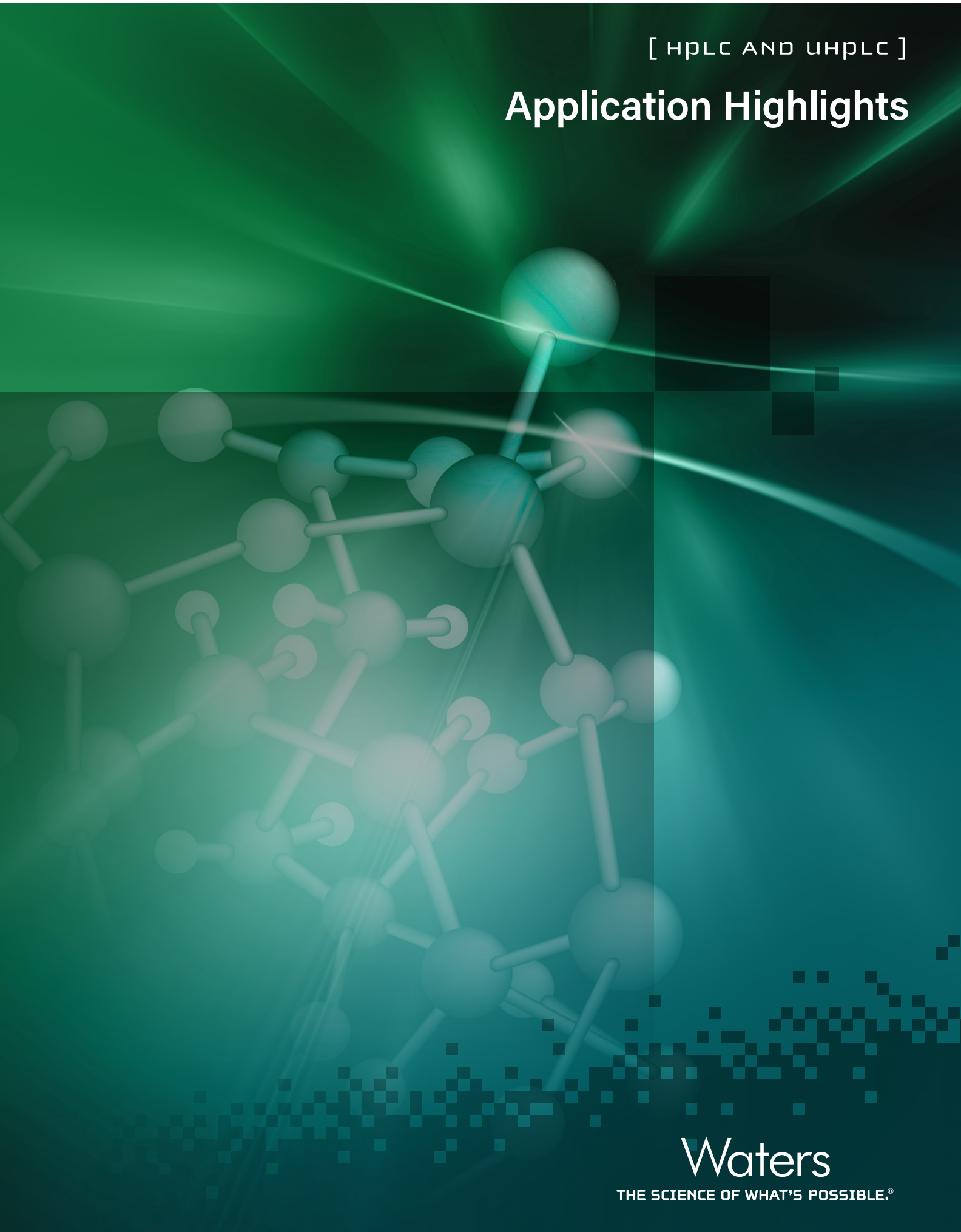
[HPLC AND UHPLC]

Application Highlights



Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Table of Contents

How to Choose a Column

Separation scientists continue to search for innovative solutions to improve chromatographic performance. With a wide array of column choices and formats, they have the ability to select the ideal column for their application. The following section introduces Waters' particle technologies and column formats to help you choose the best column to deliver throughput, resolution, and efficiency for your next chromatographic challenge.

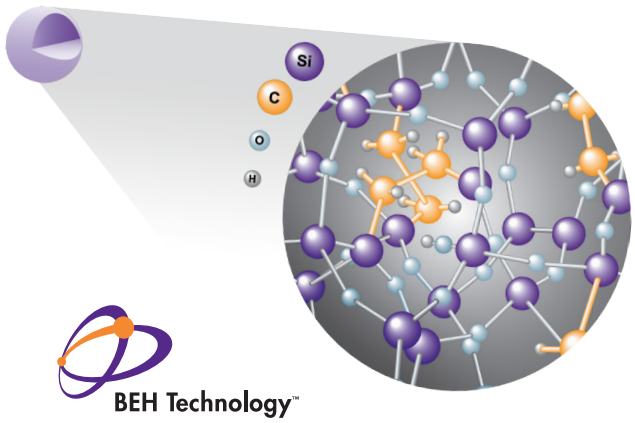
Particle Technology

Reproducibility and transferability are the cornerstones of Waters' BEH, CSH,™ HSS, and solid-core particle technologies. Our premier lines of scalable LC columns exhibit all of the chemical and physical characteristics you would expect from modern LC packing materials.

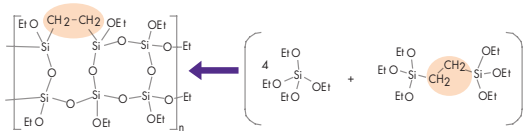
BEH Technology	CSH Technology	HSS Technology	Solid-Core Technology
▪ High retentivity for basic compounds ▪ Exceptional peak shape at elevated pH ▪ Good universal column choice for a wide variety of compounds ▪ Stable across a wide pH range ▪ For separations at high temperatures (80 °C)	▪ Good separations for basic compounds under low pH conditions ▪ Excellent MS performance with formic acid as a mobile phase modifier ▪ Fast pH switching and column equilibration	▪ High retentivity for polar organic compounds and metabolites ▪ Balanced retention of polar and hydrophobic analytes ▪ High strength silica for mechanical stability	▪ Maximum efficiency ▪ Increased sensitivity ▪ Seamless scalability from UPLC to UHPLC to HPLC

ETHYLENE BRIDGED HYBRID (BEH) PARTICLE TECHNOLOGY

Ethylene Bridged Hybrid (BEH) columns lead the industry for chromatographic versatility, chemical resistance, and mechanical stability. You can use them at extremes of pH and temperature to enhance retention and specificity for complex mixtures of acidic, alkaline, and neutral species. The BEH-particle family includes general-purpose and application-specific bonded phases that serve application areas that rely on ACQUITY UPLC,® ACQUITY® UPC,²® ACQUITY APC,® and XBridge® Columns.



Particle Synthesis



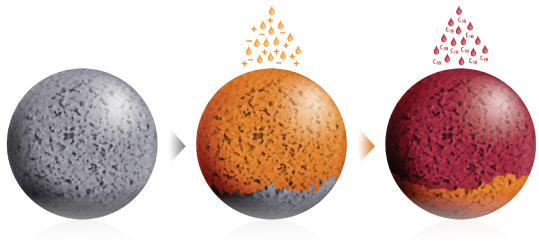
*US Patents 6,686,035; 7,223,473; 7,250,214.

Refer to "Ethylene-Bridged [BEH Technology™] Hybrids and Their Use in Liquid Chromatography" whitepaper (720001159EN) for further detail.

CHARGED SURFACE HYBRID (CSH) PARTICLE TECHNOLOGY

Columns packed with charged surface hybrid particles manifest the best attributes of BEH particles. CSH stationary phases provide chromatographic selectivity and superior performance in the presence of mobile phases of low ionic strength. The optimized surface charge, pore properties, and bonded phases make charged-surface, hybrid-based columns ideal for rapid method development. ACQUITY UPLC CSH and XSelect® CSH HPLC Columns offer easily scaled analytical solutions, from sub-2-µm to preparative-particle dimensions.

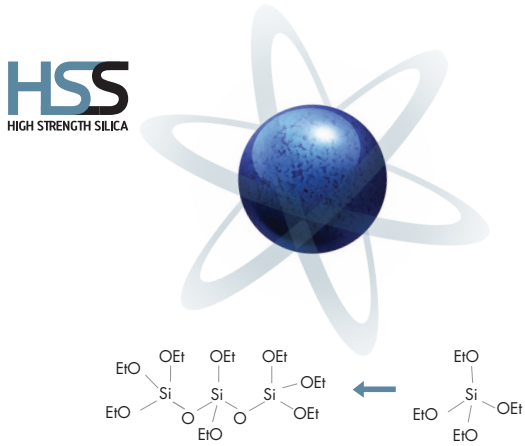
The Charged-Surface Hybrid Particle



Unbonded BEH Particle | Apply Controlled Surface Charge | Bond and End Cap

HIGH STRENGTH SILICA (HSS) PARTICLE TECHNOLOGY

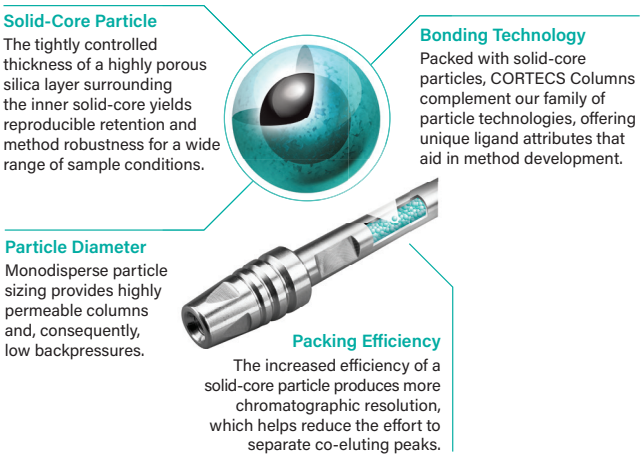
High strength silica [HSS] technology was developed specifically to complement the chromatographic performance of BEH and CSH particles. Compared with the ethylene-bridged BEH and CSH particles, the HSS particle's higher silanophilicity (100% silica) offers chromatographers significant advantages, including increased retention of polar compounds and significantly different selectivity. Additionally, as its name implies, the HSS particle possesses the mechanical strength to operate at pressures as high as 18,000 psi (1240 bar). ACQUITY UPLC HSS and XSelect HSS Columns are the first choice for proven silica-based chromatographic performance.



Polyethoxysilane (PEOS) | Tetraethoxysilane (TEOS)

SOLID-CORE PARTICLE TECHNOLOGY

Compared to columns packed with fully-porous particles, those packed with superficially porous particles demonstrate higher chromatographic efficiency and lower backpressures. The optimized porous layer that surrounds the solid-silica substrate gives rise to the key benefits of speed and efficiency. UPLC® Columns packed with CORTECS® 1.6 µm particles yield maximum efficiency when used with the ultra-low dispersion ACQUITY UPLC instrument platform. Fully scalable CORTECS Columns packed with 2.7 µm particles offer maximum flexibility, providing increased efficiencies at the backpressure limits of UHPLC and HPLC operation.



Column Configurations for Any LC System

COLUMN NOMENCLATURE

Our fully-scalable particle technologies ensure that our LC columns perform with a broad range of chromatographic instrumentation. Depending on the goals of a separation, the instrument platform used, or the sample type, you can choose the most suitable column that is matched to your system's configuration without adversely affecting the chromatographic result.

The following table serves as a guide for selecting an appropriate LC column according to instrument classification.

Nano/Micro	UPLC	UHPLC	HPLC	Preparative
ACQUITY UPLC M-CLASS BEH (1.7 µm)	ACQUITY UPLC BEH (1.7 µm)	XBridge BEH ***XP*** (2.5 µm)	XBridge BEH (3.5, 5 µm)	XBridge BEH OBD™ (5, 10 µm)
ACQUITY UPLC M-CLASS CSH (1.7 µm)	ACQUITY UPLC CSH (1.7 µm)	XSelect CSH ***XP*** (2.5 µm)	XSelect CSH ***XP*** (3.5, 5 µm)	XSelect CSH OBD (5, 10 µm)
ACQUITY UPLC M-CLASS HSS (1.8 µm)	ACQUITY UPLC HSS (1.8 µm)	XSelect HSS ***XP*** (2.5 µm)	XSelect HSS ***XP*** (3.5, 5 µm)	XSelect HSS OBD (5 µm)
—	CORTECS UPLC (1.6 µm)	CORTECS (2.7 µm)	—	—

COLUMN CONFIGURATIONS

System dispersion, the combined effect of tubing and its connections, sample valves, flow cells, and column end-fittings, is inherent in every chromatographic system. Dispersion causes sample peaks to broaden, owing to dilution, that begins at the injector and ends at the detector's outflow. As the size of particles in an LC column are reduced or the internal diameter and length of the column is decreased, the potential peak broadening in a non-optimized LC system increases. Optimum column configuration, therefore, depends mainly on the extent of sample dispersion within the LC system.

The following table summarizes the characteristics of Waters LC Systems and matches the column configuration that maintains chromatographic efficiency.





System	NANO/MICRO	UPLC	UHPLC	HPLC	PREPARATIVE
Dispersion	1 µL	<20 µL	22–29 µL	>40 µL	–
Routine Pressure	<15,000 psi	<18,000 psi	<10,000 psi	<4000 psi	<4000 psi
Particle Size	<2 µm	<2 µm	2–3 µm	3–5 µm	>5 µm
Column I.D.	75–300 µm	2.1 mm (1.0 mm)	3.0 mm (2.1 mm)	4.6 mm (3.0 mm)	>7.8 mm
Column Length	50–250 mm	<150 mm	50–150 mm	75–300 mm	50–300 mm

When you transfer LC methods, instrument bandspread is one of the most practical LC-instrument parameters to determine. Knowing the bandspread value helps you develop your own compatible methods, allowing you to seamlessly scale column dimensions or transfer methods between different instrumentation platforms and laboratory functions. The following table recommends column configurations based on nominal instrument bandspread values.

System	Bandspread*	Recommended Column Particle Sizes and I.D.s
Shimadzu Prominence UFLC	41 µL	XBridge 3.5, 5 µm XSelect 3.5, 5 µm CORTECS 2.7 µm **3.0–4.6 mm I.D.**
Alliance 2695 HPLC	29 µL	
Agilent 1260 UHPLC (600 bar)	28 µL	
Thermo Accela UHPLC	21 µL	XBridge 2.5, 3.5, 5 µm XSelect 2.5, 3.5, 5 µm CORTECS 2.7 µm **3.0 mm I.D.**
Agilent 1290 UHPLC (1200 bar)	17 µL	
ACQUITY Arc™	23 µL	XBridge 2.5, 3.5, 5 µm XSelect 2.5, 3.5, 5 µm CORTECS 2.7 µm **3.0 mm I.D.**
ACQUITY UPLC	12 µL	ACQUITY UPLC BEH 1.7 µm ACQUITY UPLC CSH 1.7 µm ACQUITY UPLC HSS 1.8 µm CORTECS UPLC 1.6 µm **2.1 mm I.D.**
ACQUITY UPLC H-Class with Column Manager	12 µL	
ACQUITY UPLC H-Class	9 µL	
ACQUITY UPLC I-Class (FTN)	7.5 µL	ACQUITY UPLC BEH 1.7 µm ACQUITY UPLC CSH 1.7 µm ACQUITY UPLC HSS 1.8 µm CORTECS UPLC 1.6 µm **1.0–2.1 mm I.D.**
ACQUITY UPLC I-Class (FL)	5.5 µL	

*These data are based on nominal values for unmodified systems. As such, they are intended for reference only. Any adjustment to a system's plumbing, connectivity, and configuration changes the instrument bandspread, affecting the quality of chromatography.

For complete experimental details, refer to full application note 720005104EN at waters.com

Analysis of Abacavir

EXPERIMENTAL

LC conditions

System: Alliance HPLC with 2489 TUV detector

Column: CORTECS C_{18}, 2.7 µm, 4.6 x 75 mm

Mobile phase A: 0.1% trifluoroacetic acid in water

Mobile phase B: 85% methanol in water

Gradient:

Time	%A	%B
0.00	95	5
6.38	70	30
10.37	10	90
11.83	10	90
12.12	95	5
15.00	95	5

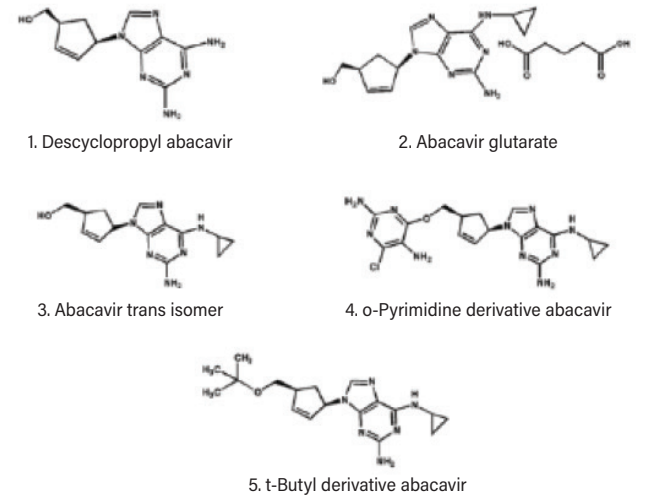
Flow rate: 1.85 mL/min

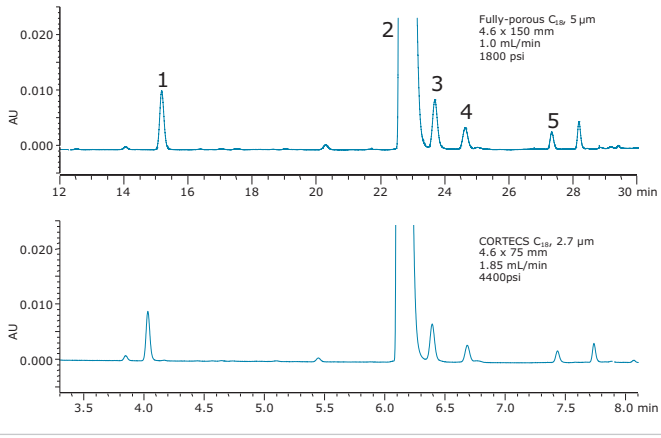
Injection volume: 4 µL

UV detection: 254 nm

Sample preparation

Abacavir-related compounds (USP reference standard)
1.0 mg/mL in 100% HPLC-grade water





ORDERING INFORMATION

Description	P/N
CORTECS C_{18}, 2.7 µm, 4.6 x 75 mm Column	186007376
Waters LCGC Certified Vial w/ Preslit Septa	186000307C

For complete experimental details, refer to full application note WA64697 at waters.com

Analysis of Abacavir Related Compounds

EXPERIMENTAL

LC conditions

System: Alliance HPLC with 2998 PDA detector

Column: CORTECS C_{18}, 2.7 µm, 4.6 x 75 mm

Mobile phase A: 0.1% trifluoroacetic acid in water

Mobile phase B: 85% methanol in water

Gradient:

Time	%A	%B	Curve
Initial	95	5	–
6.38	70	30	6
10.37	10	90	11
11.83	10	90	11
12.12	95	5	11

