Chromatography

MACHEREY-NAGEL

Modern polymeric SPE phases

CHROMABOND® HLB and HR-*Xperts*

- Well-defined portfolio of polymeric SPE phases
- Broad application range
- High performance adsorbents

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Modern polymeric CHROMABOND® SPE phases

Do you want to squeeze the best out of your samples?

CHROMABOND® HLB	Hydrophilic-lipophilic balance NVP / DVB copolymer	page 04 – 07
CHROMABOND® HR-X	Hydrophobic PS / DVB copolymer	page 08 – 09
CHROMABOND® HR-XC	Strong mixed-mode cation exchanger on PS / DVB copolymer basis	page 10 – 11
CHROMABOND® HR-XA	Strong mixed-mode anion exchanger on PS / DVB copolymer basis	page 12 – 13
CHROMABOND® HR-XCW	Weak mixed-mode cation exchanger on PS / DVB copolymer basis	page 14 – 15
CHROMABOND® HR-XAW	Weak mixed-mode anion exchanger on PS / DVB copolymer basis	page 16 – 17

Characteristics

- State-of-the-art spherical polymers with different particle sizes to suit sample volume and matrix
- Optimized pore structure and high specific surface
- High purity adsorber material
- Extremely low blind values
- High specific surface
- pH stability of 1–14

Good to know

Advantages of polymeric based adsorbents compared to silica based:

- Higher capacity of up to 30 wt % (silica gel 3–5 wt %)
- pH stability of 1–14 (silica gel ~ 2–8)
- Optimized flow rates

Benefits for you

Save time and reduce costs

- Well-defined portfolio of polymer phases to suit your application
- Excellent enrichment of neutral, acidic and basic compounds
- Outstanding price / performance ratio

Robust methodology and less pain during method development

- Good reproducibility
- Cleaner samples and protection of your HPLC and GC instruments
- High loadability and outstanding performance
- Ideal flow properties
- Consistent recoveries

No risk

- Test samples available on request.

Modern polymeric CHROMABOND® SPE phases

Selection guide

The continuous strive to improve SPE methods led to the development of our portfolio of CHROMABOND® polymer phases.

Stationary phase selection

Matrix	Aqueous (water, serum, blood, plasma, aqueous extracts)					
SPE mode	Reversed phase		Ion exchange			
Analyte characteristic	Non polar to moderate polar		Strong bases pKa >10	Acids pKa 2–8	Strong acids pKa < 1	Bases pKa 2–10
Adsorbent	HR-X	HLB	HR-XCW	HR-XA	HR-XAW	HR-XC

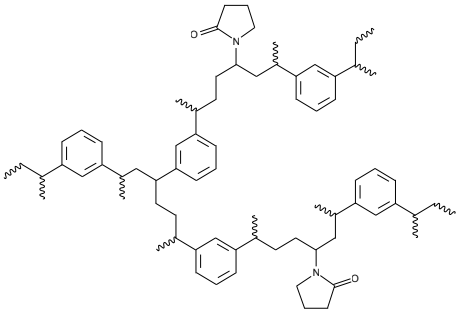
Modern polymeric CHROMABOND® SPE phases

CHROMABOND® HLB

Technical data

Hydrophilic-lipophilic balanced *N*-vinylpyrrolidone-divinylbenzene copolymer (NVP / DVB)

SPE mode:	Reversed phase
Interactions:	Hydrophobic and polar
Particle shape:	Spherical
pH stability:	1–14
Particle size:	60 µm and 30 µm
Pore size:	65 Å
Specific surface:	750 m^2/g



Special characteristics

- Applicable for a wide range of analyte polarities
- High loadability and outstanding performance
- Water wettable – even if bed runs dry, SPE can be continued

Recommended application

- Medium polar organic molecules from polar matrices
- Drugs and pharmaceuticals from urine, blood, serum and plasma
- Tetracyclines and alkaloids from serum
- Pesticides from water

Good to know

A possible replacement for:

- Oasis® HLB
- Strata™-X
- Supel™-Select HLB
- Supra-Poly® HLB
- Isolute® ENV+

Standard SPE procedure for CHROMABOND® HLB (subsequent HPLC analysis)

MN Appl. No. 306300

Column type:
CHROMABOND® HLB / 3 mL / 200 mg, REF 730924

Sample pretreatment:

Individual sample preparation in reference to the compounds and matrix. (Adjust pH value if necessary)

Conditioning:	5 mL methanol, then 5 mL dist. water
Sample application:	Slowly aspirate sample through column
Washing:	5 mL dist. water
Drying:	10 min with applied vacuum
Elution:	8 mL methanol
Evaporation:	Under nitrogen
Reconstitution:	In 1 mL dist. water + 0.1 % formic acid

Standard SPE procedure for CHROMABOND® HLB (subsequent GC analysis)

MN Appl. No. 306310

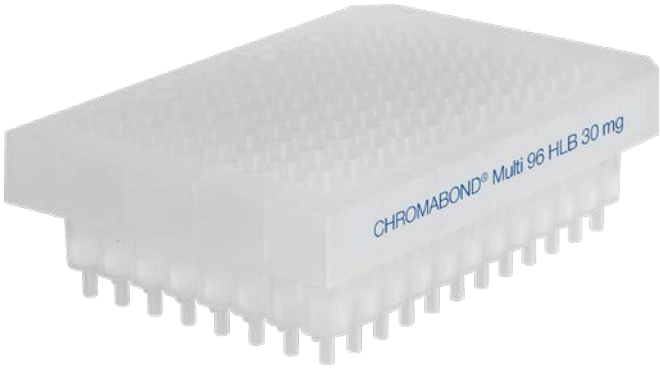
Column type:
CHROMABOND® HLB / 3 mL / 200 mg, REF 730924

Sample pretreatment:

Individual sample preparation in reference to the compounds and matrix. (Adjust pH value if necessary)

Conditioning:	5 mL solvent (e.g., ethyl acetate), 5 mL methanol, 5 mL dist. water
Sample application:	Slowly aspirate sample through column
Washing:	5 mL dist. water
Drying:	10 min with applied vacuum
Elution:	Solvent[1] (typical solvents: ethyl acetate, MTBE, methylene chloride)
Evaporation:	Under nitrogen, dry with sodium sulfate[2], adjust to final volume

