

Introduction

The enrichment of hydrophilic analytes from polar matrices is a challenge because they often cannot be retained by C₁₈/C₈-modified silica or hydrophobic polymer adsorbents. To overcome this issue MACHEREY-NAGEL developed CHROMABOND® HLB, a hydrophilic-lipophilic balanced *N*-vinylpyrrolidone-divinylbenzene copolymer. Its lipophilic backbone interacts with nonpolar hydrocarbon residues of the analytes while the linked hydrophilic groups interact with polar functional groups to provide enhanced retention.

Typical applications

Polar organic molecules from polar matrices e.g.,

- Sulfonamides
- Pesticides
- Chloramphenicol
- Iodinated contrast media

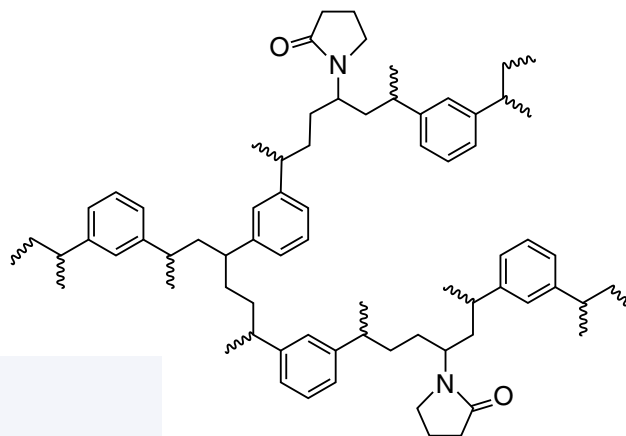
Advantages of CHROMABOND® HLB

- Applicable for a wide range of analyte polarities
- Enhanced retention for polar compounds
- High loadability and outstanding performance
- Water wettable – even if bed runs dry, SPE can be continued
- The alternative to Oasis® HLB

Technical data

Hydrophilic-lipophilic balanced *N*-vinylpyrrolidone-divinylbenzene copolymer

Particle shape:	spherical
pH stability:	1–14
Particle size:	60 µm and 30 µm
Pore size:	65 Å
Specific surface:	750 m ² /g



Standard SPE protocol (subsequent HPLC analysis)

MN Appl. No. 306300

Column:	CHROMABOND® HLB, 3 mL, 200 mg
MN REF:	730924
Column conditioning:	5 mL methanol, 5 mL dest. water
Sample application:	slowly aspirate sample through column
Washing:	5 mL dest. water
Drying:	10 min with applied vacuum
Elution:	8 mL methanol
Evaporation:	under nitrogen
Reconstitution:	in 1 mL dest. water + 0.1 % formic acid

Standard SPE protocol (subsequent GC analysis)

MN Appl. No. 306310

Column:	CHROMABOND® HLB, 3 mL, 200 mg
MN REF:	730924
Column conditioning:	5 mL solvent (e.g., ethyl acetate), 5 mL methanol, 5 mL dest. water
Sample application:	slowly aspirate sample through column
Washing:	5 mL dest. 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




Pharmaceuticals from serum

MN Appl. No. 306510

	Columns*:	CHROMABOND® HLB, 1 mL, 30 mg Oasis® HLB, 1 mL, 30 mg
	MN REF:	730921
	Column conditioning:	1 mL methanol, 1 mL dest. water
	Sample application:	1 mL serum (spiked with 50 ng of each analyte)
	Washing:	1 mL dest. water
	Drying:	10 min with applied vacuum
	Elution:	2 mL methanol
	Evaporation:	under nitrogen, 40 °C
	Reconstitution:	in 1 mL dest. water – acetonitrile (95:5, v/v)

Further analysis: LC-MS/MS, according to MN Appl. No. 128200

	Column:	EC 50/2 NUCLEOSHELL® PFP, 2.7 µm
	MN REF:	763532.20
	Eluent:	A: dest. water + 0.1 % formic acid B: acetonitrile + 0.1 % formic acid 5–95 % B in 7.5 min, 95 % B for 1 min, 95–5 % B in 0.5 min, 5 % B for 5 min
	Flow rate:	0.3 mL/min
	Temperature:	30 °C
	Detection:	MS, Selected Reaction Monitoring (SRM)
	Injection:	5 µL


Recovery rates ± RSD [%], n = 5

Compound	CHROMABOND® HLB	Oasis® HLB
Amitriptyline	77.7 ± 1.2	26.9 ± 0.8
Atenolol	71.2 ± 1.3	71.1 ± 1.2
Atropine	84.8 ± 0.9	80.5 ± 1.4
Carbamazepine	97.7 ± 0.3	57.7 ± 4.4
Chlorpheniramine	85.9 ± 2.2	91.7 ± 1.2
Clomipramine	73.6 ± 6.7	48.1 ± 0.8
Diphenhydramine	88.3 ± 2.1	94.7 ± 1.3
Indapamide	87.7 ± 3.3	49.0 ± 2.0
Ketamine	90.8 ± 1.8	88.4 ± 2.0
Ketoprofen	84.1 ± 3.1	48.9 ± 1.6

