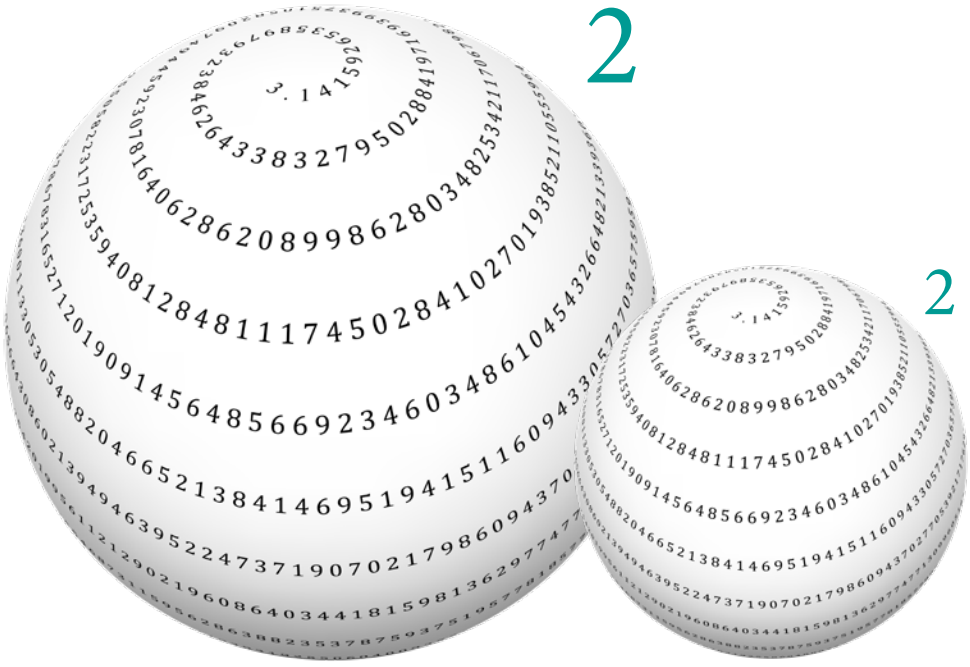
Chromatography

MACHEREY-NAGEL

NUCLEODUR® π^2



Now available in 5 µm and 3 µm particle size

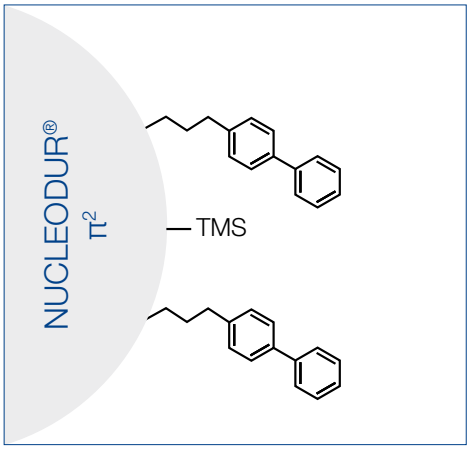
- Hydrophobic phase with alternative selectivity compared to classical C_{18} modifications
- Enhanced π-π interactions in HPLC
- Suitable for 100 % aqueous mobile phase

ADIS INTERNATIONAL
MN Since 1911

NUCLEODUR® π²

Key features

- Hydrophobic phase with alternative selectivity compared to classical C_{18} modifications
- Separation mode based on two retention mechanisms: π-π interactions and hydrophobic interactions
- Better retention of aromatic and unsaturated substances
- Excellent performance under highly aqueous conditions
- Pronounced orthogonal selectivity in acetonitrile and especially in methanol



Recommended applications

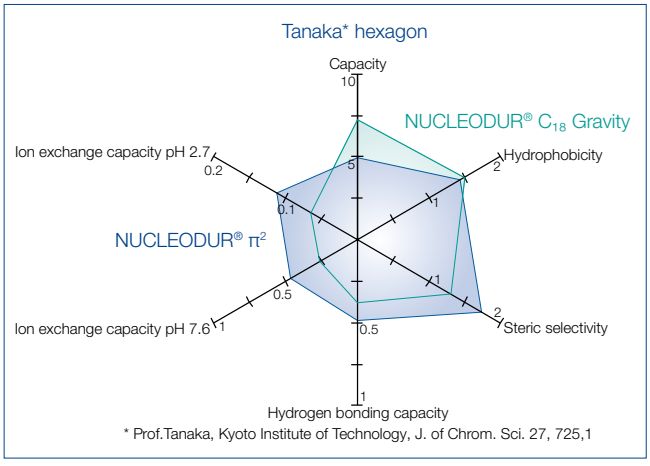
- Overall sophisticated analytical separations
- Aromatic and unsaturated compounds
- Polar compounds
- Pharmaceuticals
- Antibiotics
- Steroids
- Pesticides

USP L11

Similar phases: Pinnacle® DB Biphenyl, Ultra Biphenyl

Technical data

Biphenylpropyl modification with multi-endcapping	
pH stability:	3–10
Particle size:	3 µm and 5 µm
Pore size:	110 Å
Specific surface:	340 m^2/g
Carbon content:	17 %