[1] usually nonpolar, therefore often 10 % methanol are added
[2] e.g., with CHROMAFIX® Dry



Modern polymeric CHROMABOND® SPE phases

Applications

Tetracyclines and alkaloids from serum at pH 5

MN Appl. No. 306380

Chromatographic conditions

Columns: CHROMABOND® HLB / 1 mL / 30 mg
Oasis® HLB / 1 mL / 30 mg

MN REF: 730921

Conditioning: 1 mL methanol, then 1 mL dist. water

Application: 1 mL serum pH 5, adjusted with formic acid (spiked with 20 µg/mL of each analyte)

Washing: 1 mL dist. water

Drying: 10 min with applied vacuum

Elution: 2 mL methanol

Evaporation: Under nitrogen, 40 °C

Reconstitution: In 1 mL dist. water + 0.1 % formic acid

Recovery rates ± RSD [%], n = 4

Compound	CHROMABOND® HLB	Oasis® HLB
Berberine	85.4 ± 0.3	82.5 ± 0.6
Chlortetracycline	72.1 ± 1.4	66.3 ± 2.8
Hydrastine	88.9 ± 2.6	99.3 ± 5.7
Oxytetracycline	82.3 ± 1.4	78.7 ± 1.4
Tetracycline	78.1 ± 1.4	70.7 ± 2.6

Further analysis: HPLC, according to MN Appl. No. 128180

Column: EC 50/2 NUCLEOSHELL® RP 18plus, 2.7 µm

MN REF: 763232.20

Eluent: A: dist. water + 0.1 % formic acid
B: acetonitrile + 0.1 % formic acid
Gradient: 2–60 % B in 4 min, 60 % B for 1 min, 60–2 % B in 0.5 min, 2 % B for 3 min

Flowrate: 0.75 mL/min

Temperature: 22 °C

Detection: UV, 330 nm

Injection: 5 µL

Mycotoxins in wheat flour

MN Appl. No. 306740

Chromatographic conditions

Columns: CHROMABOND® HLB / 60 µm / 3 mL / 200 mg

MN REF: 730924

Extraction:

- Weigh 4 g homogenized sample in an empty 50 mL centrifuge tube
- Add 8 µL mycotoxin standard mixture (β = 10 µg/mL each analyte in acetonitrile)
- Add 10 mL of water / acetonitrile mixture (20:80, v/v), shake vigorously and wait 10 min
- Add CHROMABOND® QuEChERS extraction Mix XII (REF 730648), shake vigorously for 1 min and cool the mixture down in an ice bath
- Centrifuge at 4500 rpm for 20 min at 20 °C
- Take organic phase for clean-up procedure

Conditioning: 6 mL acetonitrile

Application: 1 mL sample extract was aspirated with low vacuum into a vial

Elution: 4 mL acetonitrile were aspirated with low vacuum into a vial

Evaporation: Combine cleaned sample extract and acetonitrile eluate and evaporate to dryness under nitrogen, 60 °C

Reconstitution: In 1 mL acetonitrile

Analyte	Recovery rate [%]	RSD [%], n = 5
Aflatoxin B1	88	2.6
Aflatoxin B2	91	5.0
Aflatoxin G1	85	2.6
Aflatoxin G2	88	4.5
HT-2 toxin	115	5.7
T-2 toxin	106	5.1
Zearalenone	49	3.4

Modern polymeric CHROMABOND® SPE phases

Applications

Sulfa drugs from serum

MN Appl. No. 306340

Columns*:	CHROMABOND® HLB / 60 µm / 1 mL / 30 mg Oasis® HLB / 60 µm / 1 mL / 30 mg
MN REF:	730921
Conditioning:	1 mL methanol, 1 mL dist. water
Application:	1 mL serum (spiked with 10 µg/mL of each analyte)
Washing:	1 mL dist. water
Drying:	10 min with applied vacuum
Elution:	2 mL methanol
Evaporation:	Under nitrogen, 40 °C
Reconstitution:	In 1 mL dist. water + 0.1 % formic acid

Equivalence to Oasis® HLB

CHROMABOND® HLB shows equivalent recovery rates to Oasis® HLB for the three tested sulfa drugs.

Further analysis: HPLC, according to MN Appl. No. 128130

Column:	EC 150/2 NUCLEODUR® C18 Pyramid, 3 µm
MN REF:	760261.20
Eluent:	Dist. water + 0.1 % formic acid / methanol + 0.1 % formic acid (85:15, v/v), 5 min
Flow rate:	0.6 mL/min
Temperature:	25 °C
Detection	UV, 254 nm
Injection:	5 µL

Recovery rates ± RSD [%], n = 5

Compound	CHROMABOND® HLB	Oasis® HLB
Sulfadiazine	97.3 ± 2.9	92.0 ± 3.8
Sulfamerazine	94.4 ± 1.8	92.8 ± 1.6
Sulfathiazole	90.3 ± 2.9	89.6 ± 1.5

Chloramphenicol from honey

MN Appl. No. 306350

Columns*:	CHROMABOND® HLB / 60 µm / 3 mL, 200 mg Oasis® HLB, 3 mL, 200 mg
MN REF:	730924

Sample pretreatment:

Weigh out 5 g of honey. Add 4 mL water and shake rigorously for 30 sec. Spike with 1 mL standard solution (c = 5 ng/mL in methanol) and shake rigorously for 30 sec. Add 15 mL ethyl acetate and shake rigorously for 30 sec. Centrifuge at 3000 rpm for 10 min. Take 12 mL of supernantant for eluent exchange. Evaporate extracts to dryness at 40 °C under a stream of nitrogen. Redissolve residue in 10 mL water.