Flow rate: 1.85 mL/min

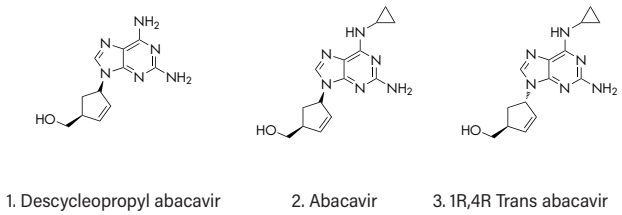
Column temp.: 30 °C

UV detection: 254 nm

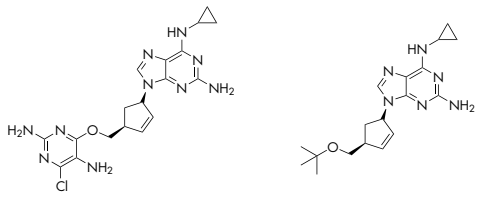
Sample preparation

Sample: Abacavir USP related compounds

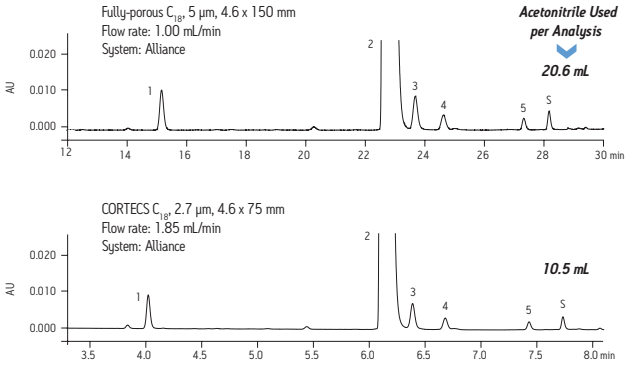
Sample diluent: Water



1. Descycleopropyl abacavir 2. Abacavir 3. 1R,4R Trans abacavir



4. O-(4-Chloro-2,5-diaminopyrimidnyl)-abacavir 5. O-t-Butyl-abacavir



ORDERING INFORMATION

Description	P/N
CORTECS C_{18}, 2.7 µm, 4.6 x 75 mm Column	186007376
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note WA64078 at waters.com

Analysis of 6-Acetylmorphine and Morphine

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC with TQD mass spectrometer

Column: XBridge BEH HILIC, 3.5 µm, 2.1 x 100 mm

Mobile phase A: 10 mM ammonium formate with 0.125% formic acid in 50/50 acetonitrile/water

Mobile phase B: 10 mM ammonium formate with 0.125% formic acid in 90/10 acetonitrile/water

Gradient:

Time	%A	%B
0.00	0.1	99.9
1.05	0.1	99.9
4.35	99.9	0.1
4.50	0.1	99.9
6.00	0.1	99.9

Flow rate: 0.6 mL/min

Column temp.: 30 °C

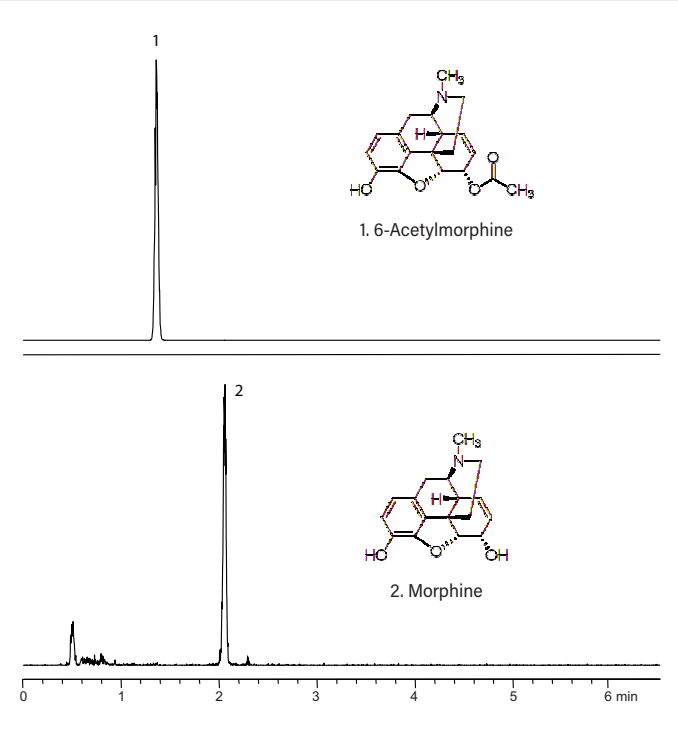
Injection volume: 10.0 µL (PLNO, 20 µL loop)

Ionization mode: ESI+

Acquisition mode: MRM (m/z): morphine 286 > 200.9; 6-acetylmorphine 328 > 164.9

Sample preparation

Sample concentration: 10 ng/mL each



ORDERING INFORMATION

Description	P/N
XBridge BEH HILIC, 3.5 µm, 2.1 x 100 mm Column	186004433
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note WA64115 at waters.com

Analysis of Acrylamide, Methacrylic Acid, and Methacrylamide

EXPERIMENTAL

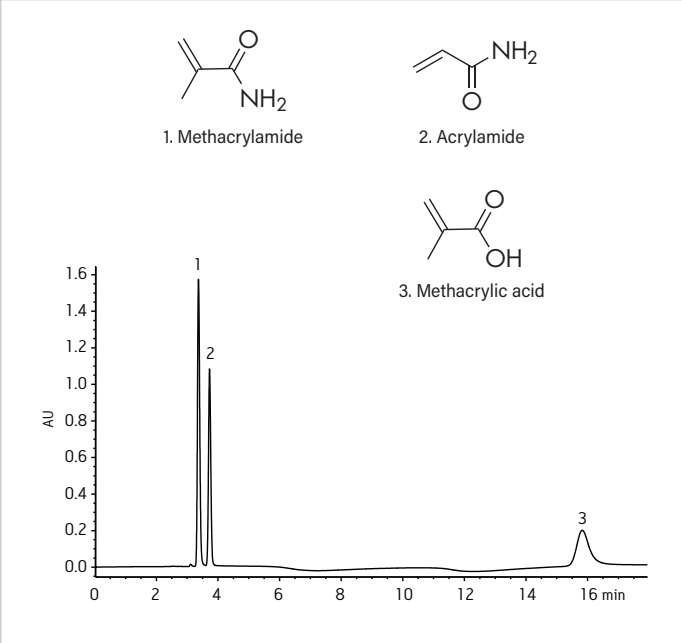
LC conditions

System:	Alliance HPLC with 2998 PDA detector
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase:	95/2.5/2.5 acetonitrile/isopropyl alcohol/ water with 5 mM ammonium acetate, pH 9.0
Separation mode:	Isocratic
Flow rate:	1.2 mL/min
Column temp.:	25 °C
Injection volume:	40.0 µL
UV detection:	210 nm

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.

Sample concentration: 30 µg/mL



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

For complete experimental details, refer to full application note 720002600EN at waters.com

Analysis of Aflatoxins in Red Pepper

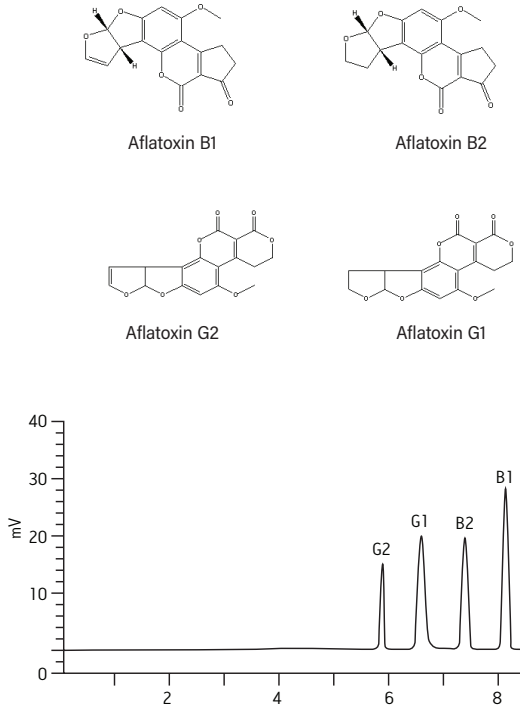
EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2475 Multi Wavelength Fluorescence
Column:	XBridge BEH C_{18}, 5 µm, 4.6 x 250 mm
Mobile phase:	Acetonitrile/water/methanol (17:54:29, v/v/v)
Separation mode:	Isocratic
Flow rate:	1 mL/min
Injection:	100 µL
FLR detection:	Excitation wavelength = 333 nm; Emission wavelength = 460 nm

Sample preparation

Add 100 mL 80:20 methanol:water (v/v) to 50 g ground red pepper and 5 g sodium chloride. Blend at high speed for 1 minute. Filter extract with fluted filter paper, take 65 mL filtrate and dilute with 60 mL phosphate buffer saline, and filter through glass microfiber filter. Load 4 mL filtered diluted extract to AflaTest Affinity Column at a rate of ~1-2 drops/second. Wash column with 10 mL 20:80 methanol:water at a rate of ~2 drops/second, and repeat once more until air comes through column. Elute AflaTest column with 1 mL HPLC-grade methanol at a rate of 1 drop/second and collect eluate, dilute with 1 mL water.



ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 5 µm, 4.6 x 250 mm Column	186003117
Waters LCMS Certified Vial	600000751CV
VICAM AflaOchra HPLC Columns, 25/pk	G1017
VICAM Glass Cuvette	34000

For complete experimental details, refer to full application note WA60195 at waters.com

Analysis of Aflatoxins Standard

EXPERIMENTAL

LC conditions

System:	Alliance 2695 with a fluorescence detector
Column:	XBridge Shield RP18, 5 µm, 4.6 x 150 mm
Mobile phase:	Water/MeOH, 70/30, v/v
Separation mode:	Isocratic
Flow rate:	0.6 mL/min
Column temp.:	40 °C
Injection volume:	10 µL
FLR detection:	Excitation wavelength = 365 nm; Emission wavelength = 455 nm

Sample preparation

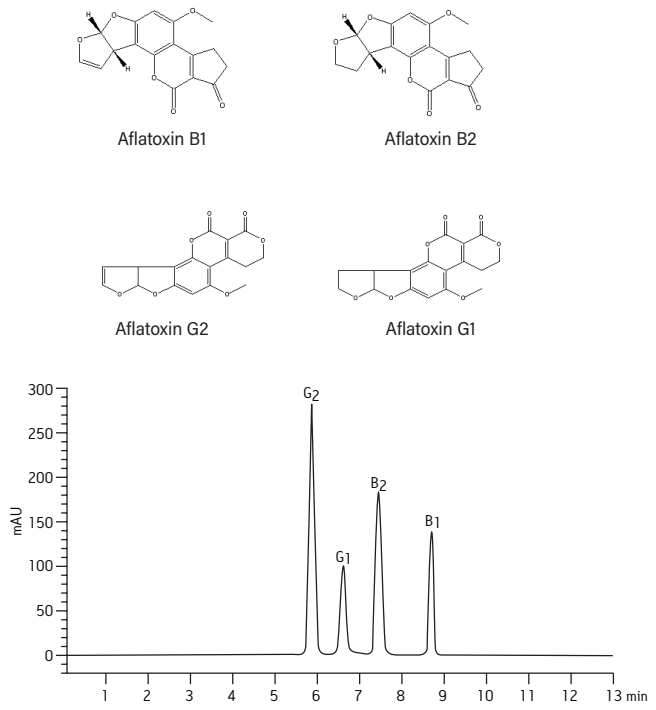
A commercial mixture of aflatoxins B1, B2, G1, and G2. A final solution was appropriately prepared to create the final concentrations:

B1: 250 pg/mL

B2: 25 pg/mL

G1: 250 pg/mL

G2: 25 pg/mL



ORDERING INFORMATION

Description	P/N
XBridge Shield RP18, 5 µm, 4.6 x 150 mm Column	186003009
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note WA64112 at waters.com

Analysis of Allantoin

EXPERIMENTAL

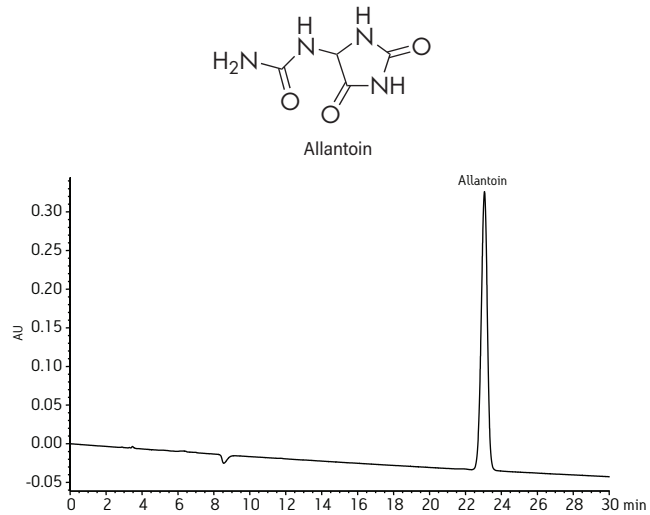
LC conditions

System:	Alliance HPLC with 2998 PDA detector
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase:	90/10 acetonitrile/water
Separation mode:	Isocratic
Flow rate:	0.5 mL/min
Column temp.:	25 °C
Injection volume:	40.0 µL
UV detection:	210 nm

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.

Sample concentration: 100 µg/mL



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

For complete experimental details, refer to full application note 720004492EN at waters.com

Analysis of Allergenic and Carcinogenic Dyes in Industrial, Cosmetics, Personal Care, and Consumer Products

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC H-Class with Xevo TQD Mass Spectrometer

Column: XBridge BEH C_{18}, 3.5 µm, 2.1 x 150 mm

Mobile phase A: Water (5 mmol/L ammonium acetate)

Mobile phase B: Acetonitrile (5 mmol/L ammonium acetate)

Gradient:

Time	%A	%B
0.00	90	10
1.00	70	30
4.00	60	40
9.00	5	95
12.00	5	95
12.10	90	10
17.00	90	10

Flow rate: 0.3 mL/min

Column temp.: 30 °C

Injection volume: 5 µL

Ionization mode: ESI+ and ESI-

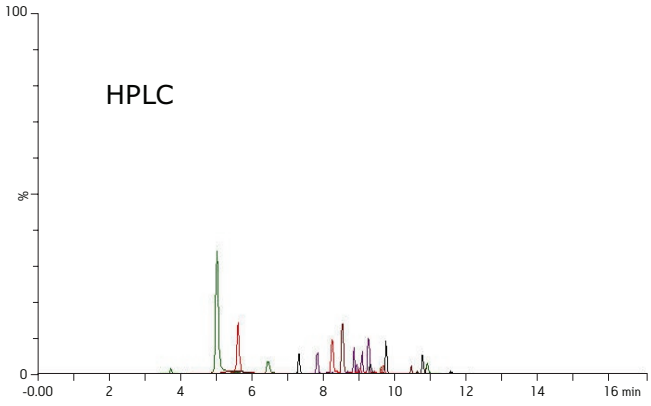
Acquisition mode: MRM

Sample preparation

Textile (0.5 g) was cut up and extracted with 20 mL of methanol for 15 minutes using an ultrasonic bath (50 °C). One hundred microliters of the extract was transferred in an LC vial and diluted with 900 µL of water.

Sample temp.: 10 °C

1. Acid red 26
2. Basic red 9
3. Basic violet 14
4. Direct red 28
5. Disperse blue 102
6. Disperse blue 106
7. Disperse blue 124
8. Disperse blue 3
9. Disperse blue 35
10. Disperse blue 7
11. Disperse brown 1
12. Disperse orange 1
13. Disperse orange 11
14. Disperse orange 149
15. Disperse orange 3
16. Disperse orange 37
17. Disperse red 1
18. Disperse red 11
19. Disperse red 17
20. Disperse yellow 1
21. Disperse yellow 23
22. Disperse yellow 3
23. Disperse yellow 39
24. Disperse yellow 49



ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 3.5 µm, 2.1 x 150 mm Column	186003023
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

Waters Corporation
34 Maple Street, Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990

For complete experimental details, refer to full application note 720004078EN at waters.com

Analysis of Amoxicillin Oral Suspension

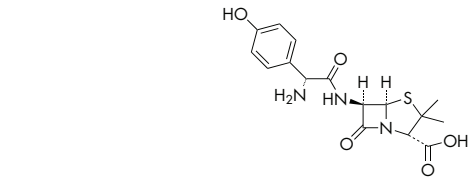
EXPERIMENTAL

LC conditions

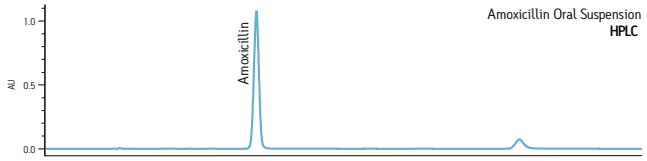
System:	Alliance 2695
Column:	XBridge BEH Shield RP18, 5 µm, 4.6 x 250 mm
Mobile phase:	98:2 diluent:acetonitrile
Diluent:	50 mM potassium phosphate, monobasic in water - pH 5.0 with potassium hydroxide
Separation mode:	Isocratic
Flow rate:	1.5 mL/min
Injection volume:	10 µL
UV detection:	230 nm

Sample preparation

Amoxicillin oral suspension powder, reconsituted in water (50 mg/mL), made up to 1 mg/mL in diluent, filtered through a 0.2 µm nylon filter prior to analysis.