Stability under aqueous conditions

MN Appl. No. 127900

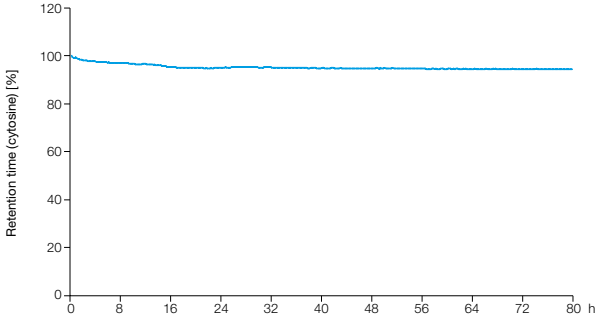
Chromatographic conditions

Column:	125/4 NUCLEODUR® π², 5 µm
MN REF:	760623.40
Eluent:	20 mmol/L potassium dihydrogen phosphate in water (adjusted to pH 3.1 by 85 % phosphoric acid)
Flow rate:	1 mL/min
Temperature:	20 °C
Detection:	UV, 220 nm
Injection:	1 µL cytosine in methanol – buffer (1:1, v/v)

Enhanced stability

Excellent performance using 100 % aqueous mobile phase.

Chromatogram



Steroids

MN Appl. No. 127910

Chromatographic conditions

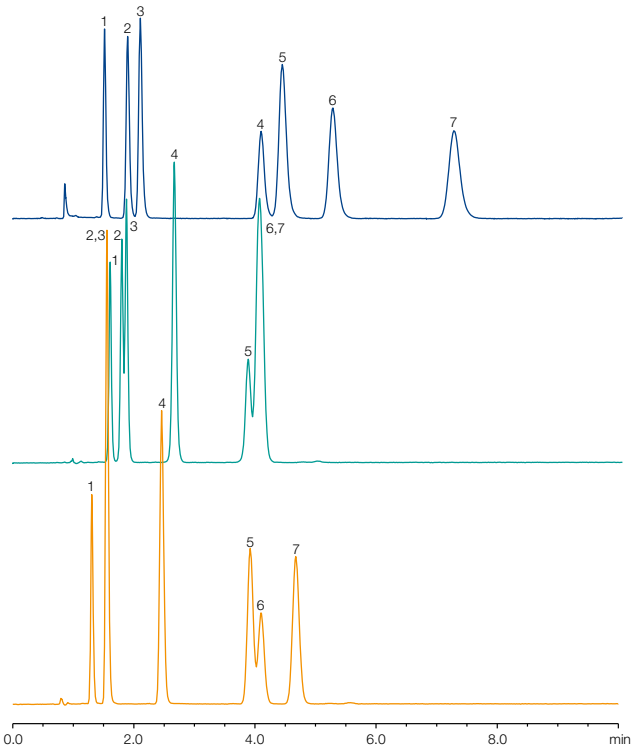
Column:	125/4 each NUCLEODUR® π², 5 µm NUCLEODUR® Phenyl-Hexyl, 5 µm NUCLEODUR® C_{18} Gravity, 5 µm
MN REF:	760623.40 (NUCLEODUR® π²)
Eluent:	acetonitrile – water (45:55, v/v)
Flow rate:	1 mL/min
Temperature:	25 °C
Detection:	UV, 230 nm
Injection:	1 µL

Analyte	Peak No.
1. Estriol	1
2. Hydrocortisone	2
3. Prednisone	3
4. β-Estradiol	4
5. Corticosterone	5
6. Cortisonacetate	6
7. Testosterone	7

Alternative selectivity

A complete separation was only achieved on NUCLEODUR® π².

Chromatograms



Amines

MN Appl. No. 128580

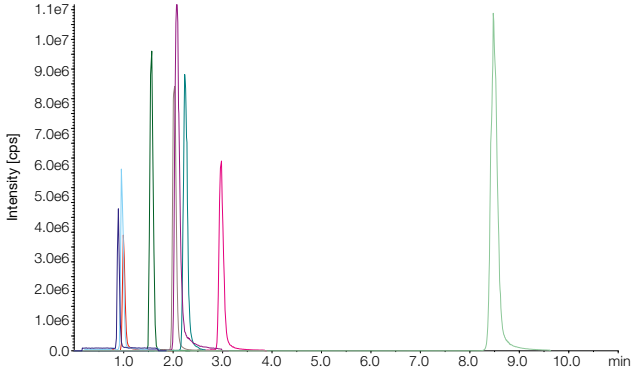
Chromatographic conditions

Column:	EC 150/2 NUCLEODUR® π², 5 µm
MN REF:	760624.20
Eluent A:	0.02 % formic acid in water
Eluent B:	0.02 % formic acid in acetonitrile
Gradient:	in 10 min from 5 % to 85 % B, hold for 10.0 min, in 1.0 min to 5 % B, hold 5 % B for 9.0 min
Flow rate:	0.3 mL/min
Temperature:	40 °C
Detection:	MS, SMRM
Injection:	5 µL

Concentration

300 ng/mL for each analyte

Chromatogram



MRM transitions

Analyte	RT [min]	$[M+H]^+$	Q_1 (Quantifier)	Q_2 (Qualifier)
Trimethylamine	0.92	60.0	44.0	45.0
2-(Diethyl-amino)-ethanol	0.96	118.0	100.1	72.0
Pyridine	1.08	80.0	53.0	
Aniline	1.49	93.9	77.1	
p-Toluidine	2.11	107.9	93.1	65.1
N,N-Dimethyl-benzylamine	2.22	136.0	91.0	65.0
Di*iso*butylamine	2.37	136.0	91.0	65.0
Dibutylamine	3.09	130.1	57.0	41.0
Dibenzylamine	8.87	198.0	91.1	65.0

