Chromatography

MACHEREY-NAGEL

CHROMABOND® HLB

CHROMABOND® HLB

- Hydrophilic-lipophilic balanced phase
- Enhanced retention for polar compounds
- Consistent recoveries

ADIS INTERNATIONAL

MN Since 1911

CHROMABOND® HLB

Introduction

The enrichment of hydrophilic analytes from polar matrices is a challenge because they often cannot be retained by C_{18}/C_8-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.

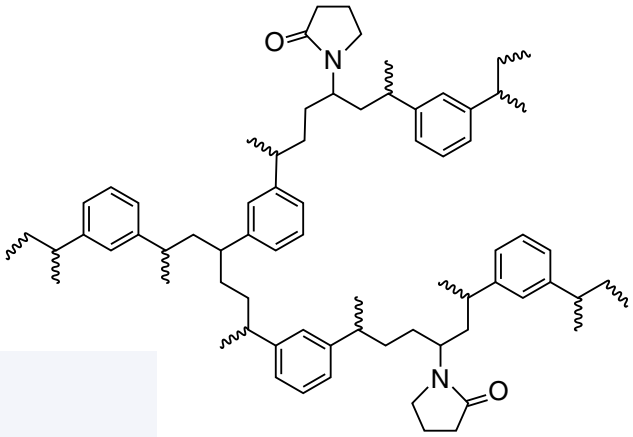
Typical applications

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

CHROMABOND® HLB

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
Propanolol	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 tab 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

CHROMABOND® HLB for polar analytes

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

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
trans-doxepin	78.5 ± 0.9	64.5 ± 0.9
cis-doxepin	81.3 ± 1.3	62.7 ± 0.6
Propanolol	93.7 ± 0.9	83.7 ± 1.2
Protriptyline	78.2 ± 2.9	60.2 ± 2.3

* 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

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® 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

Recovery rates ± RSD [%], n = 5

Compound	CHROMABOND® HLB	Oasis® HLB
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 supernantant 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® π², 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.

CHROMABOND® HLB

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
Terbuthylazine	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

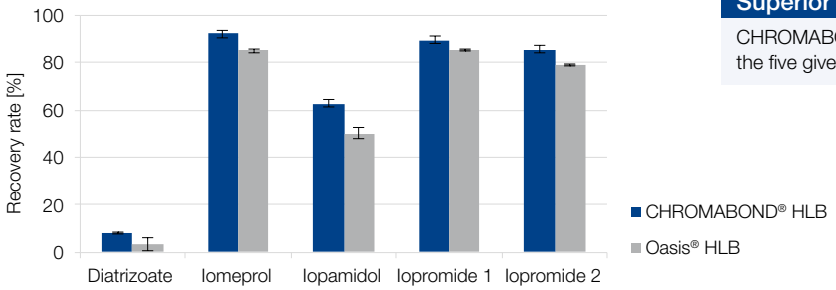
MN Appl. No. 306370

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® C_{18} 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.

CHROMABOND® HLB

Drugs from serum

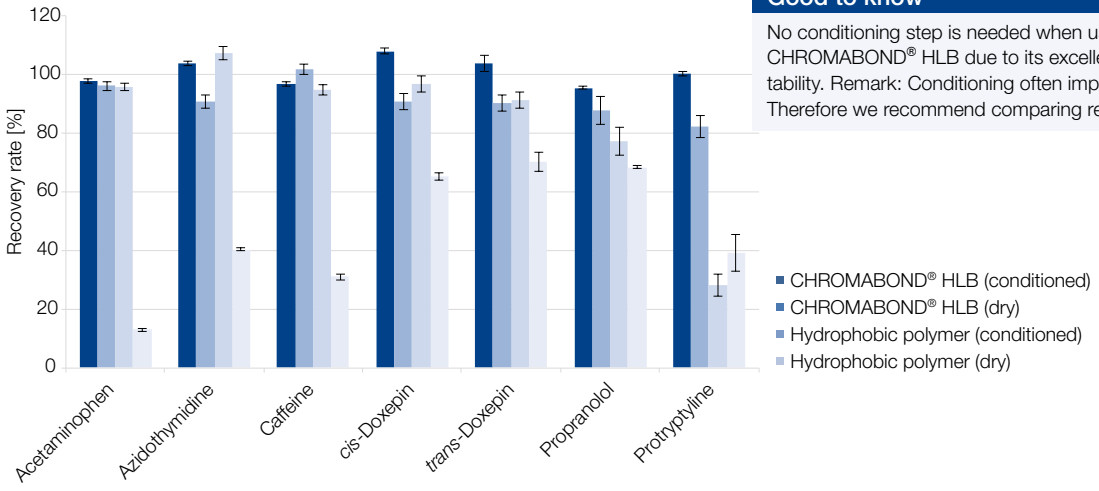
MN Appl. No. 306320

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

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

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.

*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.

CHROMABOND® HLB

Ordering information

Volume	Adsorbent weight →							Pack of
	30 mg	60 mg	100 mg	150 mg	200 mg	500 mg	1 g	
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 →	S	M	L	Pack of
Minimum adsorbent weight →	50 mg	120 mg	350 mg	
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
CHROMABOND® MULTI 96 HLB (30 µm)				
	738921.010M	738921.030M		1

Registered trademarks

Oasis®	Waters Corp. (USA)
CHROMABOND®	MACHEREY-NAGEL GmbH & Co. KG (Germany)
CHROMAFIX®	MACHEREY-NAGEL GmbH & Co. KG (Germany)
FREESTYLE®	LCTech GmbH (Germany)

Your local distributor

SC ADIS INTERNATIONAL SRL
Calea Crangasi Nr. 48, Bucuresti, Romania

T: 021.220.66.30 | M: +40773.74.23.20
office@adis-international.ro
www.adis-international.ro

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www.mn-net.com

MACHEREY-NAGEL

MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany

DE / International:
Tel.: +49 24 21 969-0
Fax: +49 24 21 969-199
E-mail: info@mn-net.com

CH:
Tel.: +41 62 388 55 00
Fax: +41 62 388 55 05
E-mail: sales-ch@mn-net.com

FR:
Tel.: +33 388 68 22 68
Fax: +33 388 51 76 88
E-mail: sales-fr@mn-net.com

US:
Tel.: +1 484 821 0984
Fax: +1 484 821 1272
E-Mail: sales-us@mn-net.com

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