Amoxicillin



ORDERING INFORMATION

Description	P/N
XBridge BEH Shield RP18, 5 µm, 4.6 x 250 mm Column	186003010
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note XBRIDGE15 at waters.com

Analysis of Antibacterials by HPLC

EXPERIMENTAL

LC conditions

System: Alliance 2695 with 2996 PDA detector

Column: XBridge BEH Phenyl, 3.5 µm, 4.6 x 100 mm

Mobile phase A: 20 mM monopotassium phosphate, pH 2.5

Mobile phase B: Acetonitrile

Gradient:

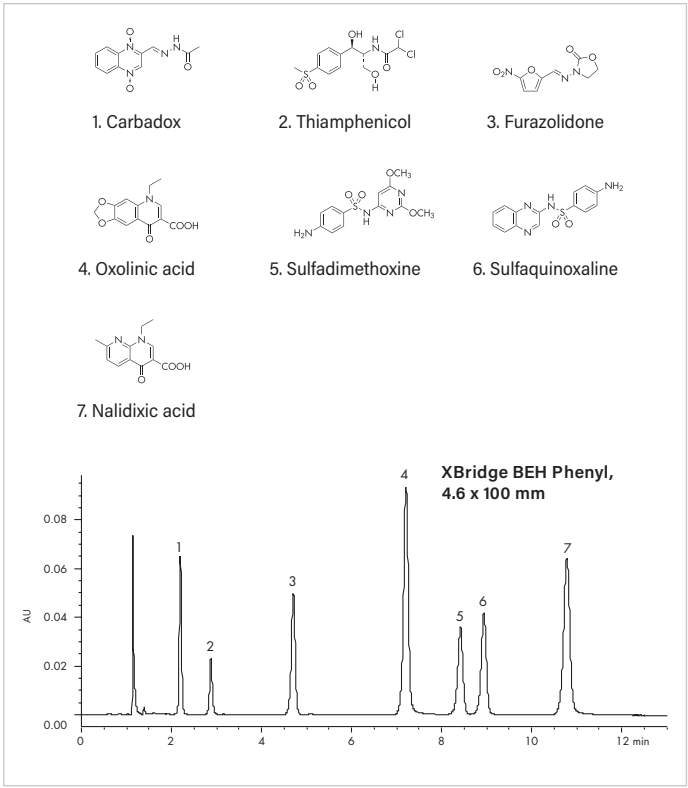
Time	%A	%B
0.00	80	20
2.00	80	20
7.00	75	25
15.00	70	30
16.00	80	20
20.00	80	20

Flow rate: 1 mL/min

Column temp.: 30 °C

Injection volume: 10 µL

UV detection: 254 nm



Sample preparation

Sample: Carbadox (10 µg/mL), Thiamphenicol (100 µg/mL), Furazolidone (10 µg/mL), Oxolinic acid (10 µg/mL), Sulfadimethoxine (10 µg/mL), Sulfaquinoxaline (10 µg/mL), Nalidixic acid (10 µg/mL) in KH_2PO_4/ACN (80/20)

Sample temp.: 15 °C

ORDERING INFORMATION

Description	P/N
XBridge BEH Phenyl, 3.5 µm, 4.6 x 100 mm Column	186003334
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note WA64696 at waters.com

Analysis of Antibacterials by UHPLC

EXPERIMENTAL

LC conditions

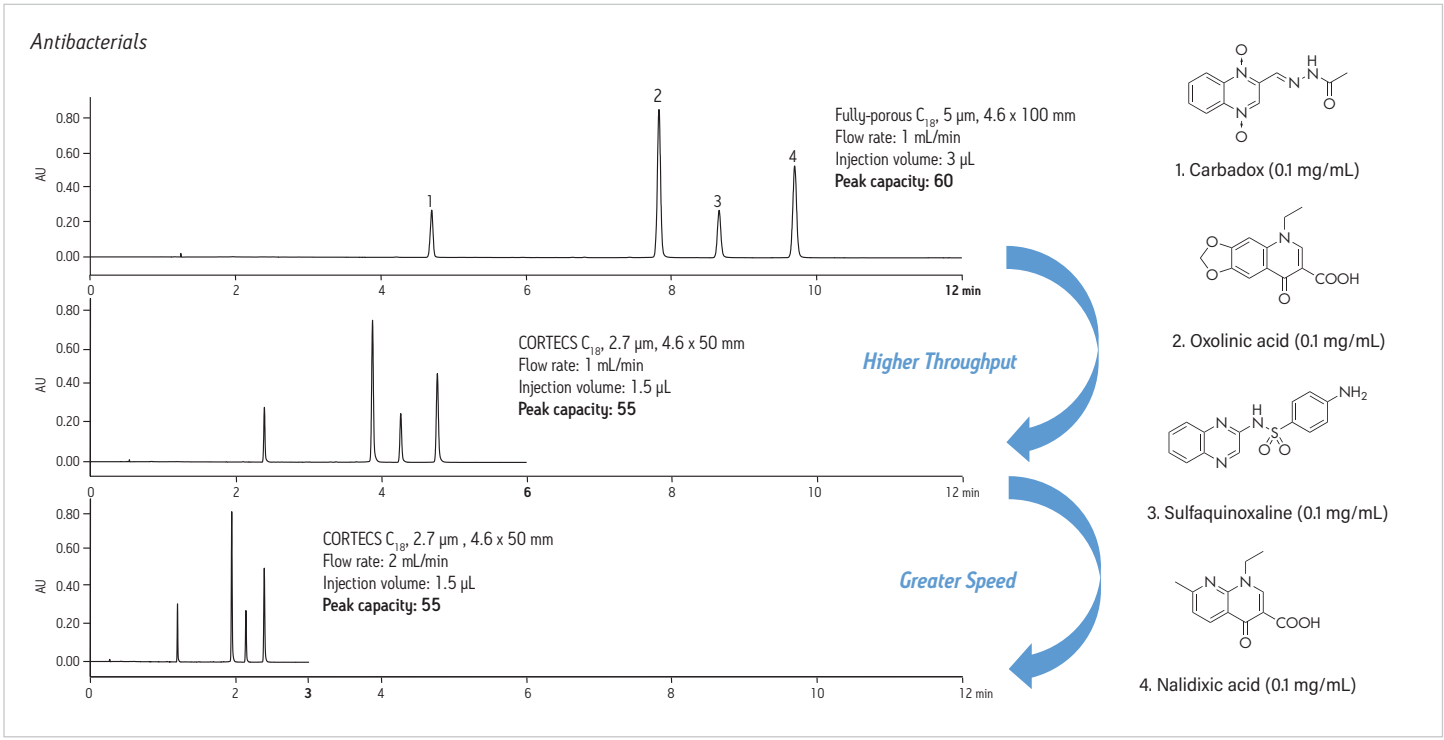
System:	ACQUITY UPLC H-Class with ACQUITY UPLC PDA Detector
Columns:	CORTECS C_{18}, 2.7 µm, 4.6 x 50 mm; Fully-porous C_{18}, 5 µm, 4.6 x 100 mm
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Gradient:	Linear from 5–50% B
Flow rate:	1 mL/min, 2 mL/min
Column temp.:	30 °C
Injection volume:	3 µL, 1.5 µL
UV detection:	254 nm

Sample preparation

Sample diluent: 20% acetonitrile in water

ORDERING INFORMATION

Description	P/N
CORTECS C_{18}, 2.7 µm, 4.6 x 50 mm Column	186007375
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



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For complete experimental details, refer to full application note WA64118 at waters.com

Analysis of Ascorbic and Isoascorbic Acid by Gradient HPLC

EXPERIMENTAL

LC conditions

System: Alliance HPLC with 2998 PDA detector

Column: XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm

Mobile phase A: 50/50 acetonitrile/water with 10 mM ammonium acetate, pH 5.0

Mobile phase B: 90/10 acetonitrile/water with 10 mM ammonium acetate, pH 5.0

Gradient:

Time	%A	%B
0.00	0.1	99.9
48.00	99.9	0.1
48.10	0.1	99.9
72.00	0.1	99.9

Flow rate: 0.5 mL/min

Column temp.: 25 °C

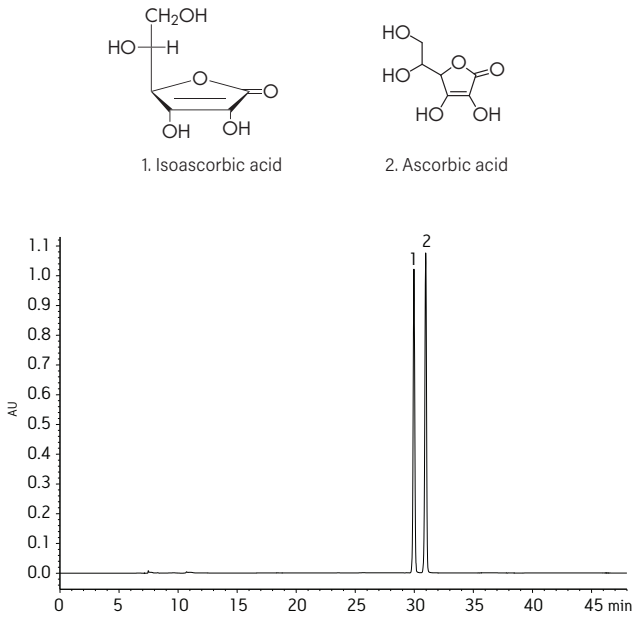
Injection volume: 60.0 µL

UV detection: 260 nm

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.

Sample concentration: 30 µg/mL



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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34 Maple Street, Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990

For complete experimental details, refer to full application note WA64119 at waters.com

Analysis of Ascorbic and Isoascorbic Acid by Isocratic HPLC

EXPERIMENTAL

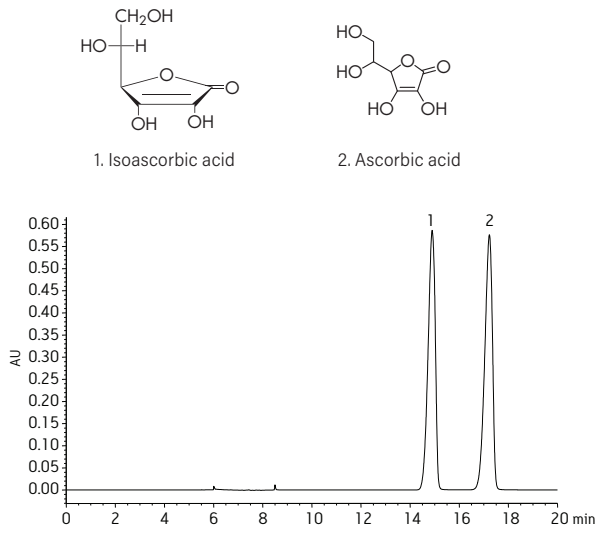
LC conditions

System:	Alliance HPLC with 2998 PDA detector
Column:	XBridge Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase:	80/20 acetonitrile/water with 2 mM monopotassium phosphate
Separation mode:	Isocratic
Flow rate:	0.5 mL/min
Column temp.:	25 °C
Injection volume:	60.0 µL
UV detection:	260 nm

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.

Sample concentration: 30 µg/mL



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

For complete experimental details, refer to full application note 720004440EN at waters.com

Analysis of Avermectins in Meat

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC with Xevo TQ-S Mass Spectrometer

Column: XSelect CSH C_{18} ***XP***, 2.5 µm, 2.1 x 100 mm

Mobile phase A: 5 mM ammonium acetate in water

Mobile phase B: 5 mM ammonium acetate in methanol

Gradient:

Time	%A	%B
0.00	30	70
5.00	3	97
8.00	3	97
8.10	30	70
10.00	30	70

Flow rate: 0.40 mL/min

Column temp.: 35 °C

Injection volume: 5 µL

Ionization mode: ESI+

Acquisition mode: MRM (m/z): abamectin 890.5 > 305.5; ivermectin 892.5 > 307.5; doramectin 916.5 > 331.2; eprinomectin 914.5 > 186.2; moxidectin 640.3 > 528.3

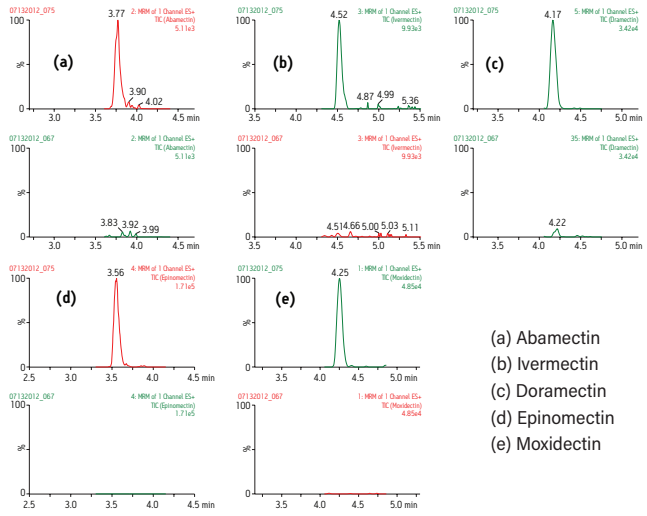
Sample preparation

Initial Extraction (QuEChERS)

Place 8 g ground beef (80% lean) and 2 mL water into a 50 mL centrifuge tube. Add 10 mL acetonitrile and shake the tube vigorously for 1 minute. Add the contents of DisQuE Pouch salts for European Committee for Standardization (CEN) QuEChERS and shake vigorously for 1 minute. Centrifuge for 15 minutes at 4000 rpm and take a 1 mL aliquot of the supernatant (top layer) for d-SPE cleanup.

d-SPE Cleanup

Transfer the 1 mL aliquot of supernatant to a 2-mL d-SPE cleanup tube that contains 150 mg magnesium sulfate and 50 mg C_{18} sorbent and shake vigorously for 1 minute. Centrifuge for 5 minutes at 12,000 rpm and take a 0.5 mL aliquot sample for LC-MS/MS analysis.



Top trace is the low level spiked sample, bottom trace is a ground beef blank.

ORDERING INFORMATION

Description	P/N
XSelect CSH C_{18} ***XP***, 2.5 µm, 2.1 x 100 mm Column	186005275
DisQuE QuEChERS Pouch	186006813
DisQuE QuEChERS 50 mL Centrifuge Tube	186004837
Waters LCMS Certified Maximum Recovery Vial w/ Preslit Septa	600000670CV

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For complete experimental details, refer to full application note 720004440EN at waters.com

Analysis of Avermectins in Milk

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC with Xevo TQ-S Mass Spectrometer

Column: XSelect CSH C_{18} ***XP***, 2.5 µm, 2.1 x 100 mm

Mobile phase A: 5 mM ammonium acetate in water

Mobile phase B: 5 mM ammonium acetate in methanol

Gradient:

Time	%A	%B
0.00	30	70
5.00	3	97
8.00	3	97
8.10	30	70
10.00	30	70

Flow rate: 0.40 mL/min

Column temp.: 35 °C

Injection volume: 5 µL

Ionization mode: ESI+

Acquisition mode: MRM (m/z): abamectin 890.5 > 305.5; ivermectin 892.5 > 307.5; doramectin 916.5 > 331.2; eprinomectin 914.5 > 186.2; moxidectin 640.3 > 528.3

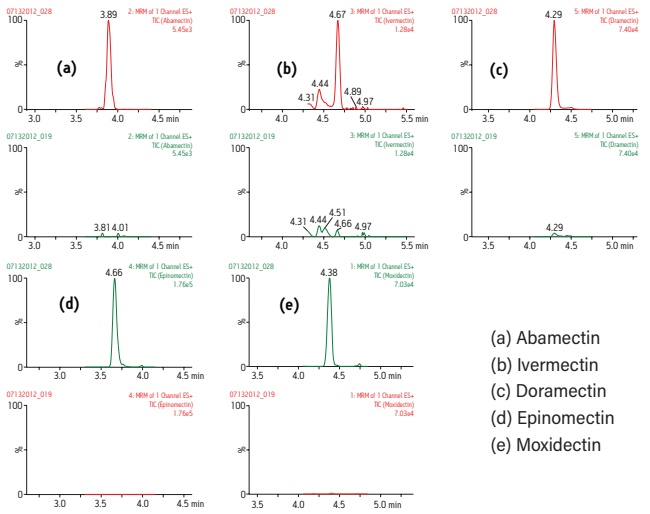
Sample preparation

Initial Extraction (QuEChERS)

Place 10 mL whole milk (pasteurized) into a 50 mL centrifuge tube. Add 10 mL acetonitrile and shake the tube vigorously for 1 minute. Add the contents of DisQuE Pouch salts for European Committee for Standardization (CEN) QuEChERS and shake vigorously for 1 minute. Centrifuge for 15 minutes at 4000 rpm and take a 1 mL aliquot of the supernatant (top layer) for d-SPE cleanup.

d-SPE Cleanup

Transfer the 1 mL aliquot of supernatant to a 2-mL d-SPE cleanup tube that contains 150 mg magnesium sulfate and 50 mg C18 sorbent and shake vigorously for 1 minute. Centrifuge for 5 minutes at 12,000 rpm and take a 0.5 mL aliquot sample for LC-MS/MS analysis.



Top trace is the low level spiked sample, bottom trace is a ground beef blank.

ORDERING INFORMATION

Description	P/N
XSelect CSH C_{18} ***XP***, 2.5 µm, 2.1 x 100 mm Column	186005275
DisQuE QuEChERS Pouch	186006813
DisQuE QuEChERS 50 mL Centrifuge Tube	186004837
Waters LCMS Certified Maximum Recovery Vial w/ Preslit Septa	600000670CV

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For complete experimental details, refer to full application note 720002825EN at waters.com

Analysis of Basic Compounds

EXPERIMENTAL

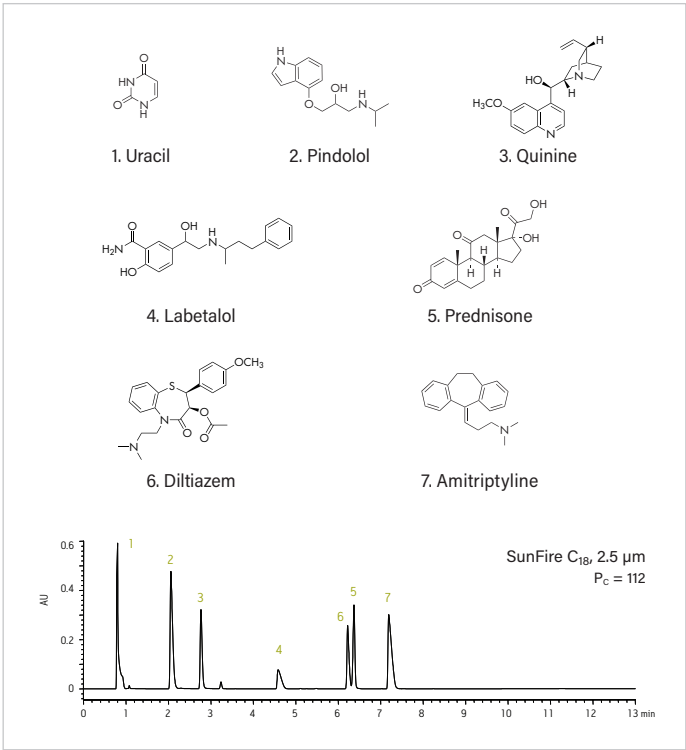
LC conditions

System:	Alliance 2695 with 2998 PDA detector
Column:	SunFire C_{18}, 2.5 µm, 4.6 x 75 mm
Mobile phase A:	10 mM ammonium formate, pH 3
Mobile phase B:	100% acetonitrile

Gradient:

Time	%A	%B
0.00	85	15
13.00	35	65
15.00	35	65
15.10	85	15
24.00	85	15

Flow rate:	1 mL/min
Column temp.:	30 °C
Injection volume:	10 µL
UV detection:	260 nm



Sample preparation

Standard mixture of basic compounds (1) uracil, (2) pindolol, (3) quinine, (4) labetalol, (5) prednisone, (6) diltiazem, (7) amitriptyline.