Conditioning:	3 mL methanol (dispensing speed 1 mL/min), 5 mL dist. water (disp. speed 1 mL/min)
Application:	9 mL water sample (disp. speed 3 mL/min over sample loop)
Washing:	10 mL dist. water (disp. speed 3 mL/min)
Drying:	100 mL air (disp. speed 100 mL/min)
Elution:	5 mL ethyl acetate /!methanol (80:20, v/v)
Drying:	100 mL air (disp. speed 100 mL/min)
Evaporation:	under nitrogen, 40 °C
Reconstitution:	in 1 mL dist. water / acetonitrile (95:5, v/v)

The SPE application was performed with a FREESTYLE® SPE automation system.

Further analysis: LC-MS/MS, according to MN Appl. No. 128140

Column:	EC 150/2 NUCLEODUR® π^2, 5 µm
MN REF:	760624.20
Eluent:	A: dist. water B: acetonitrile 5–95 % B in 7.5 min, 95 % B for 1 min, 95–5 % B in 1 min, 5 % B for 5 min
Flow rate:	0.3 mL/min
Temperature:	35 °C
Detection:	MS, Selected Reaction Monitoring (SRM)
Injection:	5 µL

Recovery rates ± RSD [%], n = 5

Compound	CHROMABOND® HLB	Oasis® HLB
Chloramphenicol-d5	90.9 ± 5.4	90.0 ± 9.3

Good to know

Antibiotics and pesticides contamination of agricultural products such as honey has been an issue in the recent years and resulted in stricter guidelines in food safety control.

*Same conditions for all used columns. Due to a better comparability CHROMABOND® HLB and Oasis® HLB adsorbents (60 µm) were packed into equal column hardware. The shown chromatograms may not be representative of other applications.

Modern polymeric CHROMABOND® SPE phases

Applications

Pesticides from tap water

MN Appl. No. 306360

Columns*:	CHROMABOND® HLB / 60 µm / 3 mL / 200 mg Oasis® HLB / 60 µm / 3 mL / 200 mg
MN REF:	730924
Conditioning:	5 mL methanol, 5 mL dist. water
Application:	1000 mL tap water (spiked with 50 ng of each analyte)
Washing:	10 mL dist. water
Drying:	5 min with applied vacuum (-15 psi)
Elution:	6 mL acetonitrile
Evaporation:	Under nitrogen, 40 °C
Reconstitution:	In 1 mL dist. water / acetonitrile (95:5, v/v)

Further analysis: LC-MS/MS, according to MN Appl. No. 128150

Column:	EC 50/2 NUCLEOSHELL® PFP, 2.7 µm
MN REF:	763532.20
Eluent:	A: dist. water + 0.1 % formic acid B: acetonitrile + 0.1 % formic acid 5–95 % B in 15 min, 95 % B for 5 min, 95–5 % B in 1 min, 5 % B for 9 min
Flow rate:	0.3 mL/min
Temperature:	40 °C
Detection:	MS, Selected Reaction Monitoring (SRM)
Injection:	5 µL

Recovery rates ± RSD [%], n = 5

Compound	CHROMABOND® HLB	Oasis® HLB
Acetamiprid	73.3 ± 5.0	112.1 ± 9.9
Atrazine	110.3 ± 17.8	114.0 ± 11.6
Azoxystrobin	74.7 ± 5.4	98.1 ± 10.8
Carbaryl	65.7 ± 5.4	69.1 ± 7.1
Chlorotoluron	82.7 ± 5.7	101.2 ± 3.8
Chlorpyrifos	50.3 ± 5.4	47.0 ± 3.7
Clofentezine	27.8 ± 2.7	21.4 ± 3.7
Clothianidin	69.4 ± 6.5	52.9 ± 2.9
Coumaphos	69.8 ± 4.8	82.3 ± 5.2
Cyanazine	99.8 ± 9.3	85.1 ± 7.2
Desethylatrazine	94.8 ± 15.1	87.4 ± 11.4
Desisopropylatrazine	92.5 ± 7.6	N/A
Diazinon	71.5 ± 7.9	73.3 ± 4.7
Difenoconazole	83.9 ± 6.5	28.8 ± 5.0
Diuron	70.0 ± 4.8	80.1 ± 8.4
Ethoprophos	72.4 ± 9.3	85.4 ± 7.2
Hexazinone	88.4 ± 7.7	104.3 ± 7.4
Imazalil	27.3 ± 15.7	N/A
Imidacloprid	93.4 ± 5.1	40.3 ± 5.2
Isoproturon	100.2 ± 4.2	102.8 ± 13.0
Linuron	84.5 ± 7.6	88.3 ± 9.5
Methabenzthiazuron	72.5 ± 5.3	48.0 ± 3.7
Methomyl	78.8 ± 5.4	83.6 ± 5.6
Metobromuron	73.8 ± 5.6	85.6 ± 9.3
Metolachlor	79.0 ± 5.2	89.2 ± 5.0
Monolinuron	75.4 ± 6.2	97.9 ± 7.2
Myclobutanil	101.8 ± 11.4	88.7 ± 14.5
Phosalone	63.8 ± 7.7	74.0 ± 4.0
Piperonylbutoxide	101.4 ± 8.6	99.7 ± 7.9
Propazine	102.1 ± 13.6	90.9 ± 9.4
Propyzamide	84.8 ± 7.1	86.4 ± 10.6
Terbuthylazine	107.9 ± 13.3	100.0 ± 13.6
Thiacloprid	74.1 ± 6.3	86.5 ± 10.8

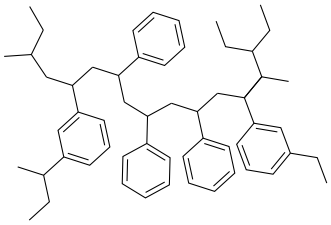
Modern polymeric CHROMABOND® SPE phases

CHROMABOND® HR-X

Technical data

Hydrophobic polystyrene-divinylbenzene copolymer (PS / DVB)

SPE mode:	Reversed phase
Interactions:	Hydrophobic and π–π
Particle shape:	Spherical
pH stability:	1–14
Particle size:	85 µm and 45 µm
Pore size:	55–60 Å
Specific surface:	1000 m^2/g
RP capacity:	390 mg/g (caffeine in water)



Recommended application

- Pharmaceuticals / active ingredients from tablets, creams and water
- Drugs and pharmaceuticals from urine, blood, serum and plasma
- Trace analysis of pesticides, herbicides, phenols, PAH and PCBs from water

Good to know

A possible replacement for:

- Nexus
- ENVI-Chrom P
- Bakerbond H_2O-phobic DVB
- Strata™-X

Standard protocol for CHROMABOND® HR-X

MN Appl. No. 304310

Column type:
CHROMABOND® HR-X / 3 mL / 200 mg, REF 730931

Sample pretreatment:

Individual sample preparation in reference to the compounds and matrix (adjust pH value if necessary).