Anthocyanins in blueberry juice

MN Appl. No. 128260

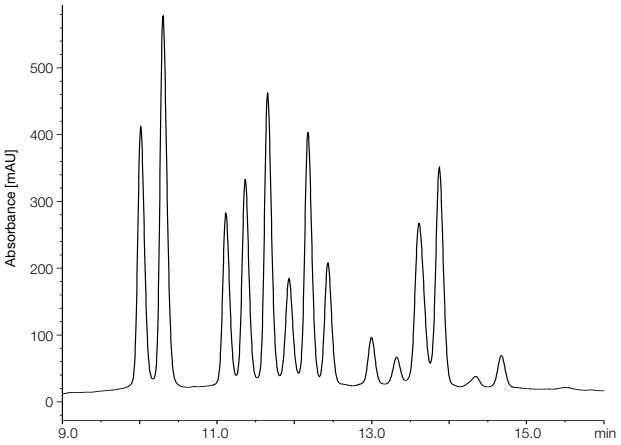
Chromatographic conditions

Column:	EC 250/4 NUCLEODUR® π², 5 µm
MN REF:	760625.40
Eluent A:	5 % formic acid in water
Eluent B:	methanol
Gradient:	5–95 % B in 30 min, hold for 10 min, back to 5 % B in 1 min, hold for 9 min
Flow rate:	0.75 mL/min
Temperature:	65 °C
Detection:	Vis, 520 nm
Injection:	20 µL

Concentration

Blueberry juice was diluted 10 times with eluent A, following filtration with CHROMAFIL® Xtra PET-20/13 (MN REF 729222)

Chromatogram



MRM transitions

Analyte	RT [min]
Delphinidin 3-galactoside	10.5
Delphinidin 3-glucoside	10.8
Delphinidin 3-arabinoside	11.6
Cyanidin 3-galactoside	11.9
Cyanidin 3-glucoside	12.2
Petunidin 3-galactoside	12.5
Petunidin 3-glucoside	12.7

Analyte	RT [min]
Cyanidin 3-arabinoside	13.0
Petunidin 3-arabinoside	13.6
Peonidin 3-galactoside	13.9
Malvidin 3-galactoside + Peonidin 3-glucoside	14.2
Malvidin 3-glucoside	14.4
Malvidin 3-arabinoside	14.9

Triarylmethane dyes

MN Appl. No. 128430

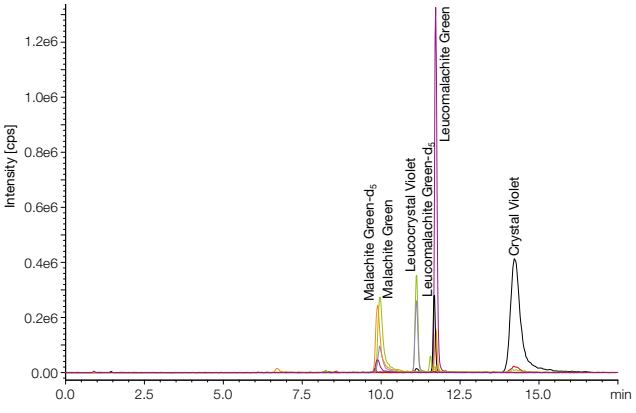
Chromatographic conditions

Column:	EC 100/3 NUCLEODUR® π², 3 µm
MN REF:	760636.30
Eluent A:	5 mmol/L ammonium acetate + 0.1 % formic acid in water
Eluent B:	acetonitrile
Gradient:	in 10 min from 20 % to 85 % B, hold for 5.0 min, in 1.0 min to 20 % B, hold 20 % B for 5.0 min
Flow rate:	0.4 mL/min
Temperature:	25 °C
Detection:	MS, MRM
Injection:	5 µL

Concentration

0.25 ng/mL in eluent A for each analyte

Chromatogram



MRM transitions

Analyte	RT [min]	[M+H]⁺	Q_1 (Quantifier)	Q_2 (Qualifier)
Malachite Green	10.1	328.9	313.1	207.9
Malachite Green-d_5	10.1	333.9	318.3	213.1
Leucocrystal Violet	11.2	373.9	358.0	238.2
Leucomalachite Green-d_5	11.7	335.9	321.1	243.1

Analyte	RT [min]	[M+H]⁺	Q_1 (Quantifier)	Q_2 (Qualifier)
Leucomalachite Green	11.8	330.9	316.2	239.1
Crystal Violet	14.8	372.2	356.3	251.1

Isothiazolinones

MN Appl. No. 128440

Chromatographic conditions

Column:	EC 50/4.6 NUCLEODUR® π², 3 µm
MN REF:	760633.46
Eluent A:	0.1 % formic acid in water
Eluent B:	acetonitrile – methanol (80:20, v/v)
Gradient:	in 0.5 min from 0 % to 5.0 % B, in 2.0 min from 5 % to 95 % B, hold 95 % B for 0.5 min, in 0.1 min to 0 % B, hold 0 % B for 1.9 min
Flow rate:	1.3 mL/min
Temperature:	30 °C
Detection:	MS, MRM
Injection:	5 µL