ORDERING INFORMATION

Description	P/N
SunFire C_{18}, 2.5 µm, 4.6 x 75 mm Column	186003419
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note WA60707 at waters.com

Analysis of Basic Drugs in River Water

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC H-Class with Xevo TQD Mass Spectrometer

Column: CORTECS HILIC, 2.7 µm, 2.1 x 100 mm

Mobile phase A: Water + 0.1% acetic acid/ammonium acetate buffer (1 g/L water)

Mobile phase B: Acetonitrile

Gradient:

Time	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.280	2	98	–
4.50	0.280	30	70	6
10.80	0.280	30	70	6
11.25	0.280	2	98	6
14.40	0.280	2	98	6
14.50	0.280	2	98	6

Flow rate: 0.28 mL/min

Column temp.: 45 °C

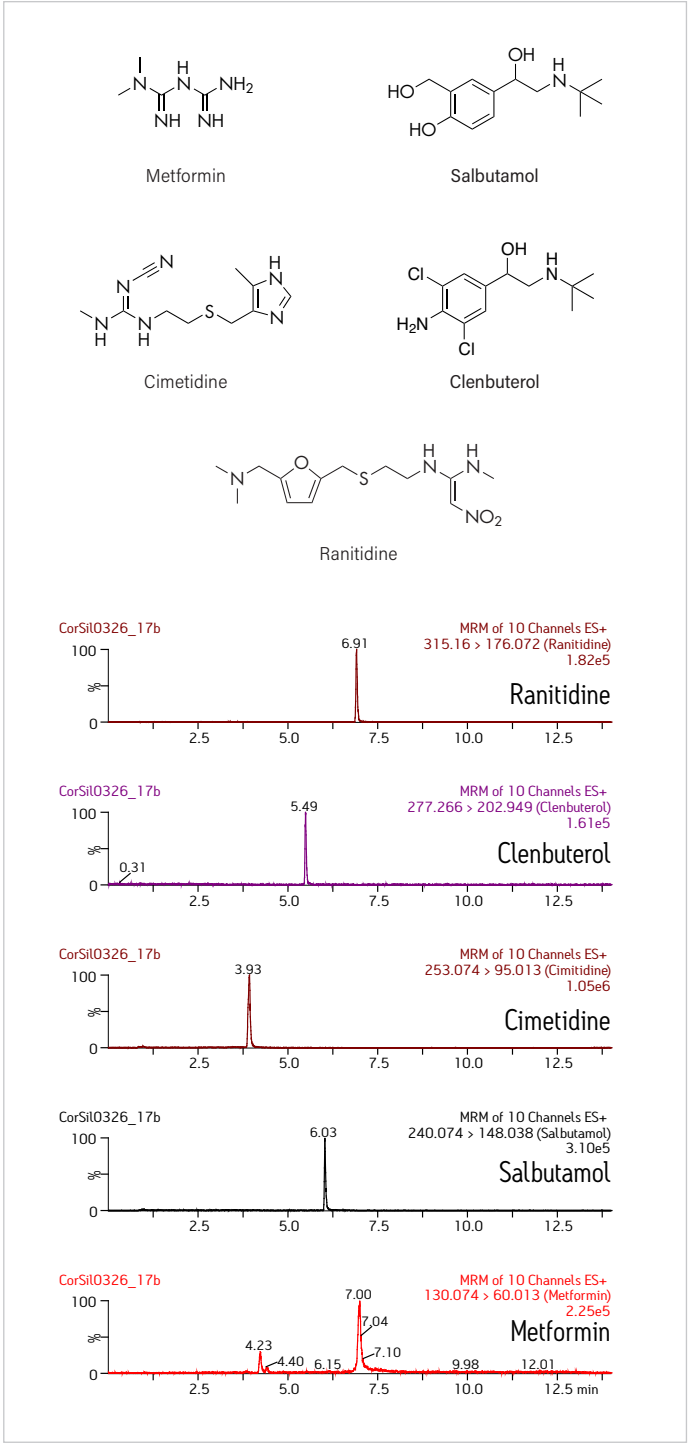
Ionization mode: ESI+

Acquisition mode: MRM

Sample preparation

Sample: Basic drugs from river water

Two hundred milliliters river water (pH 5.0 with acetic acid) prepared using Oasis MCX 6 cc Vac Cartridge, 150 mg sorbent per cartridge, 30 µm particle size.



ORDERING INFORMATION

Description	P/N
CORTECS HILIC, 2.7 µm, 2.1 x 100 mm Column	186007382
Oasis MCX 6 cc Vac Cartridge	186000256
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note WA64694 at waters.com

Analysis of Basic Impurities

EXPERIMENTAL

LC conditions

System: Alliance HPLC with 2998 PDA detector

Column: CORTECS C_{18}+, 2.7 µm, 4.6 x 150 mm

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.1% formic acid in acetonitrile

Gradient:

Time	%A	%B	Curve
0.0	75	25	–
11.3	65	35	6
12.0	65	35	6
12.1	75	25	6
15.0	75	25	6

Flow rate: 1.5 mL/min

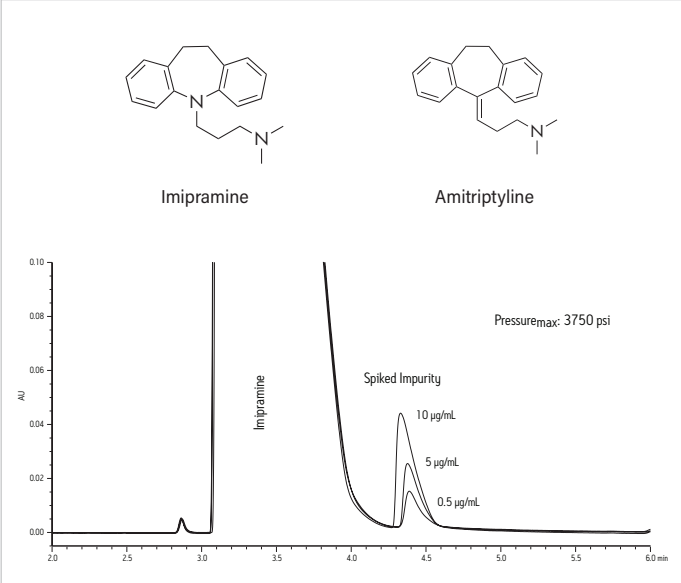
Column temp.: 30 °C

Injection volume: 36 µL

UV detection: 254 nm

Sample preparation

Sample: Imipramine (0.5 mg/mL), with various concentrations of amitriptyline (10 µg/mL, 5 µg/mL, and 0.5 µg/mL) prepared in water



ORDERING INFORMATION

Description	P/N
CORTECS C_{18}+, 2.7 µm, 4.6 x 150 mm Column	186007408
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720005062EN at waters.com

Analysis of Basic Impurities *(Solid-Core Technology Comparison)*

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC with PDA detector and TQD mass spectrometer

Columns: CORTECS C_{18}+, 2.7 µm, 3.0 x 50 mm;
Competitor solid-core C_{18}, 2.6 µm, 3.0 x 50 mm

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.1% formic acid in acetonitrile

Gradient:

Time	%A	%B
0.00	75	25
3.00	65	35
3.10	75	25
4.10	75	25

Flow rate: 0.8 mL/min

Column temp.: 30 °C

Injection volume: 10.0 µL

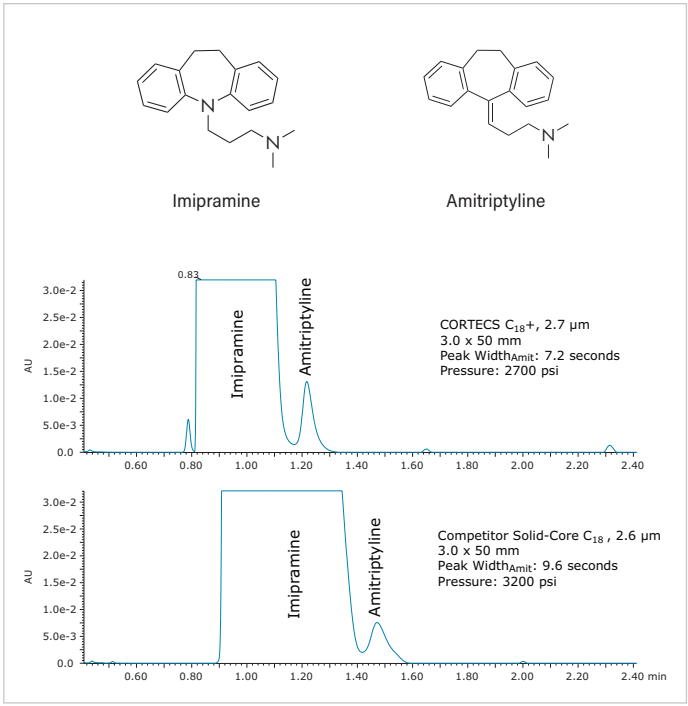
UV detection: 254 nm

Ionization mode: ESI+

Acquisition mode: SIR (m/z) 278.4

Sample preparation

0.5 mg/mL imipramine and 0.5 µg/mL amitriptyline aqueous solution.



ORDERING INFORMATION

Description	P/N
CORTECS C_{18}+, 2.7 µm, 3.0 x 50 mm Column	186007400
Waters LCMS Certified Max Recovery Vial	600000749CV

For complete experimental details, refer to full application note XBRIDGE17 at waters.com

Analysis of Beta Blockers

EXPERIMENTAL

LC conditions

System:	Alliance 2695 with 2996 PDA detector
Column:	XBridge BEH Shield RP18, 3.5 µm, 4.6 x 100 mm
Mobile phase A:	Water
Mobile phase B:	Methanol
Mobile phase C:	100 mM ammonium bicarbonate, pH 9.0

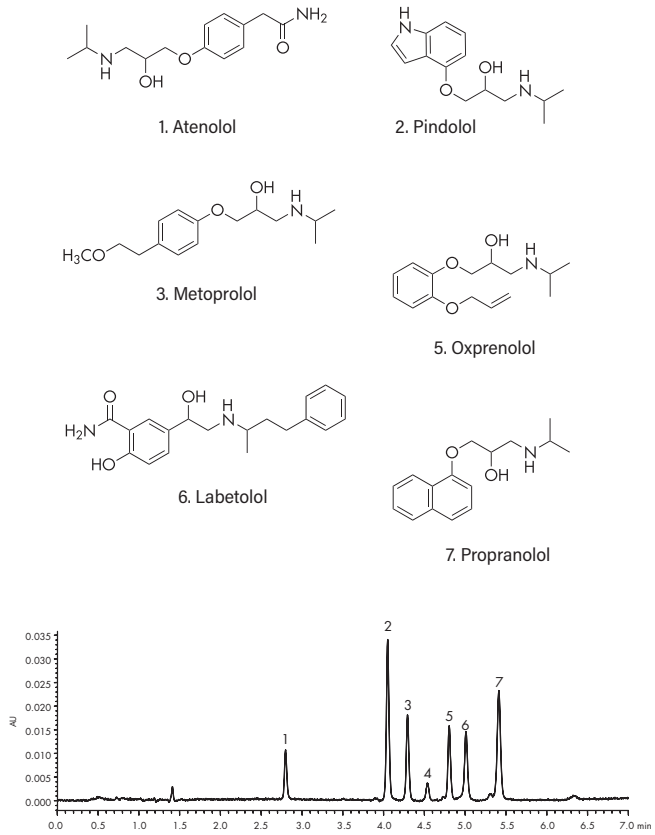
Gradient:

Time	%A	%B	%C
0.00	85	5	10
3.00	55	35	10
10.00	55	35	10
11.00	85	5	10
15.00	85	5	10

Flow rate:	1.0 mL/min
Column temp.:	30 °C
Injection volume:	10 µL
UV detection:	280 nm

Sample preparation

Sample concentration and Diluent: 10 µg/mL in H_2O



ORDERING INFORMATION

Description	P/N
XBridge BEH Shield RP18, 3.5 µm, 4.6 x 100 mm Column	186003044
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720004129EN at waters.com

Anaysis of Budesonide Nasal Spray

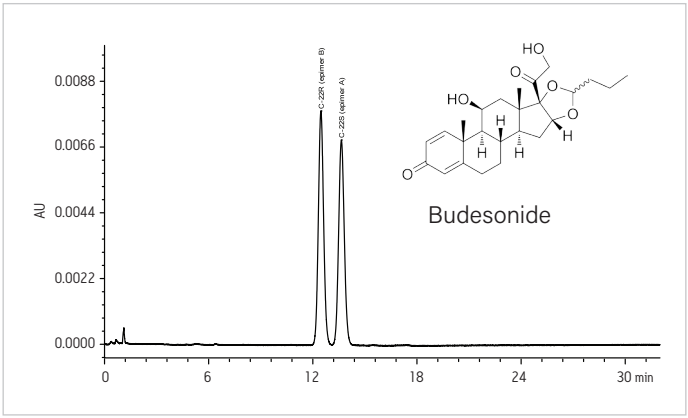
EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2489 UV/Visible detector
Column:	XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm
Mobile phase:	Acetonitrile and Solution A (32:68)
Solution A:	3.17 mg/mL of monobasic sodium phosphate and 0.23 mg/mL of phosphoric acid; pH 3.2 +/- 0.1
Separation mode:	Isocratic
Flow rate:	1.5 mL/min
Injection volume:	20 µL
UV detection:	254 nm

Sample preparation

An amount equivalent to 1.0 g of Rhinocort AQUA (budesonide) nasal spray was accurately weighed and transferred to a 50-mL volumetric flask. Sixteen milliliters of acetonitrile was added to this flask. This mixture was mechanically shaken in the Burrell Wrist-Action shaker, Model 75 for 15 minutes. The mixture was diluted with Solution A to volume and mechanically shaken for an additional 10 minutes. This mixture was then subjected to centrifugation at 3220 rcf (4000 rpm) for 15 minutes. The supernatant was aliquoted into a 2-mL Waters Certified Glass Screw Cap Vial with bonded pre-slit PTFE/silicone septum (p/n 186000307C). Final concentration of the working sample was 12.8 µg/mL.



ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm Column	186003116
Waters LCGC Certified Vial w/ Preslit Septa	186000307C

For complete experimental details, refer to full application note WA64077 at waters.com

Analysis of Buprenorphine and Buprenorphine Glucuronide

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC with TQD detector

Column: XBridge BEH HILIC, 3.5 µm, 2.1 x 100 mm

Mobile phase A: 10 mM ammonium formate in water, 0.125% formic acid in 50:50 acetonitrile:water

Mobile phase B: 10 mM ammonium formate in water, 0.125% formic acid in 90:10 acetonitrile:water

Gradient:

Time	%A	%B
0.00	0.1	99.9
1.05	0.1	99.9
4.35	99.9	0.1
5.00	99.9	0.1
5.01	0.1	99.9
6.00	0.1	99.9

Flow rate: 0.3 mL/min

Column temp.: 30 °C

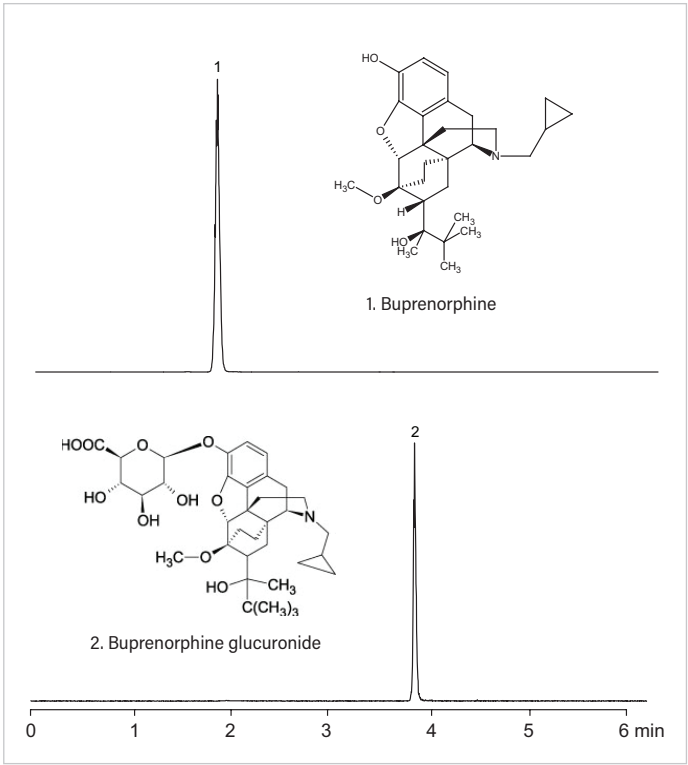
Injection volume: 5 µL

Acquisition mode: MRM (m/z): buprenorphine 468.3 > 54.7; buprenorphine glucuronide 644.3 > 468.3

Sample preparation

Sample concentration: 50 ng/mL each

Sample diluent: 75:25 ACN:MeOH with 0.2% HCOOH



ORDERING INFORMATION

Description	P/N
XBridge BEH HILIC, 3.5 µm, 2.1 x 100 mm Column	186004433
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note XBRIDGE6 at waters.com

Analysis of Caffeine and Metabolites Using XBridge BEH C_{18} Columns

EXPERIMENTAL

LC conditions

System: Alliance 2695 with 2996 PDA detector

Column: XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm

Mobile phase A: 5 mM ammonium acetate, pH 7.5

Mobile phase B: Acetonitrile

Gradient:

Time	%A	%B
0.00	98	2
15.00	80	20
18.00	80	20
19.00	98	2
25.00	98	2

Flow rate: 1.0 mL/min

Column temp.: 25 °C

Injection volume: 10 µL

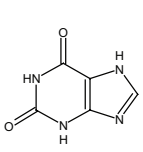
UV detection: 273 nm

Sample preparation

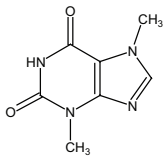
Sample concentration: 20 µg/mL in water

ORDERING INFORMATION

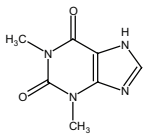
Description	P/N
XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm Column	186003116
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



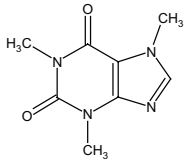
1. Xanthine



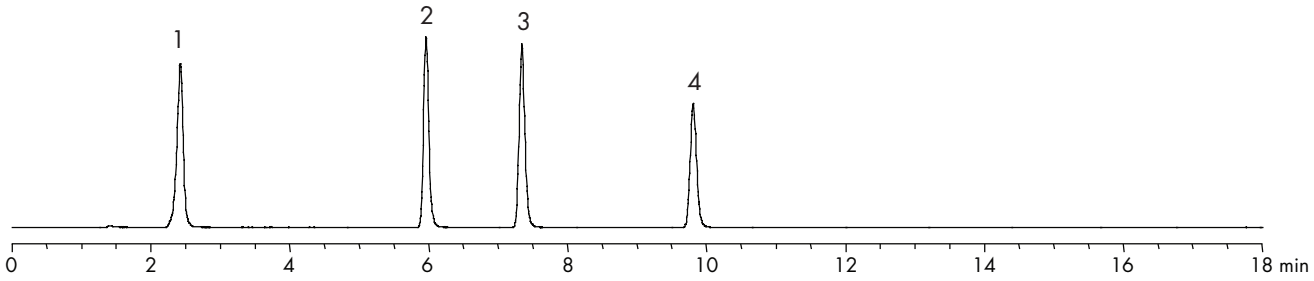
2. Theobromine



3. Theophylline



4. Caffeine



For complete experimental details, refer to full application note XBRIDGE11 at waters.com

Analysis of Caffeine Metabolites Using XBridge BEH Phenyl Columns

EXPERIMENTAL

LC conditions

System: Alliance 2695 with 2996 PDA detector

Columns: XBridge BEH Phenyl, 3.5 µm, 4.6 x 100 mm

Mobile phase A: Water

Mobile phase B: Acetonitrile

Mobile phase C: 100 mM ammonium bicarbonate

Gradient:

Time	%A	%B	%C
0.00	89	1	10
9.00	66	24	10
10.00	89	1	10
20.00	89	1	10

Flow rate: 1.0 mL /min

Column temp.: 30 °C

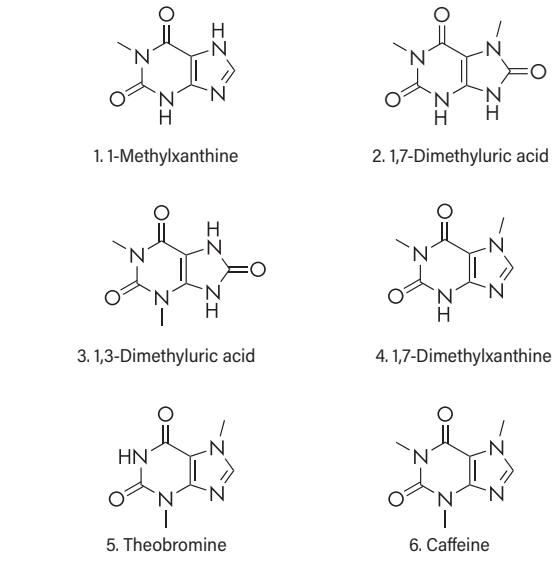
Injection volume: 10 µL

UV detection: 280 nm

Sample preparation

Sample: Caffeine (10 µg/mL), Theobromine (10 µg/mL), 1-Methylxanthine (10 µg/mL), 1,3-Dimethyluric acid (10 µg/mL), 1,7-Dimethylxanthine (10 µg/mL), 1,7-Dimethyluric acid (10 µg/mL) in H_2O/NH_4HCO_3 (90/10)

Sample temp.: 15 °C



ORDERING INFORMATION

Description	P/N
XBridge BEH Phenyl, 3.5 µm, 4.6 x 100 mm Column	186003334
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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34 Maple Street, Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990

For complete experimental details, refer to full application note 720002732EN at waters.com

Analysis of Carbonyl Compounds in Drinking Water

EXPERIMENTAL

LC conditions

System: Alliance HPLC with UV detection

Column: XBridge BEH Phenyl, 3.5 µm, 4.6 x 150 mm

Mobile phase A: Water/tetrahydrofuran (THF) 90:10

Mobile phase B: Acetonitrile

Gradient: Eluent gradient for EPA methods 554 and 8315 Option 1.