Conditioning:	5 mL methanol, then 5 mL water (do not let run the column dry!)
Sample aspiration:	The prepared sample is passed through the column by vacuum or pressure (max. 1000 mL sample volume)
Washing:	5 mL water / methanol (95:5, v/v)
Drying:	With nitrogen or air
Elution:	3 x 2 mL methanol

Further analysis:

Evaporation and reconstitution (if necessary); HPLC or GC

These conditions are a starting point for SPE method development. Further optimization may be required to improve results.

Modern polymeric CHROMABOND® SPE phases

Applications

Determination of pyrrolizidine alkaloids

MN Appl. No. 306620

Chromatographic conditions

Columns: CHROMABOND® HR-X / 85 µm / 3 mL / 200 mg

MN REF: 730921

Pretreatment: The following analysis were performed with standard solutions

Conditioning: 5 mL methanol, 5 mL water

Application: 10 mL neutralized standard solution with a flow rate of 3 mL/min

Washing: 2 x 5 mL of water with a flow rate of 3 mL/min

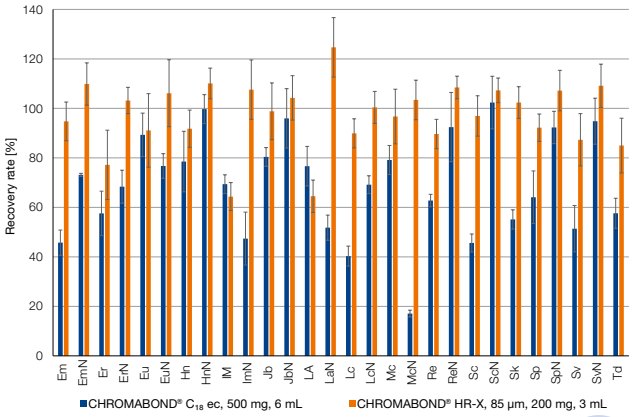
Drying: 5–10 min with vacuum

Elution: 5 mL methanol

Eluent exchange: Add 1.0 mL water as keeper. Evaporate eluate to a volume of 0.5 mL at 40 °C under a stream of nitrogen and fill up to 1.0 mL with water / methanol (95:5, v/v).

Further analysis:

HPLC determination of recovery rates with EC 150/2 NUCLEOSHELL® RP 18plus, 2.7 µm (REF 763236.20) in reference to MN Appl. No. 127480



Superior to silica based RP phase

CHROMABOND® HR-X shows higher recovery rates for most tested pyrrolizidine alkaloids than CHROMABOND® C18 ec under the given conditions.

Enrichment of opiates

MN Appl. No. 306710

Chromatographic conditions

Columns: CHROMABOND® HR-X / 45 µm / 3 mL / 60 mg

MN REF: 730936P45

Pretreatment: 400 µL methanolic standard solution were diluted with 50 mmol/L phosphate buffer pH 7.0 to 20 mL 2.5 mL of this solution are equal to 5 ng of each analyte

Conditioning: 3 x 1 mL methanol, 3 x 1 mL water, then 3 x 1 mL 50 mmol/L phosphate buffer pH 7.0

Aspiration: 2.5 mL of pretreated sample solution is passed through the column at a flow of 1–2 mL/min

Washing: 3 x 1 mL 50 mmol/L phosphate buffer pH 7.0, 3 x 1 mL water

Drying: 5 mL air by pushing with a syringe

Elution: 3 x 1 mL 0.1 % formic acid in methanol

Solvent change: Eluate is evaporated to dryness at 30 °C under a stream of nitrogen and then redissolved in organic solvent suited for the subsequent analysis.

Further analysis:

HPLC determination of recovery rates with EC 100/2 NUCLEOSHELL® Biphenyl, 2.7 µm (REF 763634.20) in reference to MN Appl. No. 128880

Compound	Recovery rate [%]	Standard deviation [%]
Ecgonine methyl ester	94	0
Morphine	77	3
Dihydrocodeine	101	1
Codeine	97	1
6-Acetylmorphine	89	1
Benzoylecgonine	102	0
6-Acetylcodeine	100	0
Cocaine	109	1
Noscapine	95	1
Papaverine	98	2

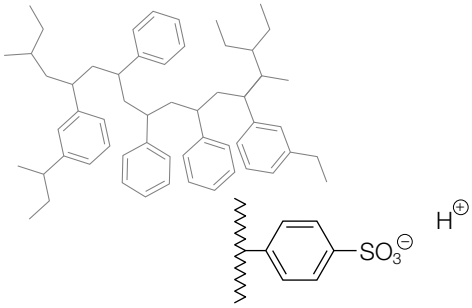
Modern polymeric CHROMABOND® SPE phases

CHROMABOND® HR-XC

Technical data

Strong cation exchanger based on polystyrene-divinylbenzene copolymer (PS / DVB)

SPE mode:	Ion exchange and reversed phase (mixed-mode)
Interactions:	Ionic, hydrophobic and π–π
Particle shape:	Spherical
pH stability:	1–14
Particle size:	85 µm and 45 µm
Pore size:	65–75 Å
Specific surface:	800 m^2/g
RP capacity:	300 mg/g (caffeine in water)
Exchange capacity:	1.0 meq/g, pKa < 1



Recommended application

- Basic active ingredients from heavily matrix-contaminated samples, e. g., urine, plasma, serum
- Fungicides from food
- Basic analytes, e. g., amines
- Bases with pKa 2–10

Good to know

A possible replacement for:

- Oasis® MCX
- Strata™-X-C
- StyreScreen® DBX
- HyperSep™ Retain CX

Standard protocol for CHROMABOND® HR-XC

MN Appl. No. 304790

Column type:
CHROMABOND® HR-XC / 3 mL / 200 mg, REF 730952

Sample pretreatment:

Individual sample preparation in reference to the compounds and matrix (adjust pH value if necessary).