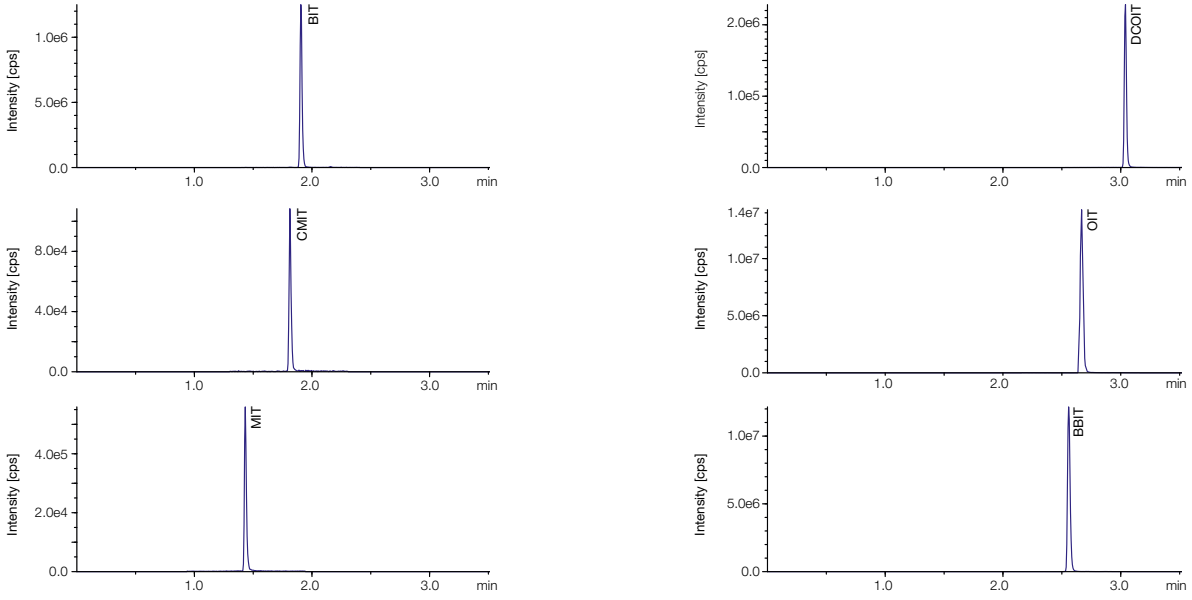
Concentration

100 ng/mL in water for each analyte

MRM transitions

Analyte	RT [min]	$[M+H]^+$	Q_1 (Quantifier)	Q_2 (Qualifier)
2-Methyl-4-isothiazolin-3-one (MIT)	1.43	116.1	53.1	45.0
5-Chloro-2-methyl-4-isothiazolin-3-one (CMIT)	1.81	149.9	87.0	135.0
1,2-Benzisothiazol-3(2H)-one (BIT)	1.91	152.0	109.0	134.1
2-Butyl-1,2-benzisothiazolin-3-one (BBIT)	2.52	208.1	152.0	109.0
2-Octyl-4-isothiazolin-3-one (OIT)	2.67	214.2	102.0	43.1
4,5-Dichloro-2-octyl-isothiazolinone (DCOIT)	3.04	282.1	169.8	73.0

Chromatograms



Drug analytes

MN Appl. No. 128380

Chromatographic conditions

Column:	EC 50/4.6 NUCLEODUR ® π², 3 µm
MN REF:	760633.46
Eluent A:	0.1 % formic acid in water
Eluent B:	0.1 % formic acid in methanol
Gradient:	in 4.5 min from 5 % to 90 % B, hold for 0.5 min, in 0.5 min to 5 % B, hold 5 % B for 4.5 min
Flow rate:	1.3 mL/min
Temperature:	30 °C
Detection:	MS, MRM
Injection:	5 µL

