anthracene	soil	CN/SiOH	145
	soil, sludge	SA	146
	water	C18 PAH	142
		NH_2/C18	141
Dibenzazepines	serum	CN	91
Dibenzo[a,i]carbazole	soil	SB/SiOH + SA	148
Dibenzofuran	soil	SB/SiOH + SA	148
Dibenzothiophene	soil	SB/SiOH + SA	148
Dibenzothiophenesulfone	soil	SB/SiOH + SA	148
Dibromoacetic acid	water	HR-P	195
4,4-Dibromobenzophenone	honey	C18 ec + Florisil®	130

Substance index

Substance index

Substance index

Substance index

Substance index

Substance	Sample matrix	SPE phase	Page
6-Monoacetylmorphine	blood	C18 ec.	97
	blood, serum	Drug	95
	hair	C18 ec.	99
	plasma, blood	C18 ec.	96
Monoaromatic hydrocarbons	diesel fuel	SiOH	183
Monobromoacetic acid	water	HR-P	195
Monochloroacetic acid	water	HR-P	195
Monochloronitrobenzene	water	C18 ec.	186
Monoglycerides	chloroform extracts	NH_2	56
Monolinuron	water	Easy	164, 166
		HR-P	163, 165, 167
Monuron	water	Easy	164
Morphine	blood	C18 ec.	97
	blood, serum	Drug	95
	hair	C18 ec.	99
	plasma	Easy	96
	plasma, blood	C18 ec.	96
	serum	Drug	101
	serum, urine, hair	Drug	102
Morphine + glucuronides	urine	C18 ec.	98
Moxifloxacin	blood, surface water	Tetracycline	89
Mycotoxins	apple juice	C18 + NH_2	129
	cereal, nuts, peanut butter	SiOH	126
	cereals, food	SiOH	128
	corn flour	C18 ec.	126
	liver	C18 ec.	127
	maize	Phenyl	127
	milk	C18 ec.	127
	porcine serum	SiOH	128

N

Nadolol	serum	C18 ec.	78
Nalidixic acid	crude extracts	C18 ec.	88
Naloxone	plasma	CN	100
Naphthalene	soil	CN/SiOH	145
	soil, sludge	SA	146
	water	C18 PAH	142
		NH_2/C18	141
Naphthalenesulfonate isomers	water	HR-P	200
Naphthoic acid isomers	soil	SB/SiOH + SA	148
2-Naphthol	soil	SB/SiOH + SA	148
Naphthol isomers	water	HR-P	184
Naphthylamine isomers	water	HR-P	187, 189
2-Naphtylamine	textile materials	XTR	188
Narcotic antagonist naloxone	plasma	CN	100
Neutral lipids	chloroform extracts	NH_2	56
Niacinamide	aqueous solutions	C18 ec.	107

Substance index

Substance index

Substance index

Substance index

Substance	Sample matrix	SPE phase	Page
Simetryn	water	HR-P	155, 163
Sodium benzoate	orange juice	C18 ec + OH (Diol)	122
Sodium laurylsulfate	water	SiOH + Al_2O_3	203
α-Solanine	potatoes, potato products	C18 ec.	111
Spirogermanium	serum	C18 ec.	81
Steroids	ointment	SiOH	105
	plasma	C18 ec.	105
	urine, serum, plasma	C18 ec.	105
Steryl esters	reaction mixtures	C18 ec.	122
Stevioside	leaves	C18	63
Stobadin	serum	C18	104
Streptomycin	milk	C18 ec.	113
Sulfachlorpyridazine	meat, kidney	SA	114
Sulfadiazine	meat, kidney	SA	114
	surface water	Easy	204
Sulfadimethoxine	meat, kidney	SA	114
Sulfadimidine	meat, kidney	SA	114
	surface water	Easy	204
Sulfadoxine	meat, kidney	SA	114
Sulfaguanidine	meat, kidney	SA	114
Sulfamate	bacterial culture	HR-P	49
Sulfamerazine	meat, kidney	SA	114
Sulfamethizole	meat, kidney	SA	114
Sulfamethoxypyridazine	meat, kidney	SA	114
Sulfamide	bacterial culture	HR-P	49
Sulfanilamide	meat, kidney	SA	114
Sulfapyridine	meat, kidney	SA	114
Sulfathiazole	honey	C18 ec.	112
	meat, kidney	SA	114
Sulfonamides	meat, kidney	SA	114
Sulfonates, aromatic	water	HR-P	200, 202
Sulphate removal	water	PS-Ba^{2+}	197
Surfactants	fractionation	SiOH + Al_2O_3	203
	water	HR-P	202
Sympathomimetics	biological samples	C18 ec.	79
	calves urine	C18 ec.	79