Conditioning:	5 mL methanol, then 5 mL water (do not let run the column dry!)
Sample aspiration:	The prepared sample is passed through the column by vacuum or pressure
Washing 1:	2 mL 0.1 M HCl in water
Washing 2: / Elution 1:	2 mL methanol (elution of neutral and acidic compounds)
Drying:	With nitrogen or air
Elution 2:	5 mL methanol / 5 % NH_3 (elution of basic compounds)

Further analysis:

Evaporation and reconstitution (if necessary); HPLC or GC

These conditions are a starting point for SPE method development. Further optimization may be required to improve results.

SPE hardware formats

Check out our different hardware types, e. g., CHROMAFIX® cartridges

Applications

Enrichment of benzodiazepines

MN Appl. No. 306720

Chromatographic conditions

Columns: CHROMABOND® HR-XC 45 µm / 3 mL / 60 mg

MN REF: 730956P45

Pretreatment: 400 µL methanolic standard solution were diluted with phosphate buffer pH 6.0 to 20 mL
2.5 mL of this solution are equal to 5 ng of each analyte

Conditioning: 2 mL methanol, 2 mL phosphate buffer pH 6.0

Aspiration: 2.5 mL of pretreated sample solution is passed through the column at a flow of 1–2 mL/min.

Washing: 2 mL phosphate buffer pH 6.0, 2 mL methanol / water (30:70, v/v), 3 mL 0.1 mol/L hydrochloric acid, 2 mL methanol / water (30:70, v/v), 0.1 mL methanol followed by 1 min drying, 2 mL methanol / water (30:70, v/v)

Drying: 5 min with a slight nitrogen stream

Elution: 2 x 1.5 mL 25 % aqueous ammonia solution / ethylacetate (2:100, v/v)

Solvent change: Eluate is evaporated to dryness at 30 °C under a stream of nitrogen and then redissolved in organic solvent suited for the subsequent analysis.

Further analysis:

HPLC determination of recovery rates with EC 150/2 NUCLEOSHELL® Bluebird RP 18, 2.7 µm (REF 763436.20) in reference to MN Appl. No. 128890

Compound	Recovery rate [%]
Nortetrazepam	85
Tetrazepam	85
α-Hydroxytriazolam	87
Zaleplon	84
Nitrazepam	92
Oxazepam	104
Nordiazepam	83
N-Desmethylflunitrazepam	90
Lorazepam	89
Clonazepam	88
Desalkylflurazepam	102
Temazepam	103
Flunitrazepam	89
Lormetazepam	109
Clobazam	90
Diazepam	98

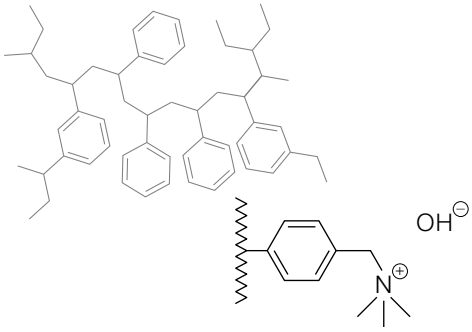
Modern polymeric CHROMABOND® SPE phases

CHROMABOND® HR-XA

Technical data

Strong anion exchanger based on polystyrene-divinylbenzene copolymer (PS / DVB)

SPE mode:	Ion exchange and reversed phase (mixed-mode)
Interactions:	Ionic, hydrophobic and π-π
Particle shape:	Spherical
pH stability:	1–14
Particle size:	85 µm and 45 µm
Pore size:	55–65 Å
Specific surface:	850 m^2/g
RP capacity:	350 mg/g (caffeine in water)
Exchange capacity:	0.25 meq/g, pKa ~ 18



Recommended application

- Acidic active ingredients from heavily matrix-contaminated samples, e. g., urine, plasma, serum
- Phenolic acids
- Acidic herbicides
- Weak/medium-strength acids with pKa 2–8

Good to know

A possible replacement for:

- Oasis® MAX
- Strata™-X-A
- HyperSep™ Retain AX
- StyreScreen® QAX

Standard protocol for CHROMABOND® HR-XA

MN Appl. No. 304970

Column type:
CHROMABOND® HR-XA / 3 mL / 200 mg / REF 730951

Sample pretreatment:

Individual sample preparation in reference to the compounds and matrix (adjust a basic pH value).

Conditioning:	5 mL methanol, then 5 mL water (do not let run the column dry!)
Sample aspiration:	The basic sample is passed through the column by vacuum or pressure (max. 1000 mL sample volume)
Washing 1:	2 mL 0.1 M NaOH in water
Washing 2: / Elution 1:	2 mL methanol (elution of neutral and basic compounds)
Drying:	With nitrogen or air
Elution 2:	5 mL methanol / 1-10 % formic acid (elution of acidic compounds)

Further analysis:

Evaporation and reconstitution (if necessary); HPLC or GC

These conditions are a starting point for SPE method development. Further optimization may be required to improve results.

Successful filtration

We recommend to use CHROMAFIL® Xtra syringe filters in combination with our SPE columns. For further information, please visit *www.mn-net.com/chromafil*.

Modern polymeric CHROMABOND® SPE phases

Applications

Fractions of acidic and basic analytes from serum

MN Appl. No. 305020

Chromatographic conditions

Column: CHROMABOND® HR-XA / 85 µm / 3 mL / 200 mg

MN REF: 730951

Pretreatment: 1 µg/mL analytes in serum, adjusted on basic pH with 1 N NaOH

Conditioning: 5 mL methanol, then 5 mL water (Do not let run the column dry!)

Aspiration: The prepared sample is passed through the column by vacuum

Washing: With 2.5 mL water impurities are removed

Drying: With nitrogen or air

Elution: Fraction A (basic analytes) is eluted with 5.0 mL methanol
Fraction B (acidic analytes) with 5.0 mL methanol / 10 % formic acid

Evaporation and reconstitution with 1 mL of mobile phase from subsequent HPLC.