Concentration

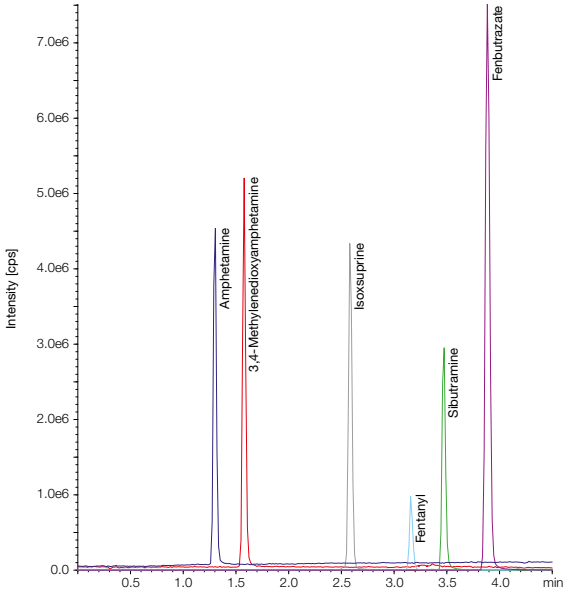
50 ng/mL each in water for each analyte

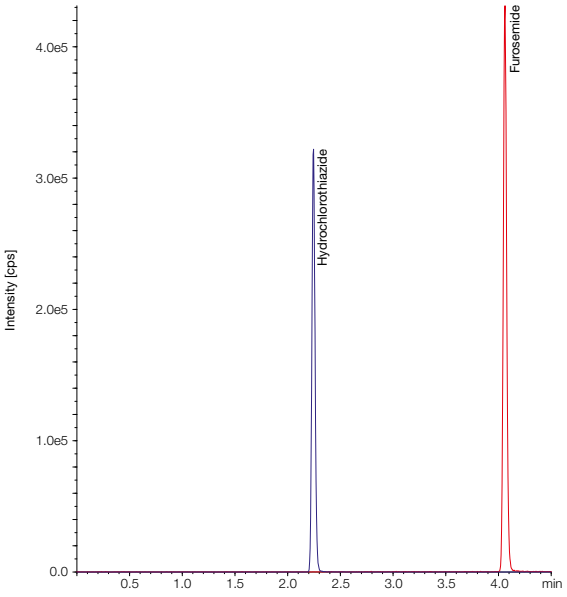
MRM transitions

Analyte	RT [min]	$[M+H]^+$	Q_1 (Quantifier)	Q_2 (Qualifier)
Amphetamine	1.30	136.0	91.1	108.9
3,4-Methylenedi-oxyamphetamine	1.58	180.0	163.1	105.0
Isoxsuprine	2.58	303.0	285.1	77.1
Fentanyl	3.16	337.0	304.9	105.1
Sibutramine	3.47	280.0	125.0	139.1
Fenbutrazate	3.89	368.2	191.1	91.1

Analyte	RT [min]	$[M-H]^-$	Q_1 (Quantifier)	Q_2 (Qualifier)
Hydrochlorothi-azide	2.24	295.9	268.7	98.9
Furosemide	4.06	329.0	283.2	255.2

Chromatograms





Nitrosamines

MN Appl. No. 128360

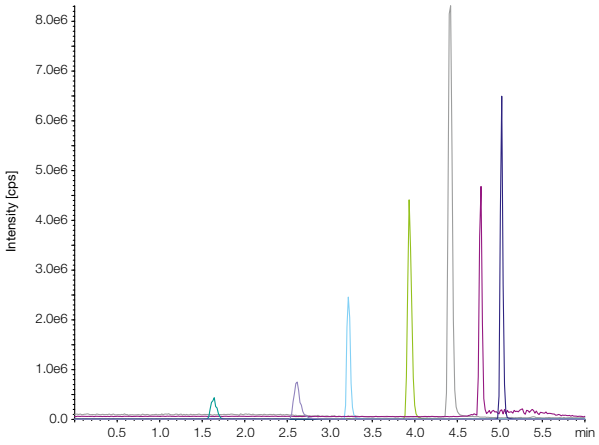
Chromatographic conditions

Column:	EC 100/2 NUCLEODUR® π², 3 µm
MN REF:	760636.20
Eluent A:	0.1 % formic acid in water
Eluent B:	0.1 % formic acid in methanol
Gradient:	hold 2.5 % B for 1.0 min, in 1 min from 2.5 % to 50 % B, hold for 1.0 min, in 0.5 min to 97.5 % B, hold for 2.5 min, in 0.1 min to 2.5 % B, hold for 3.0 min
Flow rate:	0.3 mL/min
Temperature:	40 °C
Detection:	MS, MRM
Injection:	20 µL

Concentration

100 ng/mL in water for each analyte

Chromatogram



MRM transitions

Analyte	RT [min]	[M+H]⁺	Q_1 (Quantifier)	Q_2 (Qualifier)
N-Nitroso-diethanolamine	1.64	135.1	74.0	104.1
N-Nitroso-dimethylamine	2.61	75.1	58.0	
N-Nitroso-morpholine	3.22	117.0	45.0	86.0
N-Nitroso-diethylamine	3.94	103.1	75.1	47.0
N-Nitroso-piperidine	4.41	115.1	69.1	41.0
N-Nitroso-dipropylamine	4.77	131.1	89.2	43.0
N-Nitroso-dibutylamine	5.02	159.2	57.1	103.2

THC and its metabolites

MN Appl. No. 128640

Chromatographic conditions

Column:	EC 50/4.6 NUCLEODUR® π², 3 µm
MN REF:	760633.46
Eluent A:	0.1 % formic acid in water
Eluent B:	0.1 % formic acid in acetonitrile
Gradient:	in 2.5 min from 0 % to 90 % B, hold for 0.5 min, in 0.1 min to 0 % B, hold 0 % B for 2.9 min
Flow rate:	1.3 mL/min
Temperature:	40 °C
Detection:	MS, SMRM
Injection:	5 µL

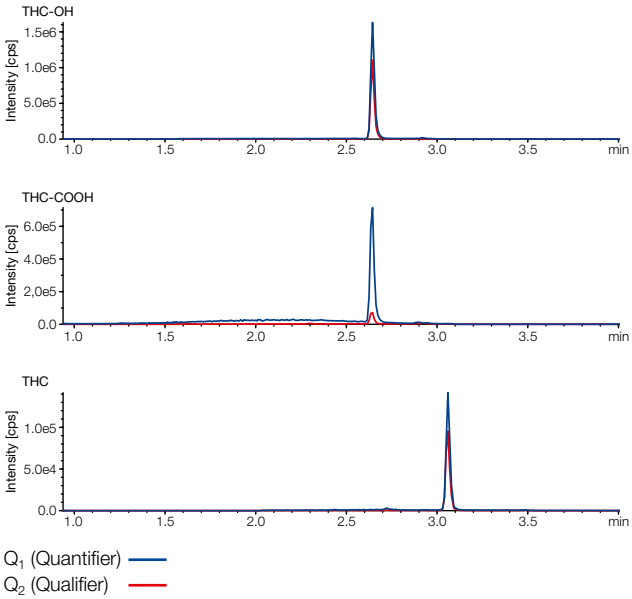
Concentration

50 ng/mL in water for each analyte

MRM transitions

Analyte	RT [min]	[M+H]⁺	Q_1 (Quantifier)	Q_2 (Qualifier)
THC-OH	2.64	331.2	99.1	43.1
THC-COOH	2.64	345.2	327.2	299.2
THC	3.06	315.2	193.1	123.1