Time	Flow	%A	%B	Curve
Initial	1.5	70	30	–
20.0	1.5	36	64	6
22.0	1.5	36	64	6
22.1	1.5	70	30	6

Eluent gradient for EPA methods TO11 and 8315 Option 2

Time	Flow	%A	%B	Curve
Initial	1.5	70	30	–
16.0	1.5	53	47	6
21.0	1.5	53	47	6
21.1	1.5	70	30	6

Flow rate: 1.5 mL/min

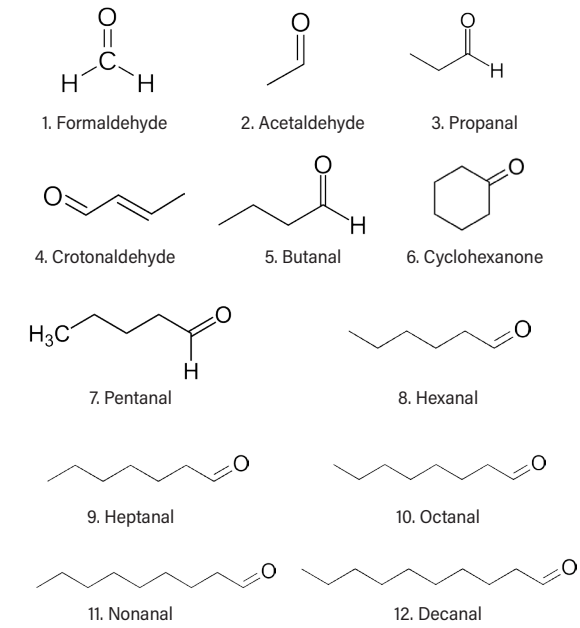
Column temp.: 35 °C

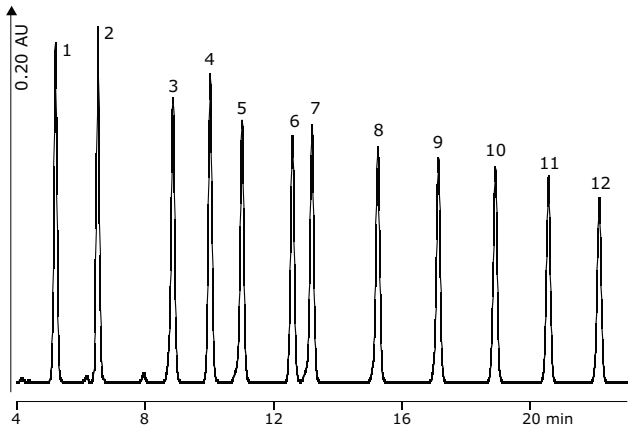
Injection: 20 µL each of AccuStandard mix (M- 8315-R1- DNPH and M- 8315-R2- DNPH) diluted 1:5 in 40:60 water/acetonitrile

UV detection: 360 nm

Sample preparation

DNPH reagent added to 100 mL sample, extract with Oasis HLB or use methylene chloride extraction option.





ORDERING INFORMATION

Description	P/N
XBridge BEH Phenyl, 3.5 µm, 4.6 x 150 mm Column	186003335
Oasis HLB, 3 cc, 60 mg Cartridge	WAT094226
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note XBRIDGE14 at waters.com

Analysis of Catechins

EXPERIMENTAL

LC conditions

System: Alliance 2695 with 2996 PDA detector

Columns: XBridge BEH Phenyl, 3.5 µm, 4.6 x 150 mm

Mobile phase A: Water

Mobile phase B: Methanol

Mobile phase C: 0.2% formic acid in water

Gradient:

Time	%A	%B	%C
0.00	84	15	1
8.00	84	15	1
15.00	62	37	1
20.00	62	37	1
26.00	84	15	1
30.00	84	15	1

Flow rate: 0.8 mL/min

Column temp.: 30 °C

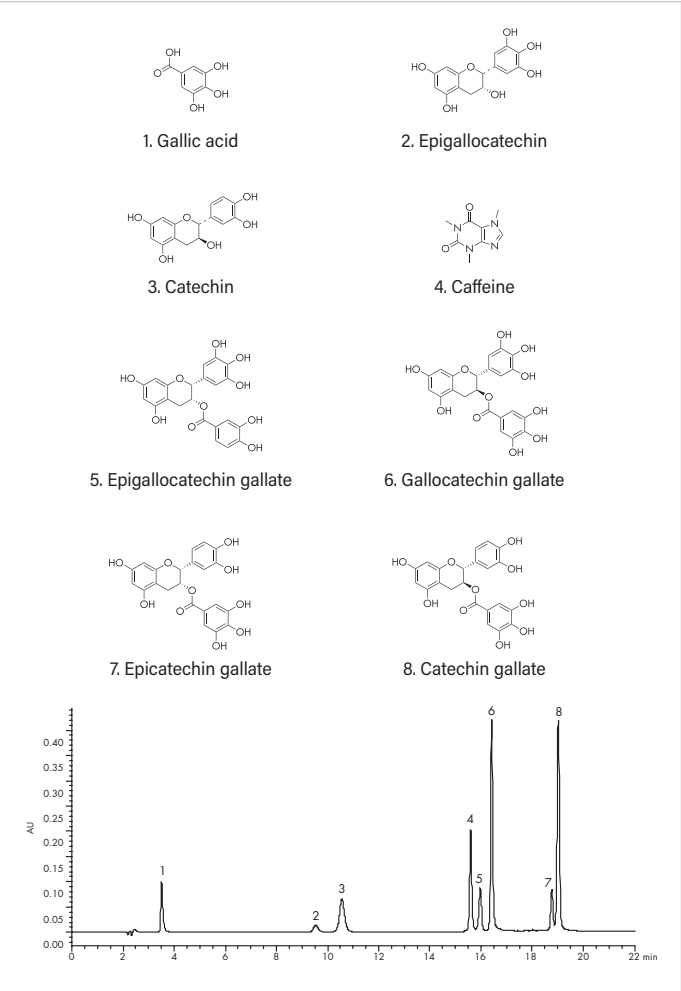
Injection volume: 20 µL

UV detection: 280 nm

Sample preparation

Sample: Gallic acid (10 µg/mL), Epigallocatechin (50 µg/mL), Catechin (50 µg/mL), Caffeine (10 µg/mL), Epigallocatechin gallate (50 µg/mL), Gallocatechin gallate (50 µg/mL), Epicatechin gallate (10 µg/mL), Catechin gallate (50 µg/mL), in H_2O/ MeOH (85/15)

Sample temp.: 15 °C



ORDERING INFORMATION

Description	P/N
XBridge BEH Phenyl , 3.5 µm, 4.6 x 150 mm Column	186003335
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note XBRIDGE7 at waters.com

Analysis of Catecholamines Using XBridge BEH C_{18} Columns

EXPERIMENTAL

LC conditions

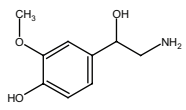
System:	Alliance 2695 with 2996 PDA detector
Column:	XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm
Mobile phase:	20 mM sodium phosphate buffer, pH 2.5/methanol (97/3)
Separation mode:	Isocratic
Flow rate:	1.5 mL/min
Column temp.:	25 °C
Injection volume:	10 µL
UV detection:	210 nm

Sample preparation

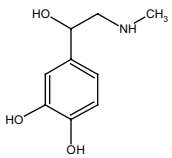
Sample concentration: 20 µg/mL in water

ORDERING INFORMATION

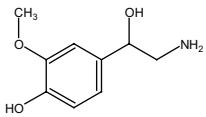
Description	P/N
XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm Column	186003116
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



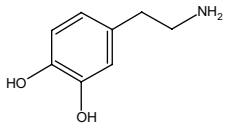
1. Norepinephrine



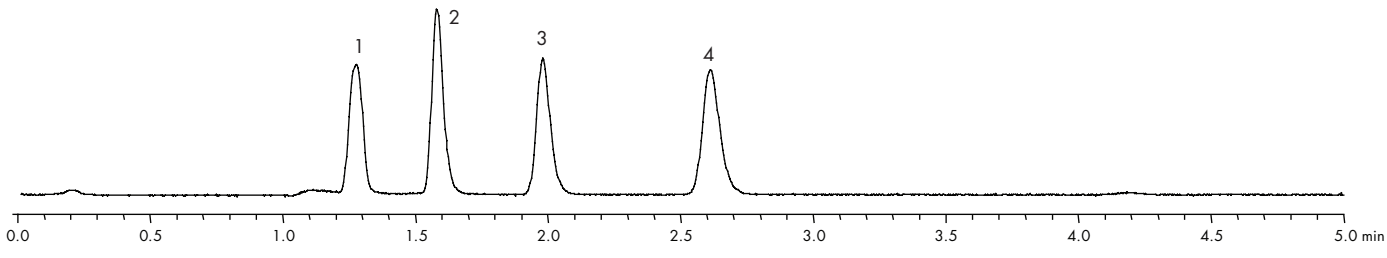
2. Epinephrine



3. Normetanephrine



4. Dopamine



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For complete experimental details, refer to full application note XBRIDGE18 at waters.com

Analysis of Catecholamines Using XBridge BEH Shield RP18 Columns

EXPERIMENTAL

LC conditions

System: Alliance 2695 with 2996 PDA detector

Column: XBridge BEH Shield RP18, 3.5 µm, 4.6 x 100 mm

Mobile phase A: Water

Mobile phase B: Acetonitrile

Mobile phase C: 100 mM ammonium formate, pH 3.0

Gradient:

Time	%A	%B	%C
0.00	90	0	10
5.00	90	0	10
15.00	65	25	10
16.00	65	25	10
17.00	90	0	10
20.00	90	0	10

Flow rate: 1.0 mL /min

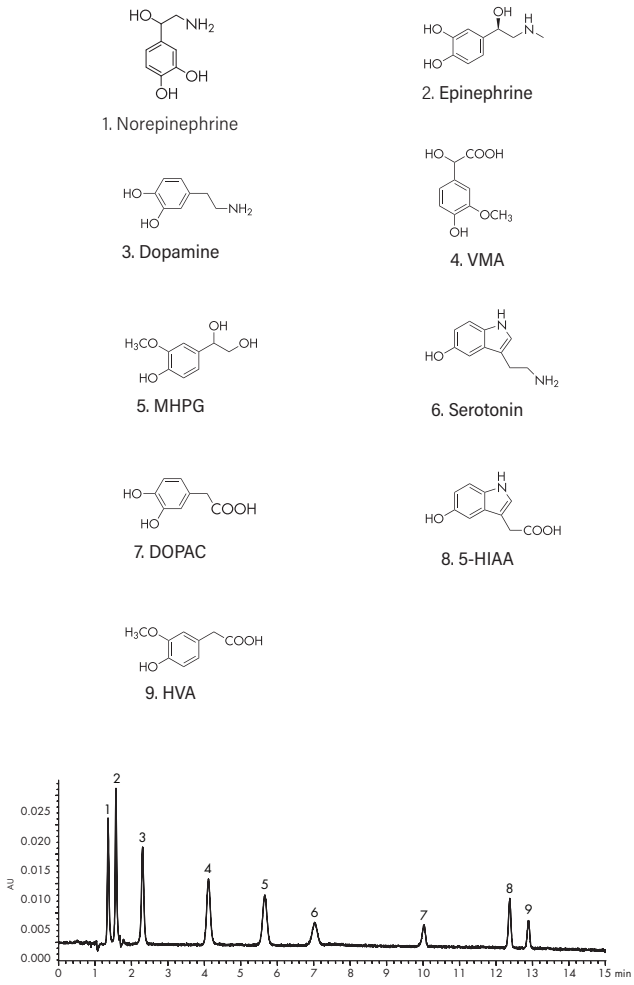
Column temp.: 30 °C

Injection volume: 10 µL

UV detection: 280 nm

Sample preparation

Sample concentration and diluent: 10 µg/mL in H_2O



ORDERING INFORMATION

Description	P/N
XBridge BEH Shield RP18, 3.5 µm, 4.6 x 100 mm Column	186003044
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note WA64102 at waters.com

Analysis of Cellulosic Hydrolysates by ELSD

EXPERIMENTAL

LC conditions

System: Alliance HPLC with 2424 ELSD

Column: XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm

Mobile phase A: 80/20 acetonitrile/water with 0.2 % triethylamine

Mobile phase B: 30/70 acetonitrile/water with 0.2 % triethylamine

Gradient:

Time	%A	%B
0.00	100	0
21.00	40	60
21.01	100	0
33.00	100	0

Flow rate: 1.0 mL/min

Column temp.: 35 °C

Injection volume: 15.0 µL

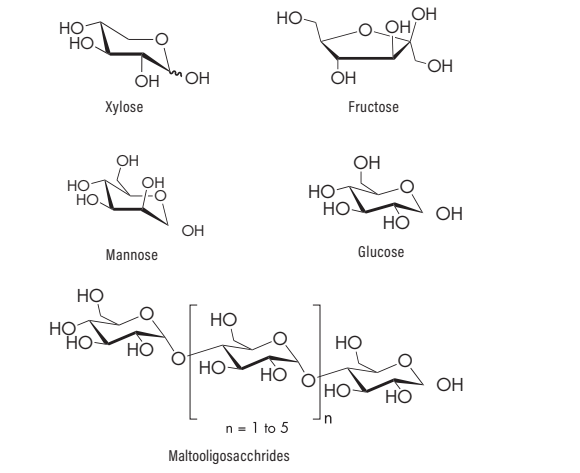
ELSD pressure: 30 psi

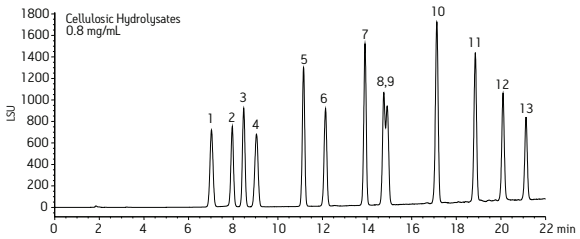
Drift tube temp.: 50 °C

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.

Sample concentration: 0.8 mg/mL each





1. Xylose
2. Fructose
3. Mannose
4. Glucose
5. Maltooligosacchrides
6. Sucrose
7. Cellobiose
8. Melezitose
9. Raffinose
10. Maltotetraose
11. Maltopentaose
12. Maltohexaose
13. Maltoheptaose

ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note XBRIDGE2 at waters.com

Analysis of Cephalosporin Antibiotics

EXPERIMENTAL

LC conditions

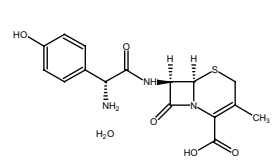
System:	Alliance 2695 with 2996 PDA detector
Column:	XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm
Mobile phase:	0.5% acetic acid/acetonitrile (85/15)
Separation mode:	Isocratic
Flow rate:	1.0 mL/min
Column temp.:	25 °C
Injection volume:	10 µL
UV detection:	254 nm

Sample preparation

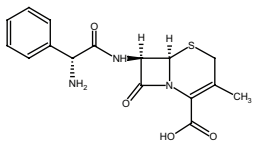
Sample concentration: 20 µg/mL in water

ORDERING INFORMATION

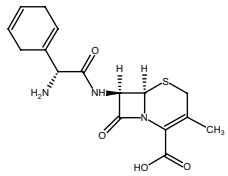
Description	P/N
XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm Column	186003116
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



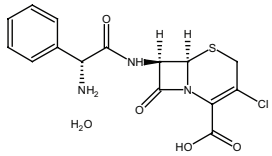
1. Cefadroxile



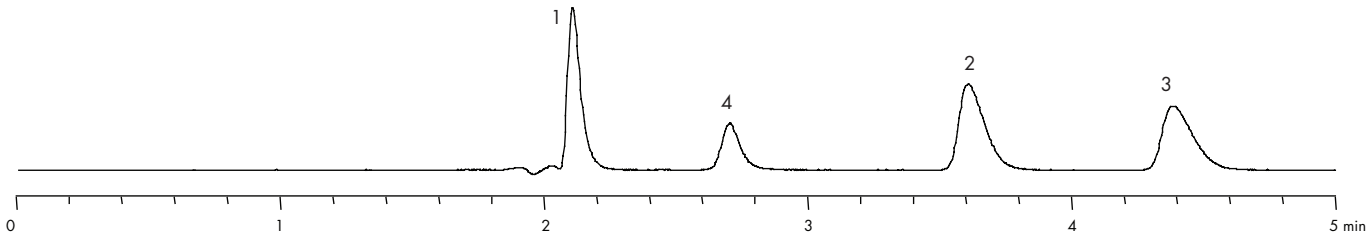
2. Cephalexin



3. Cephradine



4. Cefaclor



For complete experimental details, refer to full application note 720004461EN at waters.com

Analysis of Clarithromycin

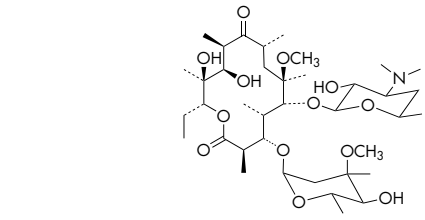
EXPERIMENTAL

LC conditions

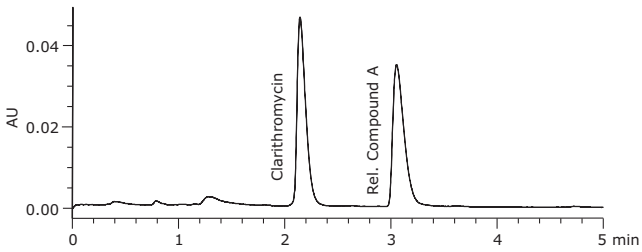
System:	Alliance 2695
Column:	XSelect CSH C_{18} ***XP***, 2.5 µm, 4.6 x 75 mm
Mobile phase:	65:35 methanol:67 mM monobasic potassium phosphate, pH 4.0 with phosphoric acid
Separation mode:	Isocratic
Flow rate:	1.0 mL/min
Column temp.:	50 °C
Injection volume:	10 µL
UV detection:	210 nm

Sample preparation

Clarithromycin sample: clarithromycin and clarithromycin related compound A USP standards were prepared in the mobile phase to a concentration of 0.5 mg/mL. The sample was placed in a TruView Maximum Recovery Vial for injection.