Washing: 1.6 mL acetonitrile, 20 µL/s

Subsequent analysis:

Fraction A: HPLC determination on EC 125/4 NUCLEODUR® C8 Gravity, 5 µm (REF 760751.40) in reference to MN Appl. No. 118520

Fraction B: HPLC determination on EC 125/4 NUCLEODUR® C18 Gravity, 5 µm (REF 760100.40) in reference to MN Appl. No. 122230

Recovery rates:

Fraction A	Recovery [%]	Fraction B	Recovery [%]
Protriptyline	75	Suprofen	96
Nortriptyline	69	Naproxen	86
Doxepine	72	Tolmetin	85
Imipramine	80		
Amitriptyline	78		
Trimipramine	73		

Acidic pharmaceuticals from serum

MN Appl. No. 305000

Chromatographic conditions

Column: CHROMABOND® HR-XA / 85 µm / 3 mL / 200 mg

MN REF: 730951

Pretreatment: 1 µg/mL pharmaceuticals in serum, adjusted on basic pH with 1 N NaOH

Conditioning: 5 mL methanol, then 5 mL water (Do not let run the column dry!)

Aspiration: The prepared sample is passed through the column by vacuum

Washing: With the following washing mixtures impurities are removed: a) 2.5 mL water · b) 2.5 mL 0.1 N NaOH · c) 5.0 mL methanol

Drying: With nitrogen or air

Elution: Analytes are eluted with 5 mL methanol / 1 % formic acid

Evaporation to dryness and reconstitution with 1 mL of mobile phase from subsequent HPLC.

Subsequent analysis:

HPLC determination of recovery rates with EC 125/4 NUCLEODUR® C18 Gravity, 5 µm (REF 760100.40) in reference to MN Appl. No. 122840

Recovery rates:

Compound	HR-XA [%]	Oasis® MAX [%]
Ketoprofen	90	85
Fenoprop	104	123
Fenoprofen	98	69
Flurbiprofen	106	98
Ibuprofen	88	58
Carprofen	69	89
Diclofenac	95	94
Meclofenamic acid	92	93

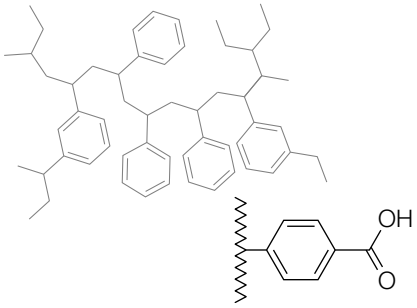
Modern polymeric CHROMABOND® SPE phases

CHROMABOND® HR-XCW

Technical data

Weak cation exchanger based on polystyrene-divinylbenzene copolymer (PS / DVB)

SPE mode:	Ion exchange and reversed phase (mixed-mode)
Interactions:	Ionic, hydrophobic and π-π
Particle shape:	Spherical
pH stability:	1–14
Particle size:	85 µm and 45 µm
Pore size:	50–60 Å
Specific surface:	850 m^2/g
RP capacity:	350 mg/g (caffeine in water)
Exchange capacity:	> 0.7 meq/g, pKa ~ 5



Recommended application

- Basic compounds like quaternary amines
- Active ingredients from heavily matrix-contaminated samples, e. g., urine, plasma, serum
- Strong bases with pKa > 10

Good to know

A possible replacement for:

- Oasis® WCX
- Strata™-X-CW

Standard protocol for CHROMABOND® HR-XCW

MN Appl. No. 305300

Column type:
CHROMABOND® HR-XCW / 3 mL / 200 mg, REF 730739

Sample pretreatment:

Individual sample preparation in reference to the compounds and matrix.

Conditioning:	5 mL methanol, then 5 mL water (do not let run the column dry!)
Sample aspiration:	The sample is passed through the column by vacuum or pressure (max. 1000 mL sample volume)
Washing 1:	2 mL 5 % aq. NH_4OH solution
Washing 2: / Elution 1:	2 mL methanol (elution of neutral and acidic compounds)
Drying:	With nitrogen or air
Elution 2:	2 x 2 mL 1-5 % formic acid in methanol (elution of strongly basic compounds)

Basic methanol (NH_3) can be used alternatively for elution 2 (e.g., for primary to tertiary amines). Here an interruption of the interactions with the cation exchanger results by a deprotonation of the analyte.

Further analysis:

Evaporation and reconstitution (if necessary); HPLC or GC

These conditions are a starting point for SPE method development. Further optimisation may be required to improve results.

HPLC columns

Are you looking for HPLC columns for subsequent analysis?
Find an overview of our HPLC columns under the following link *www.mn-net.com/hplc*.

Modern polymeric CHROMABOND® SPE phases

Applications

Tricyclic Antidepressants

MN Appl. No. 305340

Column type:
CHROMABOND® HR-XCW / 85 µm / 3 mL / 60 mg

MN REF: 730735

Pretreatment: 250 µL spiked serum, diluted with 1 mL 10 % formic acid in water

Conditioning: 3 mL MeOH

Equilibration: 3 mL water

Application: Slowly aspirate sample through the column

Washing: 1 mL 5 % formic acid in water, then 1 mL MeOH

Elution: After drying by vaccum (15 min) 3 mL 5 % formic acid in MeOH

Further analysis:

Evaporate and redissolve in a suitable solvent for HPLC on NUCLEODUR® C8 Gravity, see MN Appl. No. 118520

Recovery rates:

Compound	HR-XCW	HR-XC*	PCA**	Oasis® WCX
Doxepine	79	5	11	41
Imipramine	79	9	20	67
Amitriptyline	91	9	14	46
Trimipramine	98	7	14	27

* HR-XC: Basic analytes can not be eluted with slightly acidic organic conditions from the strong cation exchanger CHROMABOND® HR-XC, because the eluting power is not sufficient to dissociate the interaction with the ion exchanger. However, with the usage of basic methanol a complete elution can be achieved (please see also MN Appl. No. 304780).

** PCA: Due to the missing RP interactions of silica based weak cation exchanger, CHROMABOND® PCA gives only a small enrichment elution of the analytes.