Chromatograms



Q_1 (Quantifier)
Q_2 (Qualifier)

Pesticides

MN Appl. No. 128650

Chromatographic conditions

Column:	EC 50/4.6 NUCLEODUR® π², 3 µm
MN REF:	760633.46
Eluent A:	0.1 % formic acid in water
Eluent B:	0.1 % formic acid in methanol
Gradient:	in 5 min from 5 % to 100 % B, hold for 1.0 min. in 0.1 min to 5 % B, hold 5 % B for 3.9 min
Flow rate:	1.3 mL/min
Temperature:	30 °C
Detection:	MS, SMRM
Injection:	5 µL

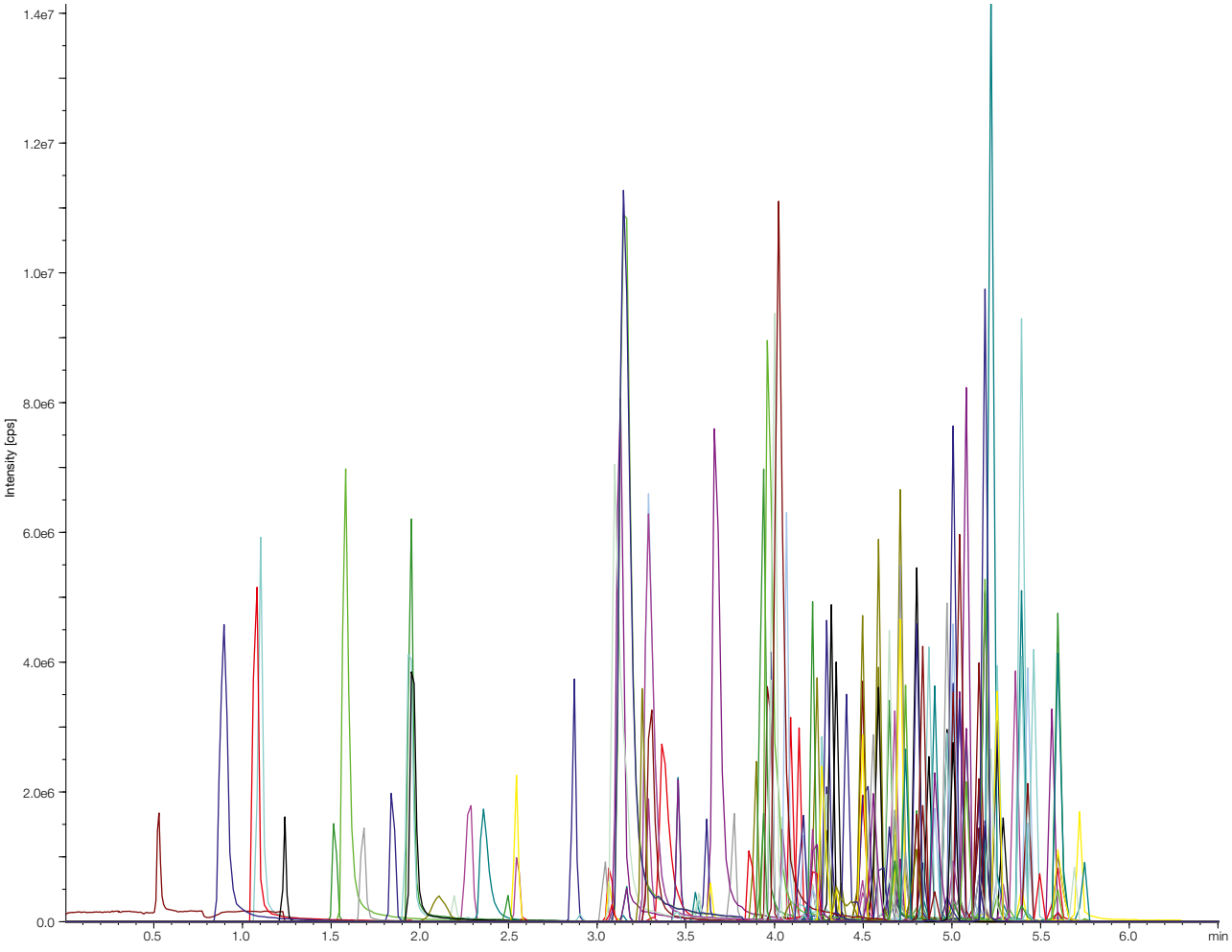
Fast analysis

LC/MS analysis of more than 170 pesticides in less than 6 minutes on NUCLEODUR® π², 3 µm.