Clarithromycin



ORDERING INFORMATION

Description	P/N
XSelect CSH C_{18} ***XP***, 2.5 µm, 4.6 x 75 mm Column	186006110
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note WA64080 at waters.com

Analysis of Cytosine, 5-Fluorocytosine, and Uracil

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC with TUV detector

Column: XBridge BEH HILIC, 3.5 µm, 2.1 x 50 mm

Mobile phase A: 95:5 acetonitrile:water with 10 mM ammonium acetate pH 5.5

Mobile phase B: 50:50 acetonitrile:water with 10 mM ammonium acetate pH 5.5

Gradient:

Time	%A	%B	Curve
0.00	99	1	6
2.00	1	99	6
2.10	99	1	6
2.50	99	1	6

Flow rate: 0.5 mL/min

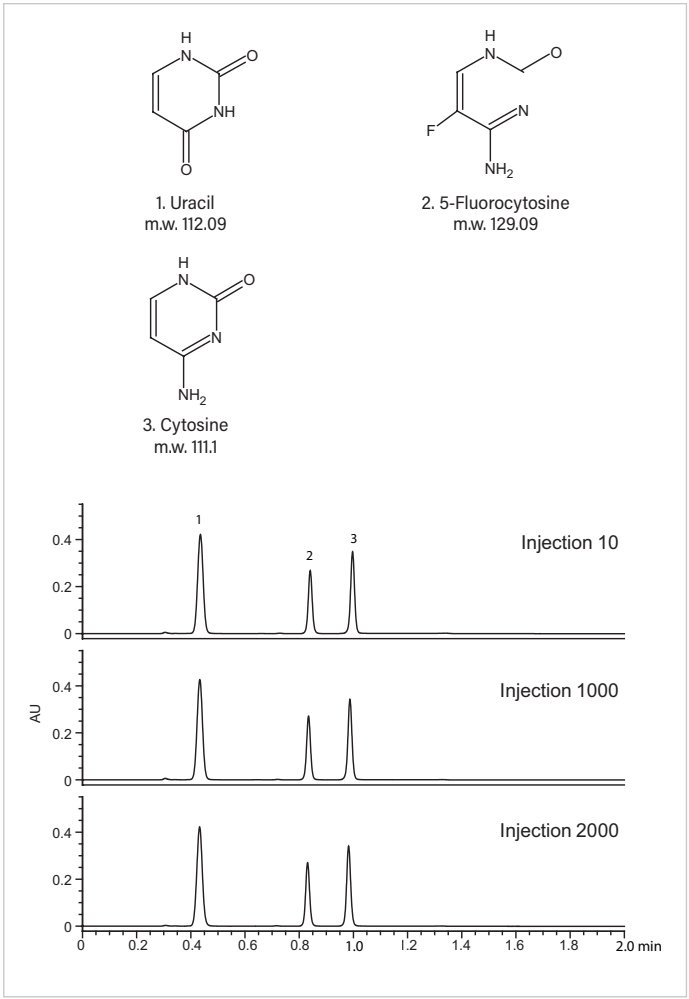
Column temp.: 30 °C

Injection volume: 2.0 µL

UV detection: 254 nm

Sample preparation

Sample diluent: 75:25 acetonitrile:methanol



ORDERING INFORMATION

Description	P/N
XBridge BEH HILIC, 3.5 µm, 2.1 x 50 mm Column	186004432
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note XBRIDGE20 at waters.com

Analysis of Dextromethorphan, Tetracaine, Triprolidine, and Warfarin

EXPERIMENTAL

LC conditions

System: Alliance 2695 with 2996 PDA detector

Column: XBridge BEH Shield RP18, 3.5 µm, 4.6 x 50 mm

Mobile phase A: 25 mM monopotassium phosphate, pH 7

Mobile phase B: Methanol

Gradient:

Time	%A	%B
0.00	60	40
7.00	20	80
7.50	60	40
10.00	60	40

Flow rate: 1.0 mL/min

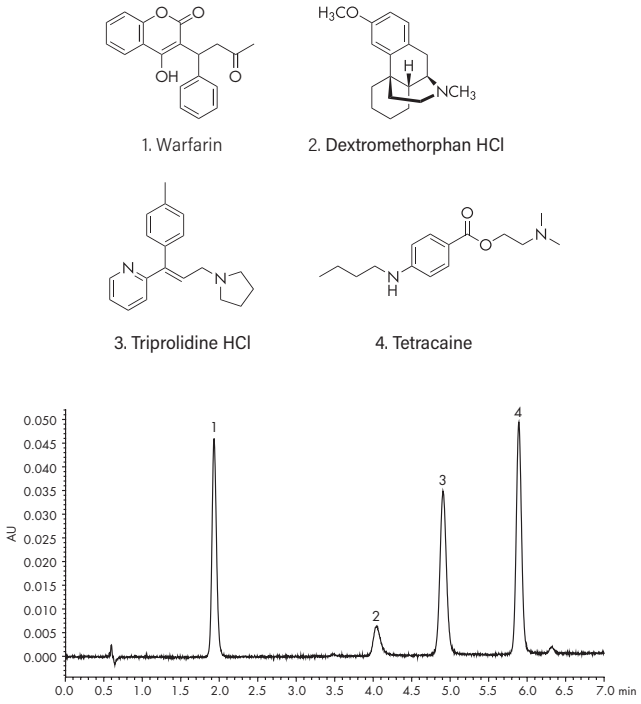
Column temp.: 30 °C

Injection volume: 10 µL

UV detection: 280 nm

Sample preparation

Sample concentration and diluent: 10 µg/mL in H_2O



ORDERING INFORMATION

Description	P/N
XBridge BEH Shield RP18, 3.5 µm, 4.6 x 50 mm Column	186003042
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note 720005552EN at waters.com

Analysis of Disperse Dyes

EXPERIMENTAL

LC conditions

System: ACQUITY Arc with ACQUITY QDa Detector

Column: XBridge BEH C_{18}, 5 µm, 2.1 x 150 mm

Mobile phase A: Ammonium acetate 10 mmol pH 3.6

Mobile phase B: Acetonitrile

Gradient:

Time	%A	%B
0.00	60	40
7.00	40	60
17.00	2	98
24.00	2	98
24.10	60	40

Flow rate: 0.30 mL/min

Column temp.: 30 °C

Injection volume: 5 µL

UV detection: 210 to 800 nm

Ionization mode: ESI+

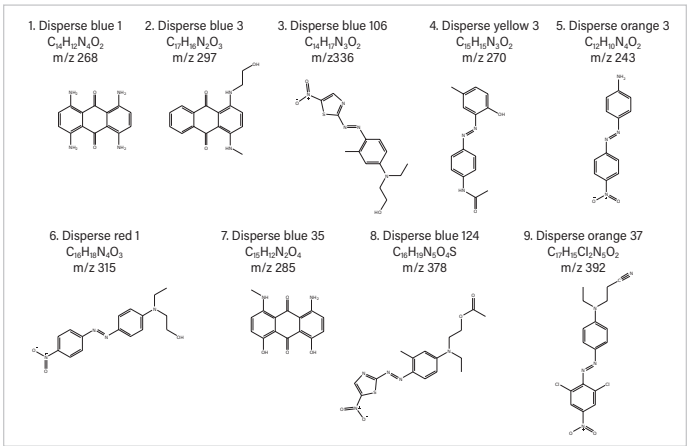
Acquisition mode: Full scan 100-600 m/z and SIR

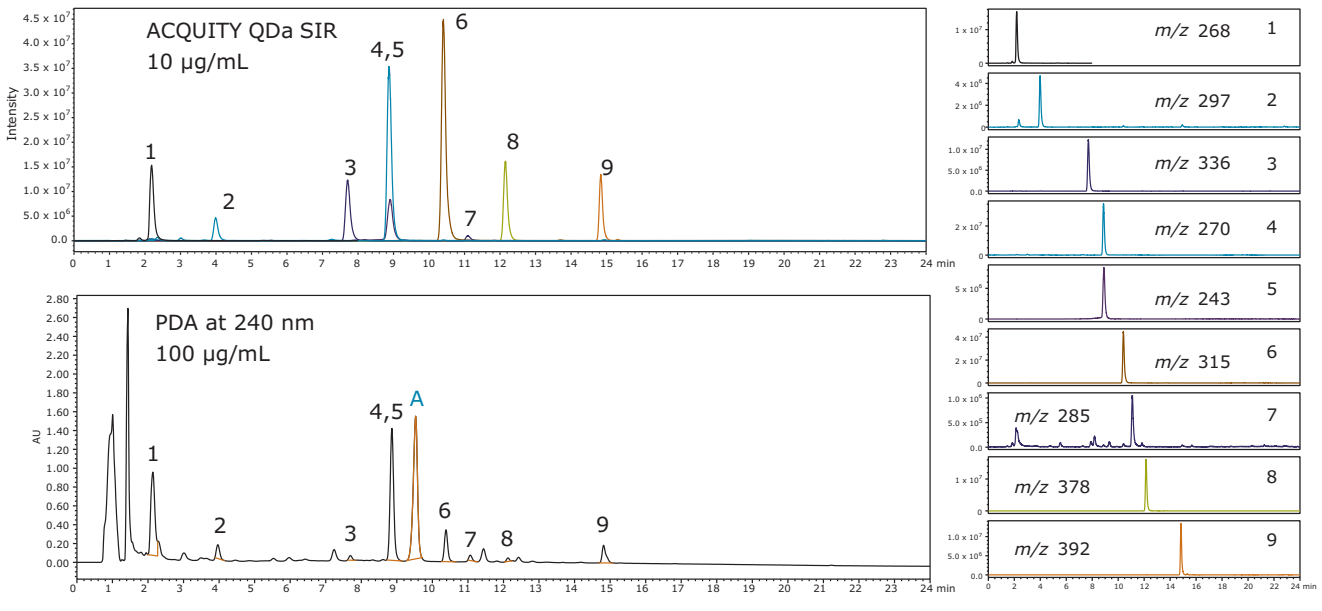
Sample preparation

Dye standards were dissolved in methanol and sequentially diluted in preparation for sample analysis.

ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 5 µm, 2.1 x 150 mm Column	186003110
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV





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For complete experimental details, refer to full application note XBRIDGE10 at waters.com

Analysis of DNPH Derivatives

EXPERIMENTAL

LC conditions

System: Alliance 2695 with 2996 PDA detector

Columns: XBridge BEH Phenyl, 3.5 µm, 4.6 x 100 mm

Mobile phase A: Water

Mobile phase B: Acetonitrile

Mobile phase C: 0.2% formic acid in water

Gradient:

Time	%A	%B	%C
0.00	40	50	10
2.67	40	50	10
6.67	0	90	10
7.33	40	50	10
11.00	40	50	10

Flow rate: 1.2 mL /min

Column temp.: 30 °C

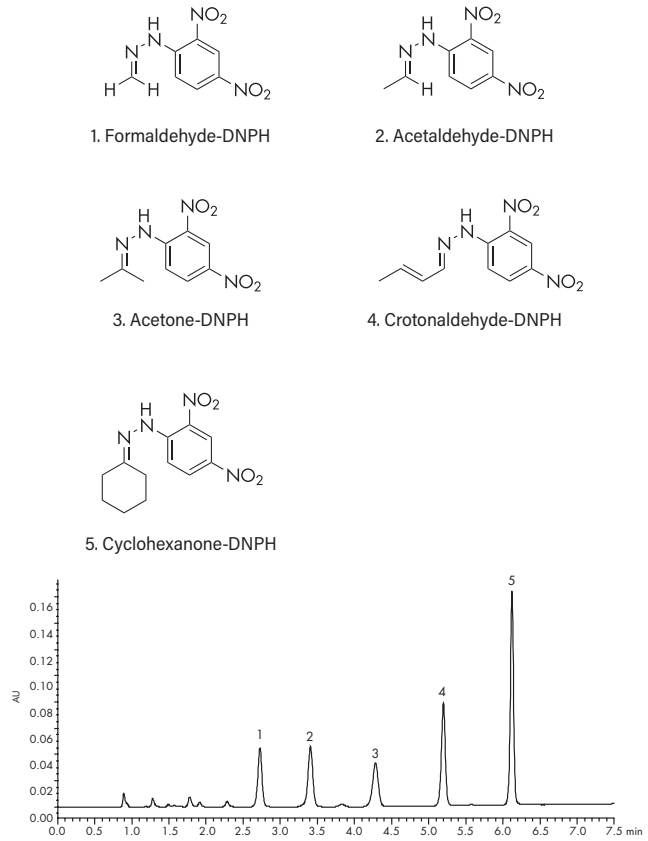
Injection volume: 10 µL

UV detection: 254 nm

Sample preparation

Sample: Acetaldehyde-DNPH (10 µg/mL), Acetone-DNPH (10 µg/mL), Cyclohexanone-DNPH (10 µg/mL), Formaldehyde-DNPH (10 µg/mL), Crotonaldehyde-DNPH (10 µg/mL) in H_2O/ACN (60/40)

Sample temp.: 15 °C



ORDERING INFORMATION

Description	P/N
XBridge BEH Phenyl, 3.5 µm, 4.6 x 100 mm Column	186003334
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720005666EN at waters.com

Analysis of Dofetilide and Related Compounds

EXPERIMENTAL

LC conditions

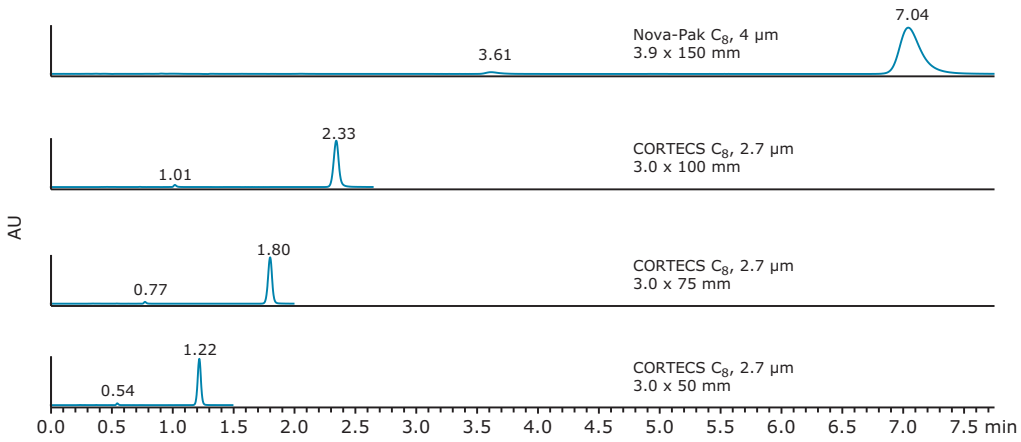
Systems:	Alliance HPLC and ACQUITY UPLC H-Class
Column:	Nova-Pak C_8, 60Å, 4 µm, 3.9 x 150 mm CORTECS C_8, 90Å, 2.7 µm, 3.0 x 100 mm CORTECS C_8, 90Å, 2.7 µm, 3.0 x 75 mm CORTECS C_8, 90Å, 2.7 µm, 3.0 x 50 mm
Mobile phase:	Acetonitrile:buffer solution (1:3)
Buffer solution:	1.36 g monobasic potassium phosphate and 5 mg ascorbic acid in 1 L water, adjusted with 0.01 M potassium hydroxide solution to pH 7.0
Separation mode:	Isocratic
Flow rate:	1.00 mL/min (4 µm column), 0.88 mL/min (2.7 µm column)
Column temp.:	30 °C
Injection volume:	50 µL (150 mm column), 19.8 µL (100 mm column), 14.8 µL (75 mm column), 9.9 µL (50 mm column)
UV detection:	230 nm

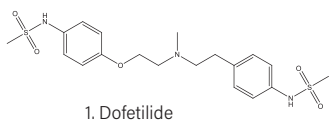
Sample preparation

A sample containing dofetilide (25 µg/mL) and dofetilide related compound A (0.5 µg/mL) was prepared with mobile phase as the diluent.

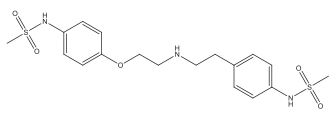
ORDERING INFORMATION

Description	P/N
Nova-Pak C_8, 4 µm, 3.9 x 150 mm Column	WAT035876
CORTECS C_8, 2.7 µm, 3.0 x 100 mm Column	186008361
CORTECS C_8, 2.7 µm, 3.0 x 75 mm Column	186008360
CORTECS C_8, 2.7 µm, 3.0 x 50 mm Column	186008359
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV





1. Dofetilide



2. Dofetilide related compound A

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For complete experimental details, refer to full application note 720004261EN at waters.com

Analysis of Donepezil Tablets

EXPERIMENTAL

LC conditions

System:	Alliance 2695
Column:	XBridge BEH C_{18}, 5 µm, 4.6 x 250 mm
Mobile phase A:	0.1% phosphoric acid in water, adjust to pH 6.5 with triethylamine
Mobile phase B:	Acetonitrile

Gradient:

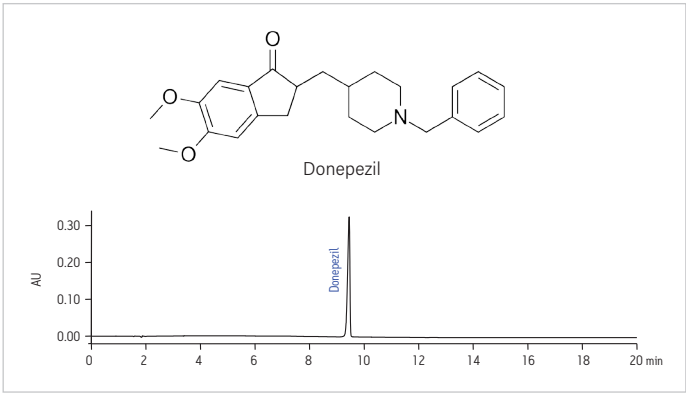
Time	%A	%B
0.00	75	25
10.00	40	60
40.00	40	60
41.00	75	25
50.00	75	25

Flow rate:	1.5 mL/min
Column temp.:	50 °C
Injection volume:	20 µL
UV detection:	286 nm

Sample preparation

Donepezil (1 mg/mL) in diluent: Waters Analytical Standard

Donepezil Tablets (1 mg/mL): Crushed tablets were weighed in a 50-mL volumetric flask and 25-mL diluent was added. The sample was sonicated for 15 minutes and made up to volume with diluent. The sample was mixed well, filtered though a 0.2 µm PTFE filter, and centrifuged at 12,000 rpm for 5 minutes.



ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 5 µm, 4.6 x 250 mm Column	186003117
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720004453EN at waters.com

Analysis of Drugs and Metabolites

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC with Xevo TQ-S Detector

Column: XBridge BEH C_{18} ***XP***, 2.5 µm, 2.1 x 50 mm

Mobile phase A: Water with 0.1% formic acid

Mobile phase B: Acetonitrile with 0.1% formic acid

Gradient:

Time	%A	%B
0.00	95	5
1.50	2	98
2.00	2	98
2.10	95	5
2.50	95	5

Flow rate: 500 µL/min

Column temp.: 30 °C

Injection volume: 5 µL

Ionization mode: ESI+

Acquisition mode: MRM

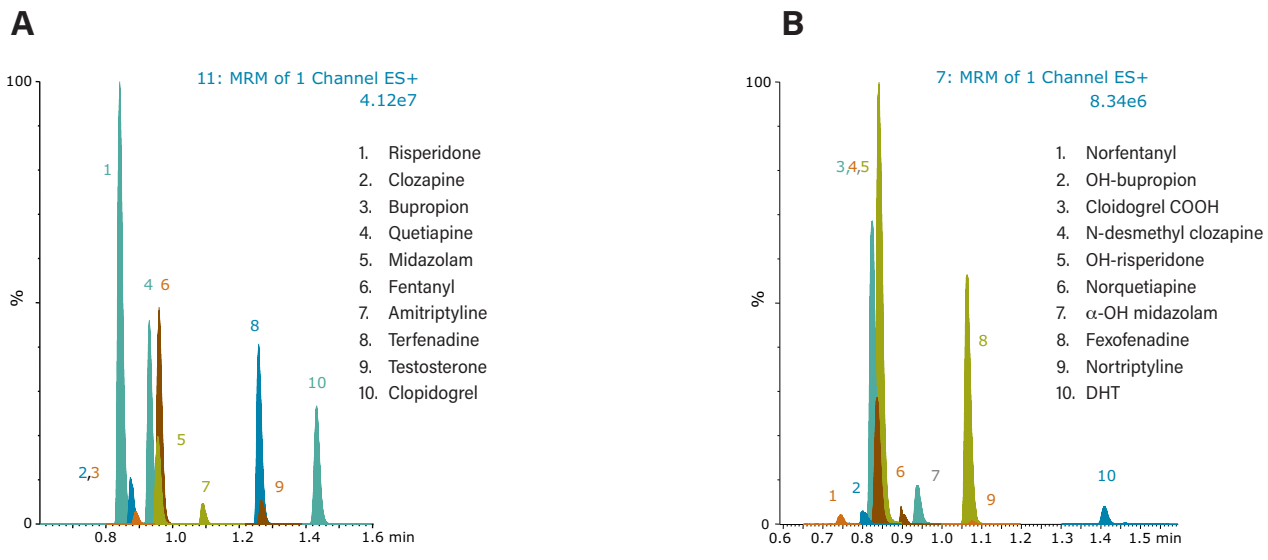
Sample preparation

Plasma samples containing parent drugs and metabolites were prepared by adding 20 µL each of working solutions of parent drugs and metabolites to 1 mL of plasma. All parent drugs were spiked at a concentration of 20 ng/mL, with the exception of testosterone, which was spiked at 100 ng/mL. Metabolites were spiked at 100%, 30%, 10%, and 0% of parent drug concentrations.

Sample temp.: 10 °C

ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18} ***XP***, 2.5 µm, 2.1 x 50 mm Column	186006029
96-well Plate with Deactivated 700 µL Glass Inserts	186000349DV



For complete experimental details, refer to full application note XBRIDGE19 at waters.com

Analysis of Drugs of Abuse

EXPERIMENTAL

LC conditions

System: Alliance 2695 with 2996 PDA detector

Column: XBridge BEH Shield RP18, 3.5 µm, 4.6 x 100 mm

Mobile phase A: Water

Mobile phase B: Acetonitrile

Mobile phase C: 100 mM ammonium bicarbonate, pH 9.6

Gradient:

Time	%A	%B	%C
0.00	63	32	5
3.00	63	32	5
7.00	45	50	5
9.00	45	50	5
10.00	63	32	5
12.00	63	32	5

Flow rate: 0.6 mL/min

Column temp.: 40 °C

Injection volume: 10 µL

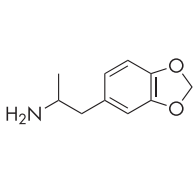
UV detection: 210 nm

Sample preparation

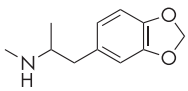
Sample concentration and diluent: 10 µg/mL in water

ORDERING INFORMATION

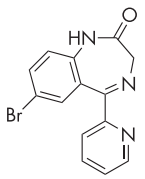
Description	P/N
XBridge BEH Shield RP18, 3.5 µm, 4.6 x 100 mm Column	186003044
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



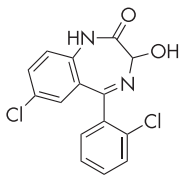
1. MDA



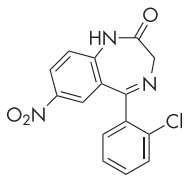
2. MDMA



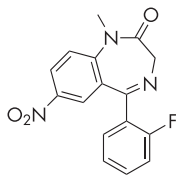
3. Bromazepam



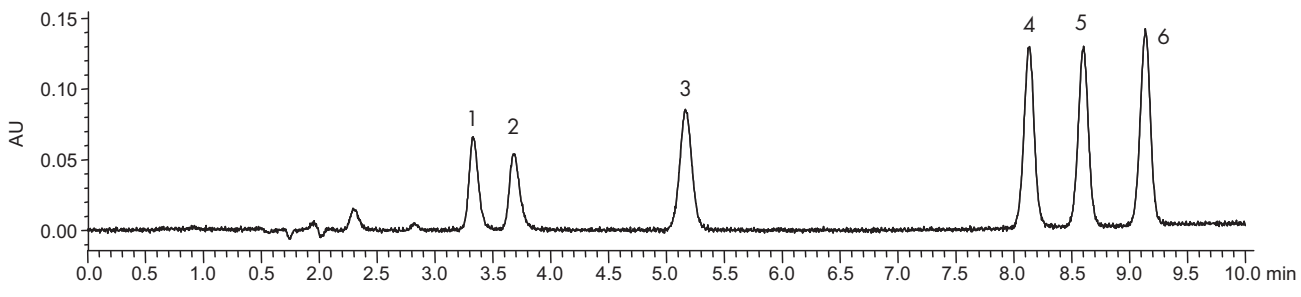
4. Lorazepam



5. Clonazepam



6. Flunitrazepam



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For complete experimental details, refer to full application note XBRIDGE1 at waters.com

Analysis of Estradiol

EXPERIMENTAL

LC conditions

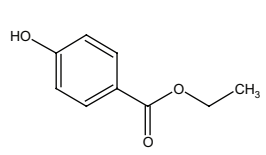
System:	Alliance 2695 with 2996 PDA detector
Column:	XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm
Mobile phase:	Water/acetonitrile (45/55)
Separation mode:	Isocratic
Flow rate:	1.0 mL/min
Column temp.:	25 °C
Injection volume:	10 µL
UV detection:	205 nm

Sample preparation

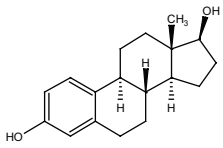
Sample concentration: 20 µg/mL in water/acetonitrile (50/50)

ORDERING INFORMATION

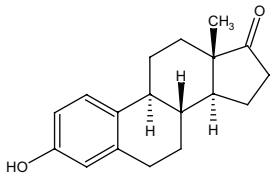
Description	P/N
XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm Column	186003116
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



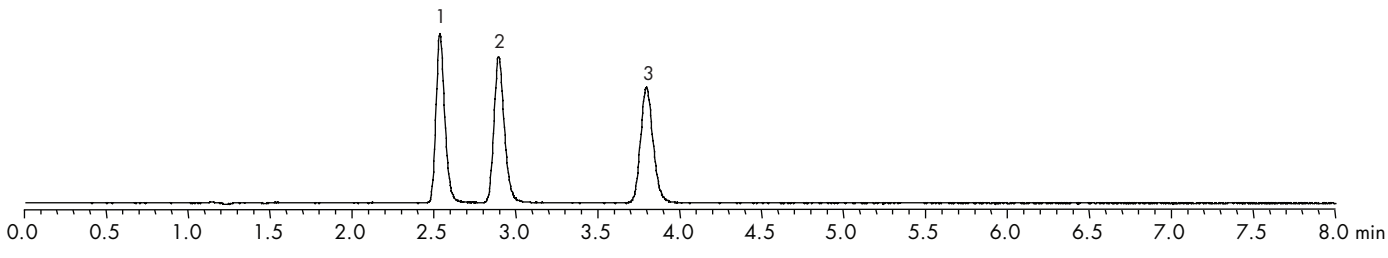
1. Ethylparaben



2. Estradiol



3. Estrone



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For complete experimental details, refer to full application note WA60197 at waters.com

Analysis of Flavonoids in Fruit Juice

EXPERIMENTAL

LC conditions

System: Alliance 2695 with 996 PDA detector

Columns: XBridge BEH Shield RP18, 5 µm, 4.6 x 150 mm
XBridge BEH C_8, 5 µm, 4.6 x 150 mm

Mobile phase A: 2% acetic acid

Mobile phase B: Acetonitrile

Gradient:

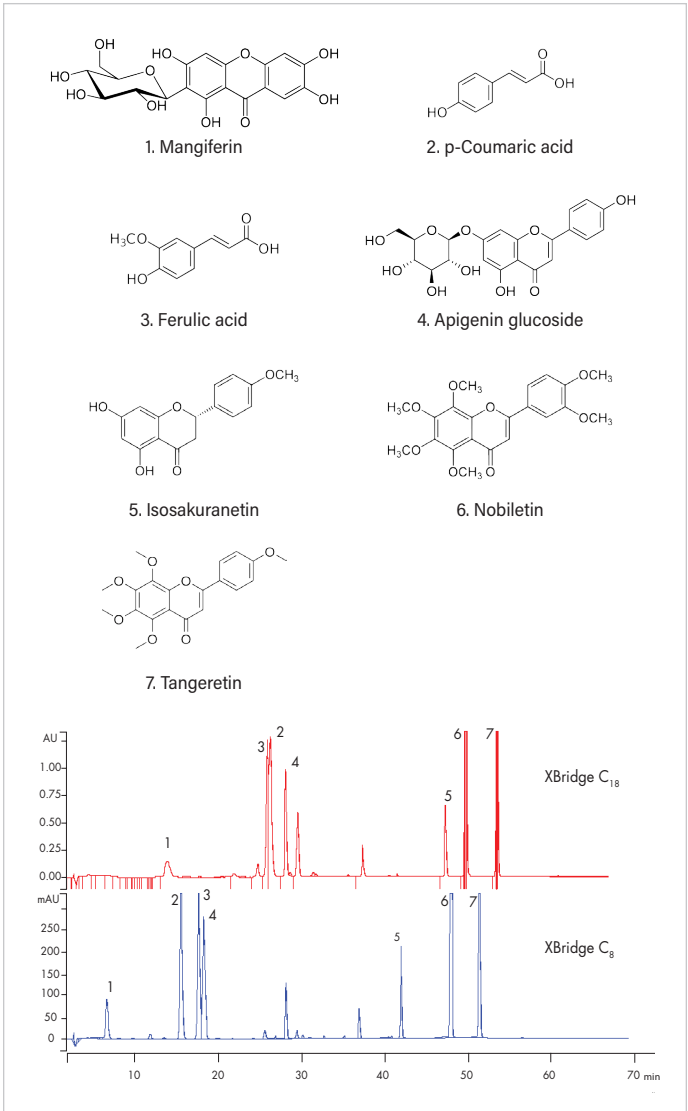
Time	%A	%B
0.00	90	10
15.00	86	14
20.00	82	18
30.00	75	25
55.00	45	55
67.00	5	95
80.00	5	95
85.00	90	10

Flow rate: 0.75 mL/min

Column temp.: Ambient

Injection: 20 µL

UV detection: 310 nm



ORDERING INFORMATION

Description	P/N
XBridge BEH Shield RP18, 5 µm, 4.6 x 150 mm Column	186003009
XBridge BEH C_8, 5 µm, 4.6 x 150 mm Column	186003017
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note WA64113 at waters.com

Analysis of 5-Fluorouracil

EXPERIMENTAL

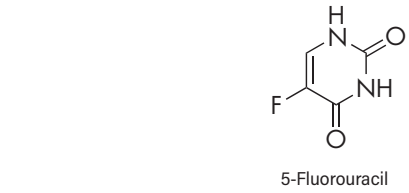
LC conditions

System:	Alliance HPLC with 2998 PDA detector
Column:	XBridge Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase:	95/2.5/2.5 acetonitrile/ isopropyl alcohol/water with 5 mM ammonium acetate, pH 9.0
Separation mode:	Isocratic
Flow rate:	0.5 mL/min
Column temp.:	25 °C
Injection volume:	50.0 µL
UV detection:	265 nm

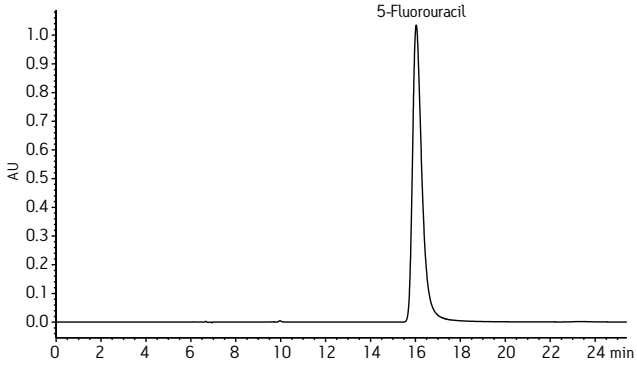
Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.

Sample concentration: 100 µg/mL



5-Fluorouracil



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note XBRIDGE16 at waters.com

Analysis of Food Additives and Preservatives

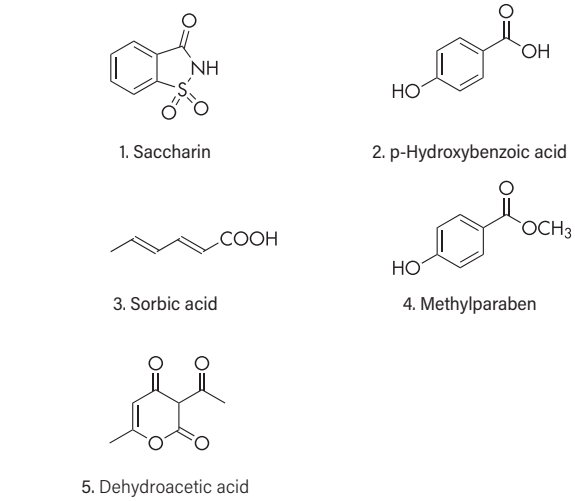
EXPERIMENTAL

LC conditions

System:	Alliance 2695 with 2996 PDA detector
Columns:	XBridge BEH Phenyl, 3.5 µm, 4.6 x 100 mm
Mobile phase A:	20 mM KH_2PO_4, pH 2.5
Mobile phase B:	Acetonitrile
Isocratic conditions:	75% A; 25% B
Flow rate:	1.0 mL/min
Column temp.:	30 °C
Injection volume:	10 µ L
UV detection:	240 nm

Sample preparation

Sample:	Saccharin (100 µg/mL), P-hydroxybenzoic acid (10 µg/mL), Dehydroacetic acid (100 µg/mL), Methylparaben (25 µg/mL), Sorbic acid (10 µg/mL) in KH_2PO_4/ACN (75/25)
Sample temp.:	15 °C



ORDERING INFORMATION

Description	P/N
XBridge BEH Phenyl, 3.5 µm, 4.6 x 100 mm Column	186003334
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note WA64094 at waters.com

Analysis of Food Sugars in Sports Drink

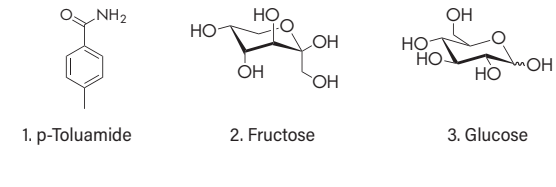
EXPERIMENTAL

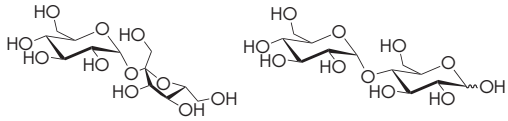
LC conditions

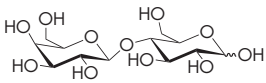
System:	Alliance HPLC with 2424 ELSD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine
Isocratic conditions:	90% A/10% B (75% acetonitrile with 0.2% triethylamine)
Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	15.0 µL
ELSD pressure:	30 psi
Drift tube temp.:	50 °C

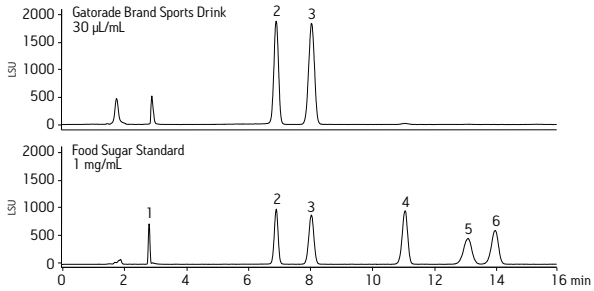
Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.









ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note WA64089 at waters.com

Analysis of Food Sugars in Bran with Raisin Cereal

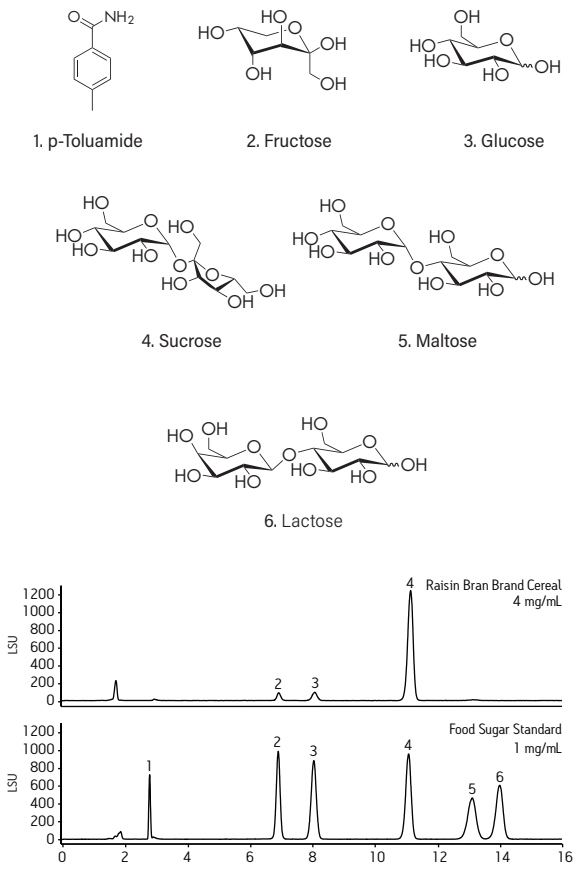
EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2424 ELSD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine
Isocratic conditions:	90% A/10% B (75% acetonitrile with 0.2% triethylamine)
Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	15.0 µL
ELSD pressure:	30 psi
Drift tube temp.:	50 °C

Sample preparation

Add 25 mL of 50:50 acetonitrile/water to ~3 g sample, homogenize, centrifuge at 3200 rpm for 30 minutes, collect supernatant and filter using 0.45 µm PVDF syringe filter.