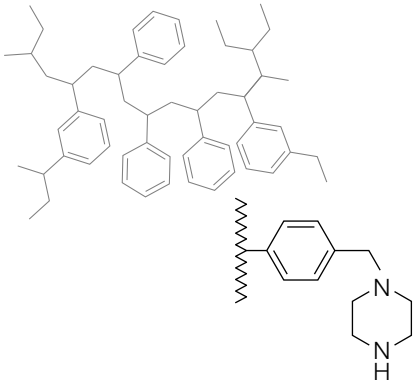
Modern polymeric CHROMABOND® SPE phases

CHROMABOND® HR-XAW

Technical data

Weak anion exchanger based on polystyrene-divinylbenzene copolymer (PS / DVB)

SPE mode:	Ion exchange and reversed phase (mixed-mode)
Interactions:	Ionic, hydrophobic and π–π
Particle shape:	Spherical
pH stability:	1–14
Particle size:	85 µm and 45 µm
Pore size:	55–65 Å
Specific surface:	850 m^2/g
RP capacity:	350 mg/g (caffeine in water)
Exchange capacity:	> 0.5 meq/g, pKa ~ 6



Recommended application

- Perfluorinated surfactants
- Acidic compounds like sulfonates
- Active ingredients from heavily matrix-contaminated samples, e. g., urine, plasma, serum
- Strong acids with pKa < 1

Good to know

A possible replacement for:

- Oasis® WAX
- Strata™-X-AW

Standard protocol for CHROMABOND® HR-XAW

MN Appl. No. 305200

Column type:
CHROMABOND® HR-XAW / 3 mL / 200 mg, REF 730748

Sample pretreatment:

Individual sample preparation in reference to the compounds and matrix.

Conditioning:	5 mL methanol, then 5 mL water (do not let the column run dry!)
Sample aspiration:	The sample is passed through the column by vacuum or pressure (max. 1000 mL sample volume)
Washing 1:	25 mM ammonium acetate in water
Washing 2: / Elution 1:	2 mL methanol (elution of neutral and basic compounds)
Drying:	With nitrogen or air
Elution 2:	2 x 2 mL 1–5 % ammonia in methanol (elution of strongly acidic compounds)

Acidic methanol (formic acid) can be used alternatively for elution 2. Here an interruption of the interactions with the anion exchanger results by a protonation of the analyte.

Further analysis:

Evaporation and reconstitution (if necessary); HPLC or GC

These conditions are a starting point for SPE method development. Further optimisation may be required to improve results.

GC columns

For more information on our high performance GC capillary columns, please visit *www.mn-net.com/optima*.

Applications

Polyfluorinated compounds (PFCs) from fresh and sea water

MN Appl. No. 306730

Chromatographic conditions

Columns: CHROMABOND® HR-XAW / 85 µm / 3 mL / 60 mg

MN REF: 730747

Pretreatment: 50 mL water sample spiked with PFC standard mixture (β = 0.5 ng for each analyt in 50 mL water), adjusted to pH value 7–8

Conditioning: 2 mL 0.1 % ammonium hydroxide in methanol, 2 mL methanol, 2 mL water

Aspiration: Pretreated sample solution is passed through the column at a flow of 5–10 mL/min

Washing: 2 mL water, 2 mL 1.0 % formic acid in acetone / acetonitrile (50:50, v/v), 2 mL methanol

Drying: No drying

Elution: 2.4 mL 0.1 % ammonium hydroxide in methanol

Solvent change: Evaporate eluate to dryness at 40 °C under a stream of nitrogen and reconstitute in 0.5 mL water / methanol (40:60, v/v)

Did you know?

Properties of PFCs:

- Persistent in the environment
- Water-, dirt- and fat-repellent; resistant against aggressive chemicals
- Often toxic; many PFCs are bioaccumulative
- Thermally and chemically stable

Daily use of PFCs:

- Fire-fighting foam
- Paper finishing
- Fibre coating
- Textile coating, e.g., seat covers, carpets, outdoor clothing
- Cookware
- Food packaging, e.g., pizza cartons, paper cups
- Building material, e.g., water resistant lacquer

Recovery rates:

Matrix	Water		Seawater	
Analyte	Recovery [%]	RSD [%, n = 3]	Recovery [%]	RSD [%, n = 3]
PFPeA	98	2.9	84	1.6
PFHxA	96	1.7	91	1.3
PFHpA	106	2.9	82	2.4
PFOA	99	2.3	86	2.5
PFNA	114	2.7	93	2.0
PFDA	110	2.6	90	2.3
PFUdA	96	5.3	85	3.5
PFDoA	84	1.6	76	2.1
PFTrDA	75	2.9	70	2.6
PFTeDA	66	4.3	74	4.0
L-PFBS	96	1.6	91	0.7
PFHxS	100	1.6	84	0.8
L-PFHpS	104	1.8	90	3.2
PFOS	103	2.0	84	2.3
L-PFDS	72	4.8	75	3.4
FOSA*	0	–	0	–
N-MeFOSAA*	3	–	0	–
N-EtFOSAA*	2	–	0	–
4:2 FTS	96	1.3	46	2.0
6:2 FTS	108	2.4	53	0.8
8:2 FTS	105	5.2	63	4.5
PFBA**	356	3.6	65	1.8
M_4-PFBA**	139	4.0	64	1.4
M_4-PFOA	101	3.7	89	2.8
M_2-PFHxA	95	2.2	84	0.5
M_4-PFHxS	96	2.2	84	1.7
M_5-PFNA	107	3.5	90	1.8
M_4-PFOS	101	2.4	82	1.2
M_2-PFDA	103	3.6	87	3.3
M_2-PFDoA	79	3.3	75	2.1
M_2-PFUdA	90	3.3	82	2.3

* Due to the organic washing steps, these analytes were eluted into waste.

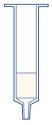
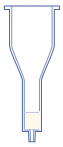
** In accordance to the properties of the analyte molecules, a not satisfying S/N ratio is received resulting in an improper integration for calculating the recovery rate.