Concentration

50 ng/mL in water for each analyte

Chromatogram



MRM transitions

Analyte	RT [min]
Cyromazine	0.72
Methamidophos	1.27
Aminocarb	1.40
Acephate	1.55

Analyte	RT [min]
Pymetrozine	1.79
Omethoate	1.88
Mexacarbate	2.20
Thiabendazole	2.26

Continued on page 9

Continued from page 8

Analyte	RT [min]
Fuberidazole	2.27
Aldicarb sulfone	2.35
Nitenpyram	2.35
Monocrotophos	2.55
Flonicamid	2.58
Oxamyl	2.58
Methomyl	2.67
Ethirimol	2.70
Dicrotophos	2.92
Fenuron	3.10
3-Hydroxycarbofuran	3.13
Mevinphos (mix of isomers)	3.20
Thiamethoxam	3.25
Clothianidin	3.26
Prometon	3.26
Dioxacarb	3.30
Secbumeton	3.30
Terbumeton	3.30
Vamidothion	3.34
Dimethoate	3.39
Pirimicarb	3.41
Simetryn	3.43
Imazalil	3.55
Spiroxamine (mix of isomers)	3.62
Aldicarb	3.65
Butocarboxim	3.66
Carbetamide	3.67
Ametryn	3.75
Fenpropimorph	3.75
Imidacloprid	3.79
Propoxur	3.94
Bendiocarb	3.96
Acetamiprid	4.00
Thidiazuron	4.00
Methoprotryne	4.01
Prometryn	4.03
Tebuthiuron	4.04
Carbofuran	4.06
Fluometuron	4.06
Terbutryn	4.09
Carbaryl	4.12
Propham	4.13
Isoprocarb	4.15
Pyracarbolid	4.16
Metribuzin	4.17

Analyte	RT [min]
Monolinuron	4.18
Chlorotoluron	4.19
Isoproturon	4.26
Ethiofencarb	4.27
Cycluron	4.33
Forchlorfenuron	4.34
Hydramethylnon	4.35
Pyrimethanil	4.35
Carboxin	4.36
Fenobucarb	4.39
Oxadixyl	4.39
Isocarbophos	4.42
Flutriafol	4.43
Siduron	4.45
Diuron	4.46
Thiacloprid	4.48
Fludioxinil	4.53
Promecarb	4.53
Spirotetramat	4.54
Halofenozide	4.55
Tricyclazole	4.55
Diethofencarb	4.56
Emamectin B1a benzoate	4.57
Spinosad (Spinosyn A)	4.57
Linuron	4.60
Metobromuron	4.60
Iprovalicarb (mix of isomers)	4.62
Ethiprole	4.63
Metalaxyl	4.63
Paclobutrazol	4.63
Spinetoram	4.64
Spinosad (Spinosyn D)	4.64
Triadimenol	4.64
Butoxycarboxim	4.65
Methabenzthiazuron	4.65
Flutolanil	4.66
Mesotrione	4.66
Methiocarb	4.68
Fipronil	4.70
Cyprodinil	4.74
Cyproconazole (mix of isomers)	4.75
Methoxyfenozide	4.75
Bupirimate	4.76
Fenamidone	4.76
Mepronil	4.77

Continued on page 10

Continued from page 9

Analyte	RT [min]
Chlorantraniliprole	4.78
Ethofumesate	4.82
Fenhexamid	4.82
Furalaxyl	4.82
Triadimefon	4.82
Neburon	4.84
Flubendiamide	4.86
Tetraconazole	4.88
Chloroxuron	4.90
Clethodim (mix of isomers)	4.90
Bifenazate	4.91
Myclobutanil	4.91
Triticonazole	4.93
Mepanipyrim	4.94
Carfentrazone-ethyl	4.99
Diflubenzuron	4.99
Hexaconazole	4.99
Tebuconazole	5.00
Triflumuron	5.04
Diniconazole	5.05
Flufenacet	5.06
Dimoxystrobin	5.10
Fenoxycarb	5.11
Penconazole	5.11
Picoxystrobin	5.12
Flusilazole	5.13
Metconazole	5.13
Butafenacil	5.15
Triflumizole	5.15
Azoxystrobin	5.16
Epoxiconazole	5.16
Mefenacet	5.20
Etaconazole (mix of isomers)	5.21
Kresoxim-methyl	5.24
Diclobutrazol	5.25
Amitraz	5.26
Benalaxyl	5.26

Analyte	RT [min]
Ipconazole (mix of isomers)	5.26
Buprofezin	5.27
Thiobencarb	5.27
Pencycuron	5.29
Bromucanozole (mix of isomers)	5.30
Tebufenpyrad	5.30
Trifloxystrobin	5.31
Cyazofamid	5.32
Fenbuconazole	5.32
Fluoxastrobin	5.32
Prochloraz	5.38
Acibenzolar-S-methyl	5.39
Propiconazole (mix of isomers)	5.40
Clofentezine	5.42
Etoxazole	5.44
Flufenoxuron	5.45
Indoxacarb	5.45
Benzoximate	5.46
Tebufenozide	5.47
Piperonyl butoxide	5.49
Propargite	5.51
Furathiocarb	5.52
Spirodiclofen	5.52
Pyraclostrobin	5.53
Pyriproxyfen	5.55
Difenoconazole (mix of isomers)	5.64
Eprinomectin	5.69
Fenpyroximate	5.71
Mandipropamid	5.71
Hexythiazox	5.72
Temephos	5.73
Fenazaquin	5.78
Quinoxyfen	5.78
Doramectin	5.84
Pyridaben	5.91
Rotenone	6.00

Chromatogram on page 8

Sulfa drugs

MN Appl. No. 128660

Chromatographic conditions

Column:	EC 50/4.6 NUCLEODUR® π², 3 µm
MN REF:	760633.46
Eluent A:	0.1 % formic acid in water
Eluent B:	0.1 % formic acid in methanol
Gradient:	in 1.0 min from 5 % to 20 % B, in 4.0 min to 95 % B, hold 95 % B for 0.5 min, in 0.1 min to 5 % B, hold 5 % B for 4.4 min
Flow rate:	1.3 mL/min
Temperature:	50 °C
Detection:	MS, MRM
Injection:	5 µL