Compound	CHROMABOND® HLB	Oasis® HLB
Nortriptyline	76.6 ± 2.1	14.9 ± 3.5
Propranolol	107.7 ± 1.4	108.3 ± 1.7
Sulfachloropyridazine	85.8 ± 1.6	84.0 ± 1.4
Sulfadoxine	99.8 ± 2.0	91.2 ± 2.0
Sulfamethoxazole	94.3 ± 1.6	81.2 ± 1.6
Sulfapyridine	64.6 ± 1.8	61.6 ± 3.9
Sulfaquinoxaline	127.1 ± 3.4	104.8 ± 2.8
Sulfamerazine	67.3 ± 0.8	63.4 ± 3.7
Trimipramine	81.5 ± 2.3	37.3 ± 1.2
Verapamil	107.5 ± 1.7	48.9 ± 0.9

Drugs from tap water

MN Appl. No. 306330

	Columns*:	CHROMABOND® HLB, 3 mL, 200 mg Oasis® HLB, 3 mL, 200 mg
	MN REF:	730924
	Column conditioning:	5 mL methanol, 5 mL dest. water
	Sample application:	1000 mL tap water (spiked with 5 µg/L of each analyte), ~10 mL/min
	Washing:	5 mL dest. water
	Drying:	10 min with applied vacuum
	Elution:	8 mL methanol
	Evaporation:	under nitrogen, 40 °C
	Reconstitution:	in 1 mL dest. water + 0.1 % formic acid

Further analysis: HPLC, according to MN Appl. No. 128110
see Drugs from serum, page 7

Recovery rates ± RSD [%], n = 5

Compound	CHROMABOND® HLB	Oasis® HLB
Azidothymidine	98.2 ± 0.7	96.9 ± 0.8
Caffeine	84.2 ± 0.9	67.9 ± 0.9
<i>trans</i> -doxepin	78.5 ± 0.9	64.5 ± 0.9
<i>cis</i> -doxepin	81.3 ± 1.3	62.7 ± 0.6
Propranolol	93.7 ± 0.9	83.7 ± 1.2
Protriptyline	78.2 ± 2.9	60.2 ± 2.3

CHROMABOND® HLB for polar analytes


CHROMABOND® HLB provides high recovery rates for polar drugs from water.




* 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.

Sulfa drugs from serum

MN Appl. No. 306340

	Columns*:	CHROMABOND® HLB, 1 mL, 30 mg Oasis® HLB, 1 mL, 30 mg
	MN REF:	730921
	Column conditioning:	1 mL methanol, 1 mL dest. water
	Sample application:	1 mL serum (spiked with 10 µg/mL of each analyte)
	Washing:	1 mL dest. water
	Drying:	10 min with applied vacuum
	Elution:	2 mL methanol
	Evaporation:	under nitrogen, 40 °C
	Reconstitution:	in 1 mL dest. water + 0.1 % formic acid

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

	Column:	EC 150/2 NUCLEODUR® C ₁₈ Pyramid, 3 µm
	MN REF:	760261.20
	Eluent:	dest. 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

Equivalence to Oasis® HLB

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




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, 3 mL, 200 mg Oasis® HLB, 3 mL, 200 mg
	MN REF:	730924
	Sample preparation:	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 supernatant for eluent exchange. Evaporate extracts to dryness at 40 °C under a stream of nitrogen. Redissolve residue in 10 mL water.
	Column conditioning:	3 mL methanol (dispensing speed 1 mL/min), 5 mL dest. water (disp. speed 1 mL/min)
	Sample application:	9 mL water sample (disp. speed 3 mL/min over sample loop)
	Washing:	10 mL dest. 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 dest. 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: dest. 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.



Pesticides from tap water

MN Appl. No. 306360

Columns*:	CHROMABOND® HLB, 3 mL, 200 mg Oasis® HLB, 3 mL, 200 mg
MN REF:	730924
Column conditioning:	5 mL methanol, 5 mL dest. water
Sample application:	1000 mL tap water (spiked with 50 ng of each analyte)
Washing:	10 mL dest. water
Drying:	5 min with applied vacuum (-15 psi)
Elution:	6 mL acetonitrile
Evaporation:	under nitrogen, 40 °C
Reconstitution:	in 1 mL dest. 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: dest. 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

Compound	CHROMABOND® HLB	Oasis® HLB
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
Terbutylazine	107.9 ± 13.3	100.0 ± 13.6
Thiacloprid	74.1 ± 6.3	86.5 ± 10.8



* 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.