Note: An LC-MS/MS method for determination of polyfluorinated compounds is shown in MN Appl. No 128900

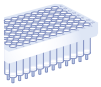
Modern polymeric CHROMABOND® SPE phases

Ordering information

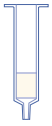
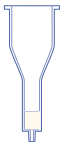
CHROMABOND® HLB

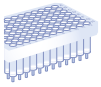
	Volume	Adsorbent weight 30 mg	60 mg	100 mg	150 mg	200 mg	500 mg	1 g	Pack of
	CHROMABOND® HLB polypropylene columns (60 µm)								
	1 mL	730921		730922					30
	3 mL		730923			730924	730925		30
	6 mL				730944	730926	730927		30
	15 mL						730928	730929	20
	CHROMABOND® HLB polypropylene columns (60 µm) · BIGpacks								
	3 mL		730923.250			730924.250			250
	6 mL					730926.250	730927.250		250
	CHROMABOND® HLB polypropylene columns (30 µm)								
	1 mL	730921P30		730922P30					30
	3 mL		730923P30			730924P30			30
	6 mL				730944P30				30
	CHROMABOND® LV-HLB (30 µm)								
	15 mL	732140	732141						30

	Size Minimum adsorbent weight	S 50 mg	M 120 mg	L 350 mg	Pack of
	CHROMAFIX® HLB cartridges (60 µm)				
		731921	731922	731923	50

	Adsorbent weight	96 x 10 mg	96 x 30 mg	96 x 60 mg	
	CHROMABOND® MULTI 96 HLB (60 µm)				
				738920.060M	1
	CHROMAFIX® MULTI 96 HLB (30 µm)				
		738921.010M	738921.030M		1

CHROMABOND® HR-X

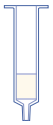
	Volume	Adsorbent weight 30 mg	60 mg	100 mg	200 mg	500 mg	1 g	Pack of
	CHROMABOND® HR-X polypropylene columns (85 µm)							
	1 mL	730934		730935				30
	3 mL		730936		730931	730937		30
	6 mL				730938	730939		30
	15 mL					730940	730941	20
	CHROMABOND® HR-X polypropylene columns (85 µm) · BIGpacks							
	3 mL				730931.250			250
	6 mL				730938.250	730939.250		250
	CHROMABOND® HR-X polypropylene columns (45 µm)							
	1 mL	730934P45		730935P45				30
	3 mL		730936P45		730931P45			30
	CHROMABOND® LV-HR-X (85 µm)							
	15 mL	732130						30

	Adsorbent weight	96 x 100 mg	
	CHROMABOND® MULTI 96 HR-X (85 µm)		
		738530.100M	1

Modern polymeric CHROMABOND® SPE phases

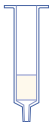
Ordering information (cont.)

CHROMABOND® HR-XC

	Volume	Adsorbent weight 30 mg	60 mg	100 mg	150 mg	200 mg	500 mg	Pack of
	CHROMABOND® HR-XC polypropylene columns (85 µm)							
	1 mL	730969		730049				30
	3 mL		730956			730952	730953	30
	6 mL				730957		730955	30
	CHROMABOND® HR-XC polypropylene columns (45 µm)							
	1 mL	730969P45		730049P45				30
	3 mL		730956P45			730952P45		30

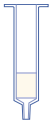
	Size Minimum adsorbent weight	S 50 mg	M 140 mg	L 400 mg	Pack of
	CHROMAFIX® HR-XC cartridges (85 µm)				
		731755	731756	731757	50

CHROMABOND® HR-XA

	Volume	Adsorbent weight 30 mg	60 mg	100 mg	150 mg	200 mg	500 mg	Pack of
	CHROMABOND® HR-X polypropylene columns (85 µm)							
	1 mL	730968		730727				30
	3 mL		730950			730951	730954	30
	6 mL				730958		730966	30
	CHROMABOND® HR-XA polypropylene columns (45 µm)							
	1 mL	730968P45		730727P45				30
	3 mL		730950P45			730951P45		30

	Size Minimum adsorbent weight	S 70 mg	M 180 mg	L 510 mg	Pack of
	CHROMAFIX® HR-XA cartridges (85 µm)				
		731768	731769	731770	50

CHROMABOND® HR-XCW

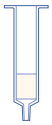
	Volume	Adsorbent weight 30 mg	60 mg	100 mg	150 mg	200 mg	500 mg	Pack of
	CHROMABOND® HR-XCW polypropylene columns (85 µm)							
	1 mL	730731		730733				30
	3 mL		730735			730739	730741	30
	6 mL				730737		730743	30
	CHROMABOND® HR-XCW polypropylene columns (45 µm)							
	1 mL	730731P45		730733P45				30
	3 mL		730735P45			730739P45		30

	Size Minimum adsorbent weight	S 60 mg	M 160 mg	L 450 mg	Pack of
	CHROMAFIX® HR-XCW cartridges (85 µm)				
		731774	731775	731776	50

Modern polymeric CHROMABOND® SPE phases

Ordering information (cont.)

CHROMABOND® HR-XAW

	Volume	Adsorbent weight 30 mg	60 mg	100 mg	150 mg	200 mg	500 mg	Pack of
	CHROMABOND® HR-XAW polypropylene columns (85 µm)							
	1 mL	730728		730729				30
	3 mL		730747			730748	730744	30
	6 mL				730749		730745	30
	CHROMABOND® HR-XAW polypropylene columns (45 µm)							
	1 mL	730728P45		730729P45				30
	3 mL		730747P45			730748P45		30

	Size Minimum adsorbent weight	S 50 mg	M 120 mg	L 360 mg	Pack of
	CHROMAFIX® HR-XAW cartridges (85 µm)				
		731771	731772	731773	50

Registered trademarks

Oasis®	Waters Corp. (USA)
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FREESTYLE®	LCTech GmbH (Germany)
Strata™	Phenomenex Inc. (USA)
Isolute®	Biotage® AB (Sweden)
Supelclean™ ENVI™	Sigma-Aldrich Inc. (part of Merck KGaA, Germany)
BakerBond®	J. T. Baker® (part of Avantor™) (USA)
Supra-Poly®	PerkinElmer® Inc. (USA)
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