Concentration

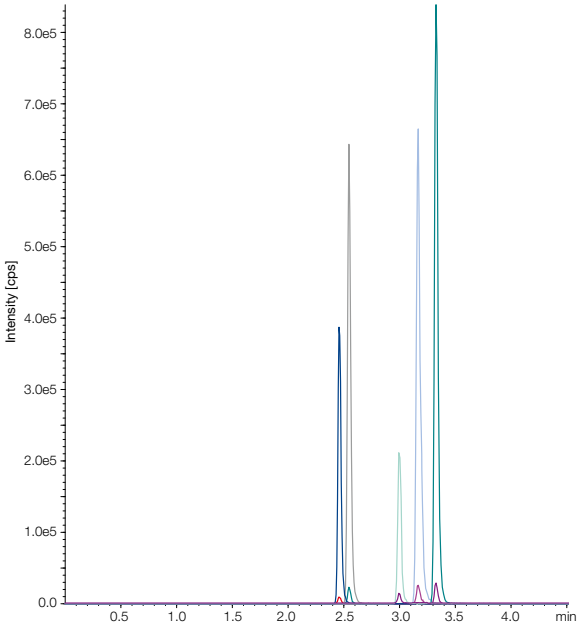
100 ng/mL in water for each analyte

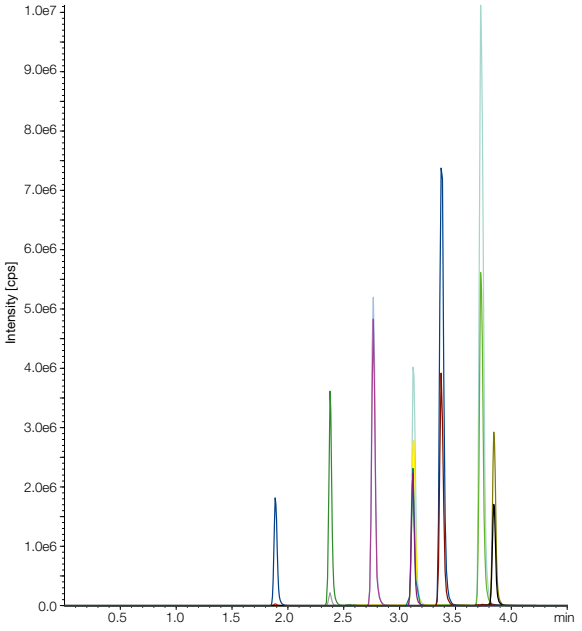
MRM transitions

Analyte	RT [min]	$[M+H]^+$	Q_1 (Quantifier)	Q_2 (Qualifier)
Sulfatiazole	2.44	256.2	156.2	92.1
Sulfapyridine	2.53	250.2	156.1	92.0
Sulfamethoxypyridazine	2.98	281.2	156.1	92.2
Sulfamonomethoxine	3.15	281.2	156.1	92.2
Sulfachlorpyridazine	3.31	285.2	156.1	92.1

Analyte	RT [min]	$[M+H]^+$	Q_1 (Quantifier)	Q_2 (Qualifier)
Sulfacetamide	1.87	215.2	156.2	92.1
Sulfadiazine	2.36	251.2	156.1	92.1
Sulfamerazine	2.75	265.1	156.1	92.1
Sulfadimidine	3.11	279.2	185.9	65.0
Sulfamethoxazole	3.11	254.2	156.1	92.1
Sulfadoxine	3.36	311.1	156.1	92.1
Sulfadimethoxine	3.72	311.1	156.1	92.1
Sulfaquinoxaline	3.84	301.2	156.1	92.1

Chromatograms





Ordering information

Length	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm	EC guard columns*
NUCLEODUR® π², 3 µm							
EC columns (pack of 1)							
2 mm ID	760633.20	760635.20	760636.20	760637.20	760638.20	760639.20	761811.20 (4/2)
3 mm ID	760633.30	760635.30	760636.30	760637.30	760638.30	760639.30	761811.30 (4/3)
4 mm ID	760633.40	760635.40	760636.40	760637.40	760638.40	760639.40	761811.30 (4/3)
4.6 mm ID	760633.46	760635.46	760636.46	760637.46	760638.46	760639.46	761811.30 (4/3)
NUCLEODUR® π², 5 µm							
EC columns (pack of 1)							
2 mm ID	760620.20	760621.20	760622.20	760623.20	760624.20	760625.20	761810.20 (4/2)
3 mm ID	760620.30	760621.30	760622.30	760623.30	760624.30	760625.30	761810.30 (4/3)
4 mm ID	760620.40	760621.40	760622.40	760623.40	760624.40	760625.40	761810.30 (4/3)
4.6 mm ID	760620.46	760621.46	760622.46	760623.46	760624.46	760625.46	761810.30 (4/3)

Selection of guard columns

Guard columns for EC columns with ID	2 mm	3 mm	4 mm	4.6 mm
*EC guard columns (pack of 3)	4 x 2 mm	4 x 3 mm	4 x 3 mm	4 x 3 mm
Required guard column holder (Column proctection system)	718966	718966	718966	718966

Guard column holder (Column proctection system)

EC guard columns

Registered trademarks

NUCLEODUR® MACHEREY-NAGEL GmbH & Co. KG (Germany)
Pinnacle® Restek Corporation (USA)

Your local distributor

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KATEN200151 NUCLEODUR pi2 en4 / 1 / 5 / 02.2018 DD · Printed in Germany