Iodinated contrast media from serum

MN Appl. No. 306370

Columns*: CHROMABOND® HLB, 1 mL, 30 mg
Oasis® HLB, 1 mL, 30 mg

MN REF: 730921

Column conditioning: 1 mL methanol, 1 mL dest. water

Sample application: 1 mL serum (spiked with 10 µg/mL of each analyte)

Washing: 1 mL dest. water

Drying: 10 min with applied vacuum

Elution: 2 mL methanol

Evaporation: under nitrogen, 40 °C

Reconstitution: in 1 mL dest. water + 5 mM ammonium formate

Further analysis: HPLC, according to MN Appl. No. 128160

Column: EC 150/2 NUCLEODUR® C₁₈ Gravity-SB, 3 µm

MN REF: 760608.20

Eluent: A: dest. water + 5 mM ammonium formate
B: methanol/acetonitrile, (1:2, v/v) + 5 mM ammonium formate
7–10 % B in 5 min, 10–100 % B in 5 min, 100 % B for 10 min

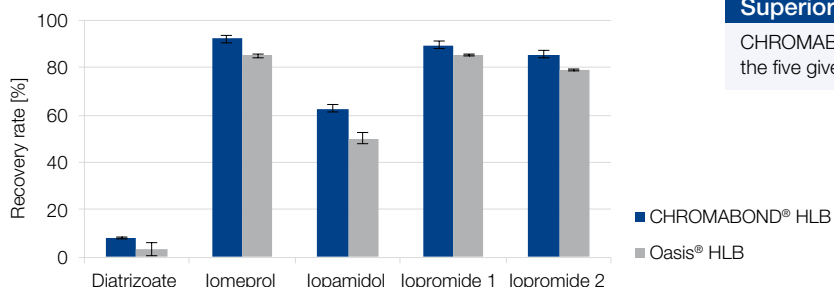
Flow rate: 0.2 mL/min

Temperature: 40 °C

Detection: UV, 254 nm

Injection: 5 µL

Recovery rates ± RSD [%], n = 5



Superior to Oasis® HLB

CHROMABOND® HLB provides higher recovery rates for the five given analytes from serum in comparison to Oasis® HLB.

Tetracyclines and alkaloids from serum at pH 5

MN Appl. No. 306380

Columns*: CHROMABOND® HLB, 1 mL, 30 mg
Oasis® HLB, 1 mL, 30 mg

MN REF: 730921

Column conditioning: 1 mL methanol, 1 mL dest. water

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

Washing: 1 mL dest. water

Drying: 10 min with applied vacuum

Elution: 2 mL methanol

Evaporation: under nitrogen, 40 °C

Reconstitution: in 1 mL dest. water + 0.1 % formic acid

Further analysis: HPLC, according to MN Appl. No. 128170

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

MN REF: 763232.20

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

Flow rate: 0.75 mL/min

Temperature: 22 °C

Detection: UV, 330 nm

Injection: 5 µL


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


* 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.

Drugs from serum

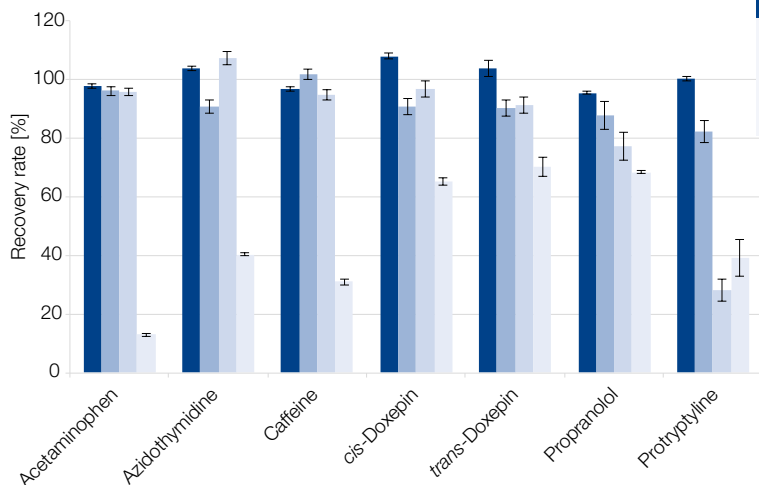
MN Appl. No. 306320

	Columns*: CHROMABOND® HLB, 1 mL, 30 mg Hydrophobic polymer, 1 mL, 30 mg MN REF: 730921 Column conditioning: 1 mL methanol, 1 mL dest. water Sample application: 1 mL serum (spiked with 10 µg/mL of each analyte) Washing: 1 mL dest. water Drying: 10 min with applied vacuum Elution: 2 mL methanol Evaporation: under nitrogen, 40 °C Reconstitution: in 1 mL dest. water + 0.1 % formic acid
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Further analysis: HPLC, according to MN Appl. No. 128110

	Column: EC 150/2 NUCLEODUR® C ₁₈ Pyramid, 3 µm MN REF: 760261.20 Eluent: A: dest. water + 0.1 % formic acid B: methanol + 0.1 % formic acid 30–45 % B in 15 min Flow rate: 0.3 mL/min Temperature: 30 °C Detection: UV, 254 nm Injection: 5 µL
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Recovery rates ± RSD [%], n = 5



Good to know

No conditioning step is needed when using CHROMABOND® HLB due to its excellent water wettability. Remark: Conditioning often improves analyte recovery. Therefore we recommend comparing results.

- CHROMABOND® HLB (conditioned)
- CHROMABOND® HLB (dry)
- Hydrophobic polymer (conditioned)
- Hydrophobic polymer (dry)



* 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.