T

2,4,5-T	soil	C18 Hydra.	176
	water	C18 ec.	174
		Easy	175
Tebuconazol	water	Easy	166
Tebutam	water	HR-P	163
Tego Betain L7	water	SiOH + Al_2O_3	203
Temozolomide	plasma, urine	C18 ec.	83
Terbacil	recovery	Easy	160
Terbumeton	water	HR-P	163

Substance index

Structure index

Liability

MACHEREY-NAGEL products are intended for general laboratory use only. They are not suited for clinical use (diagnostic, therapeutic or prognostic). MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products or due to application of our products in other fields of application. The user has to ensure that the products used are suitable for the planned applications.

Application on the human body is strictly forbidden. The user is liable of all damages resulting from such application.

MACHEREY-NAGEL products are suited for qualified personnel only.

Imprint

Editor in chief	Dr. Thomas Schüßeler
Scientific editors	Dr. Markus John, Dr. Martin Rödel
Layout	Dr. Ehrengard Renk
Photography	Dr. Detlev Lennartz, Dr. Ehrengard Renk, Christoph Textoris
Cover design	Georg Gülden

Customised SPE column request form

Inquiry to:

MACHEREY-NAGEL GmbH & Co. KG
Neumann-Neander-Str. 6 – 8 · D-52355 Düren, Germany
Tel. +49 (0)2421 969-0 · Fax +49 (0) 2421 969-199

Also see the electronic form at *www.mn-net.com.*

Name, First Name, Title . . . . . .
Company / Institute . . . . . .
Department . . . . . .
Street / P. O. Box . . . . . .
Postal Code / City / Country . . . . . .
Phone / Fax . . . . . .

CHROMABOND® special columns acc. to customers specifications

In order to handle the increasing requests for custom-made CHROMABOND® columns, MACHEREY-NAGEL offers this special service.

You can ask for an offer concerning SPE columns prepared according to your special needs (volume, sorbent weight) by simply copying this page, completing it and mailing or faxing it to us.

Type of SPE column (check appropriate box for material and volume)

available volume [ml]:	1	3	6	15	30	45	70	150
PP column	☐	☐	☐	☐	☐	☐	☐	☐
glass column	☐	☐	☐					
max. adsorbent weight [g SiOH]	0.5	1.4	3.3	5.9	11	17	30	80

Column bed
(upper bed for combinations): adsorbent: . . . . . weight: . . . . . mg

2nd (lower) column bed
(if desired): adsorbent: . . . . . weight: . . . . . mg

Frit material: ☐ glass fibre ☐ polyethylene

Required quantity of columns: . . . . . .

Remarks
(e. g. lot reservation, special requirements concerning packing or column, term of delivery required)

Interested in more products for analytical chemistry?

MACHEREY-NAGEL offers more than 25,000 products in 5 product lines

Chromatography

- HPLC columns and accessories
- GC columns and derivatisation reagents
- products for solid phase extraction (SPE) and flash chromatography
- adsorbents and plates for thin layer chromatography (TLC)

Bioanalysis

- kits for purification of nucleic acids
- kits for purification of proteins
- transfer membranes

Our bioanalysis products are powerful tools for purification of DNA/RNA from various samples and for protein purification.

Rapid tests

- indicator and test papers
- test kits for water analysis
- reagents and instruments for photometric water analysis
- rapid tests for microbiological diagnostics and hygiene assays

For many years analytical chemists, as well as chemical laymen, appreciate our special products for environmental, process and food analysis.

Filtration

- filter papers
- filter membranes
- extraction thimbles

Since 1911, MACHEREY-NAGEL has been known for high quality filter papers. Today we also supply customers worldwide with a variety of special products for specific applications.

Medical diagnostics

- test strips for urine analysis
- reflectometers

Urine test strips are indispensable tools to obtain a quick monitoring of a patient's health status.

MN on the internet:

www.mn-net.com

The MACHEREY-NAGEL internet catalogue with integrated internet shop is full of useful information about our wide product range. After your free registration you can order our products just with a click! In addition, our website offers more than 2400 applications which might actually already solve your analytical questions.

Our Chromatography Pages provide

- an integrated internet shop
- product information
- job opportunities
- introductions to HPLC, GC and bio separation
- material saftey data sheets
- troubleshooting
- **free application database with more than 2400 chromatography applications**

Also available in this Application series:

Reversed Phase HPLC Application Guide