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note WA64090 at waters.com

Analysis of Food Sugars in Ketchup

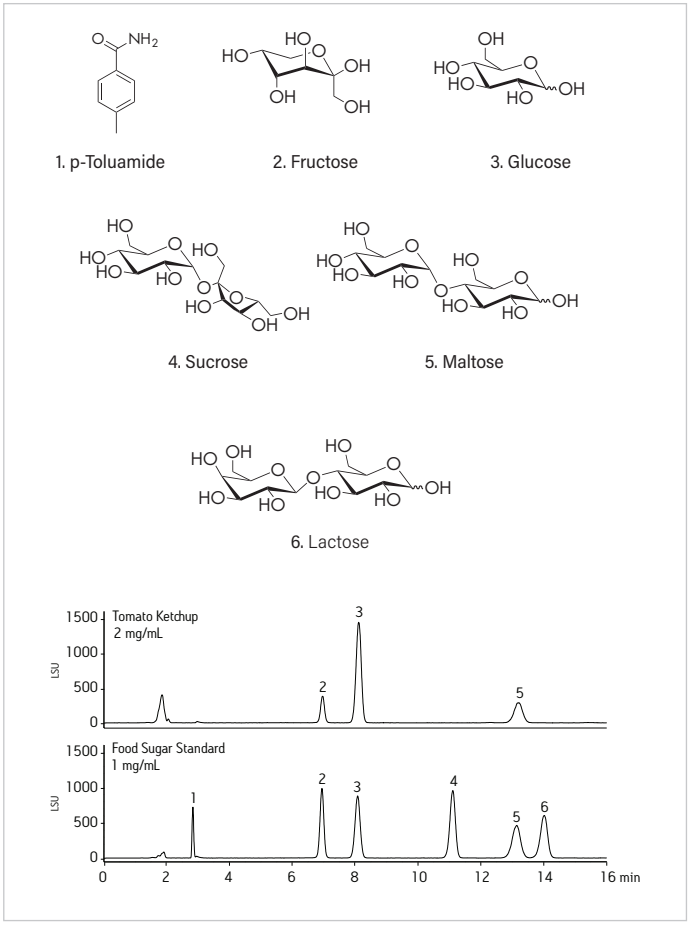
EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2424 ELSD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine
Isocratic conditions:	90% A/10% B (75% acetonitrile with 0.2% triethylamine)
Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	15.0 µL
ELSD pressure:	30 psi
Drift tube temp.:	50 °C

Sample preparation

Add 25 mL of 50:50 acetonitrile/water to ~3 g sample, homogenize, centrifuge at 3200 rpm for 30 minutes, collect supernatant and filter using 0.45 µm PVDF syringe filter.



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note WA64091 at waters.com

Analysis of Food Sugars in Milk

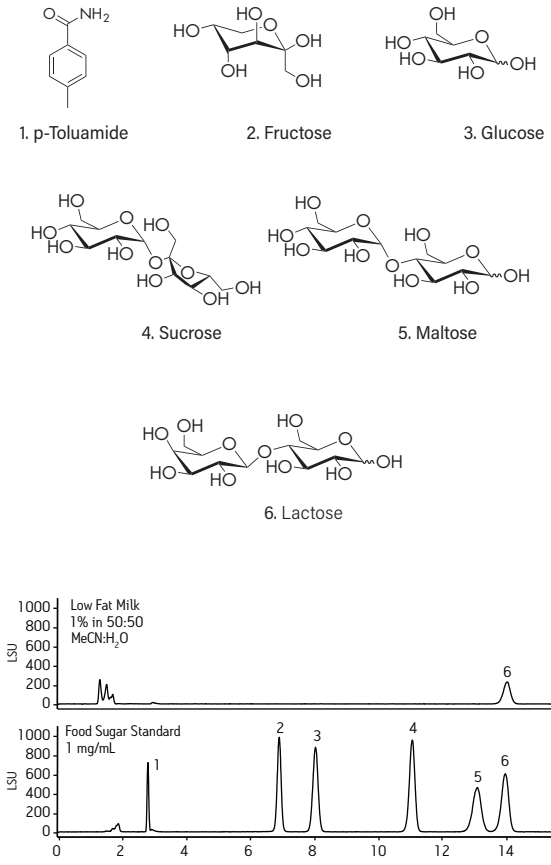
EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2424 ELSD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine
Isocratic conditions:	90% A/10% B (75% acetonitrile with 0.2% triethylamine)
Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	15.0 µL
ELSD pressure:	30 psi
Drift tube temp.:	50 °C

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note WA64092 at waters.com

Analysis of Food Sugars in Molasses

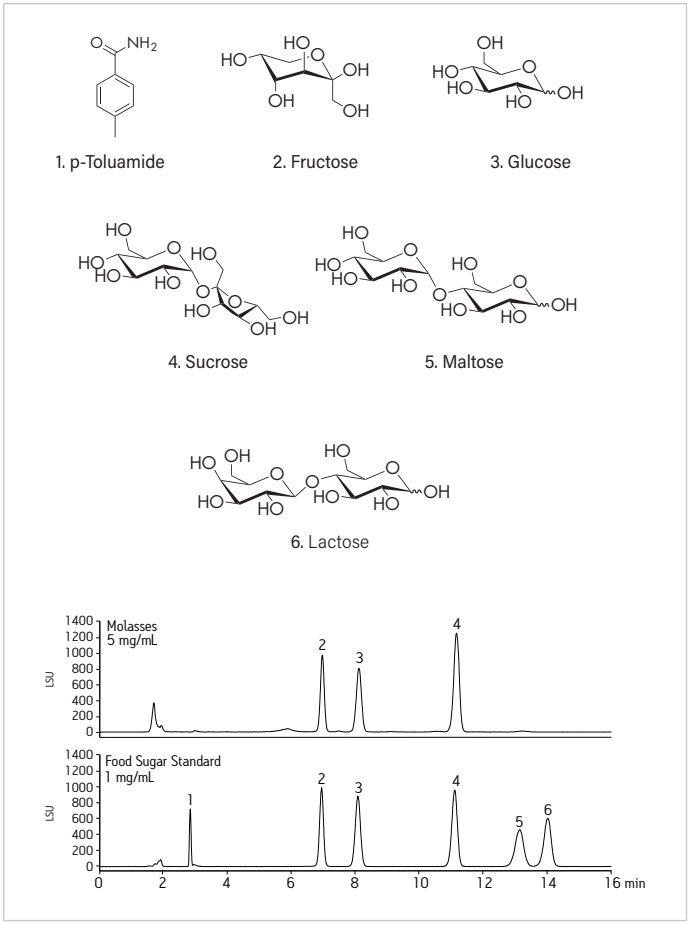
EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2424 ELSD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine
Isocratic conditions:	90% A/10% B (75% acetonitrile with 0.2% triethylamine)
Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	15.0 µL
ELSD pressure:	30 psi
Drift tube temp.:	50 °C

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note WA64096 at waters.com

Analysis of Food Sugars in Potato Chips

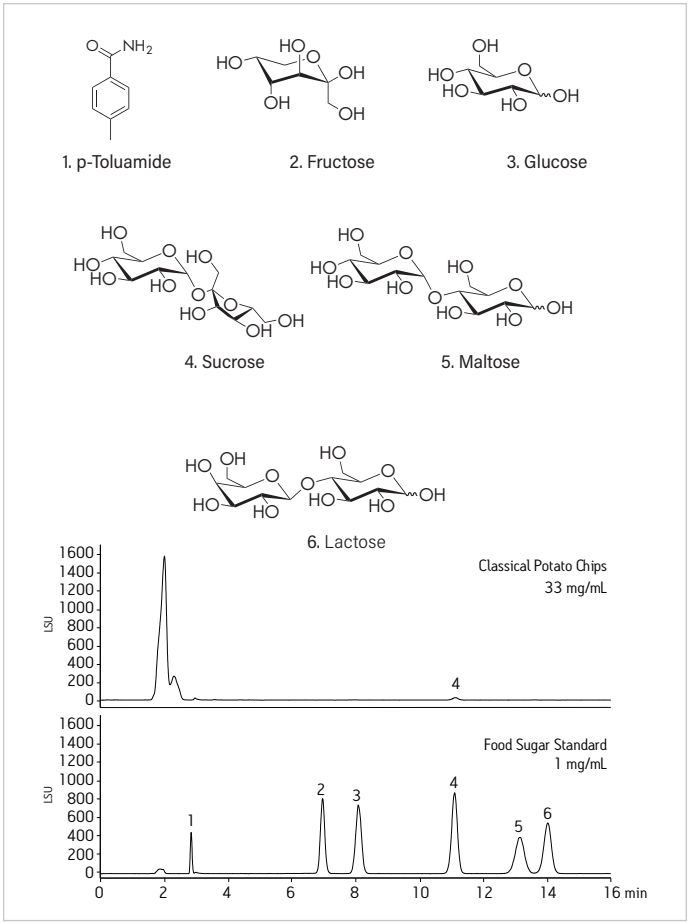
EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2424 ELSD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/wate with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine
Isocratic conditions:	90% A/10% B (75% acetonitrile with 0.2% triethylamine)
Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	15.0 µL
ELSD pressure:	30 psi
Drift tube temp.:	50 °C

Sample preparation

Add 25 mL of 50:50 acetonitrile/water to ~3 g sample, homogenize, centrifuge at 3200 rpm for 30 minutes, collect supernatant and filter using 0.45 µm PVDF syringe filter.



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note WA64093 at waters.com

Analysis of Food Sugars in Prepared Foods

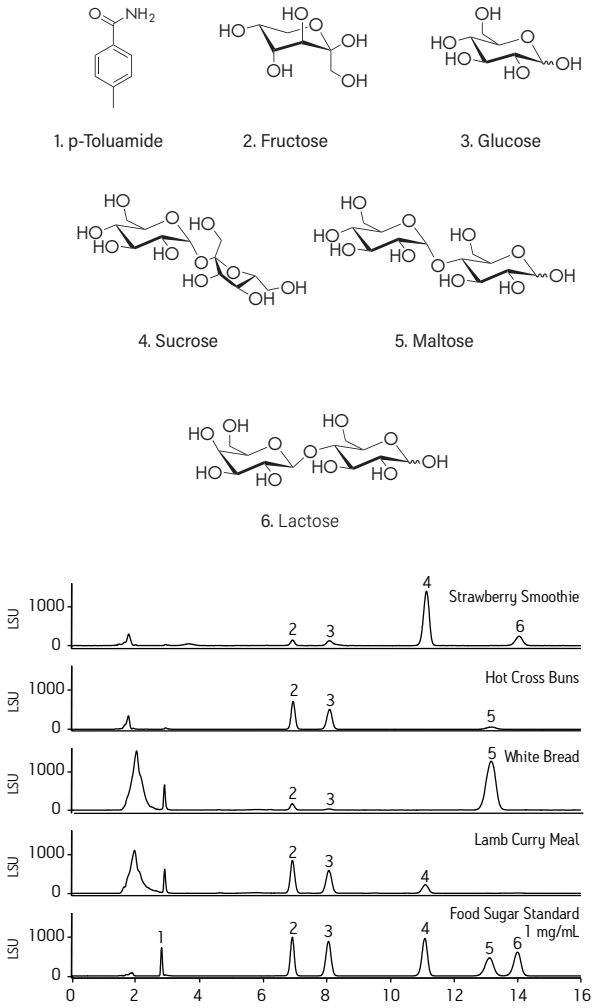
EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2424 ELSD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine
Isocratic conditions:	90% A/10% B (75% acetonitrile with 0.2% triethylamine)
Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	15.0 µL
ELSD pressure:	30 psi
Drift tube temp.:	50 °C

Sample preparation

Add 25 mL of 50:50 acetonitrile/water to ~3 g sample, homogenize, centrifuge at 3200 rpm for 30 minutes, collect supernatant and filter using 0.45 µm PVDF syringe filter.



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note WA64095 at waters.com

Analysis of Food Sugars in Wine

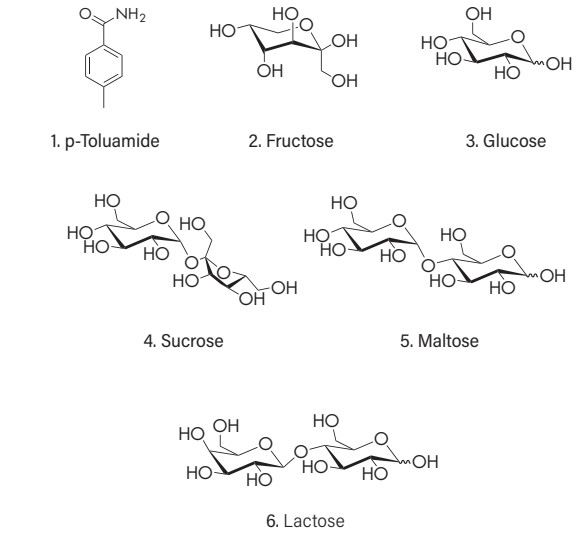
EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2424 ELSD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine
Isocratic conditions:	90% A/10% B (75% acetonitrile with 0.2% triethylamine)
Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	15.0 µL
ELSD pressure:	30 psi
Drift tube temp.:	50 °C

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note WA64088 at waters.com

Analysis of Food Sugars Standard

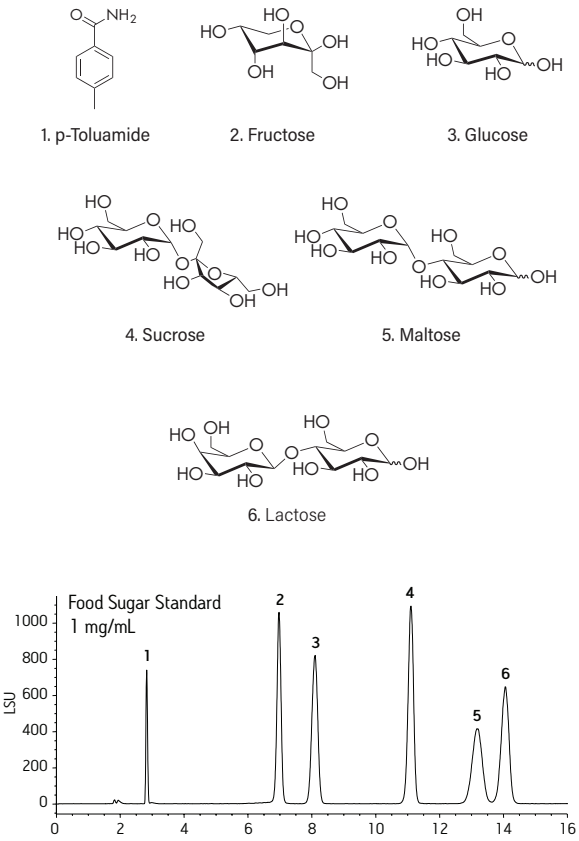
EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2424 ELSD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine
Isocratic conditions:	90% A/10% B (75% acetonitrile with 0.2% triethylamine)
Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	15.0 µL
ELSD pressure:	30 psi
Drift tube temp.:	50 °C

Sample preparation

Sample concentration: 1 mg/mL each



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note WA64101 at waters.com

Analysis of Food Sugars/Saccharides in Beer by ELSD

EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2424 ELSD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine

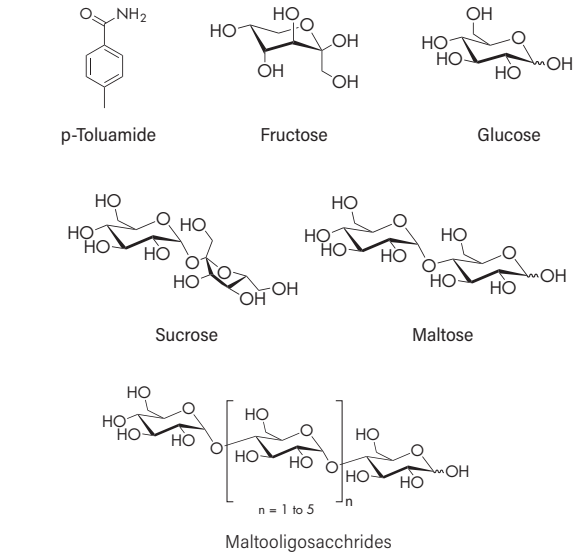
Gradient:

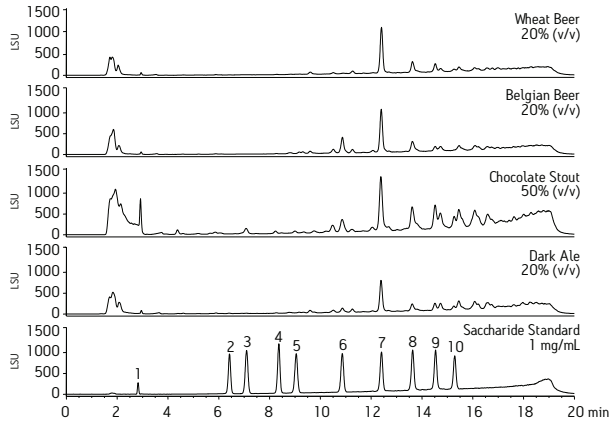
Time	%A	%B
0.00	90	10
16.00	30	70
16.01	90	10
30.00	90	10

Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	10.0 µL
ELSD pressure:	30 psi
Drift tube temp.:	50 °C

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.





1. p-Toluamide
2. Fructose
3. Glucose
4. Sucrose
5. Maltose
6. Maltotriose
7. Maltotetraose
8. Maltopentaose
9. Maltohexaose
10. Maltoheptaose

ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note WA64105 at waters.com

Analysis of Food Sugars/Saccharides in Beer by MS

EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC with 30-cm column cooler/heater and ACQUITY TQD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.1% ammonium hydroxide
Mobile phase B:	30/70 acetonitrile/water with 0.1% ammonium hydroxide

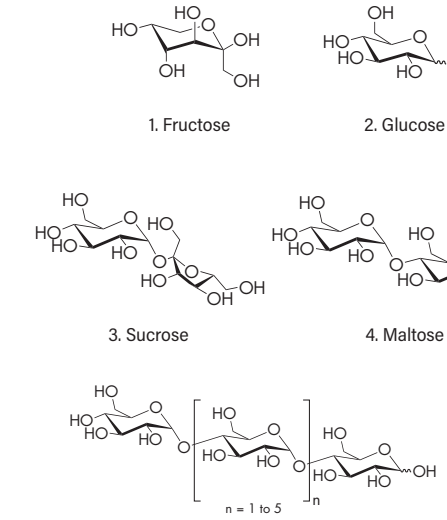
Gradient:

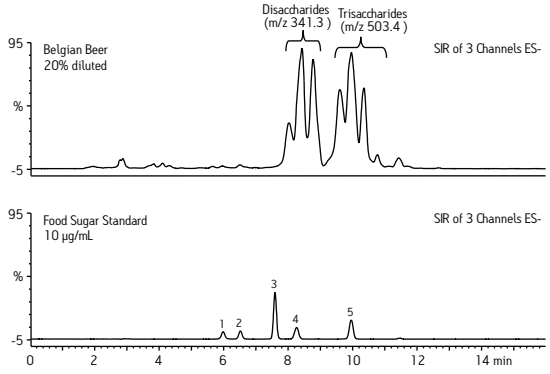
Time	%A	%B
0.00	90	10
16.00	30	70
16.01	90	10
30.00	90	10

Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	20.0 µL
Ionization mode:	ESI-
Acquisition mode:	SIR (m/z): 179.2 (fructose, glucose); 341.3 (sucrose, maltose); 503.4 (maltotriose)

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.





ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial w/ Preslit Septa	600000668CV

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34 Maple Street, Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990

For complete experimental details, refer to full application note WA64098 at waters.com

Analysis of Food Sugars/Saccharides in Cough Syrup

EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2424 ELSD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine

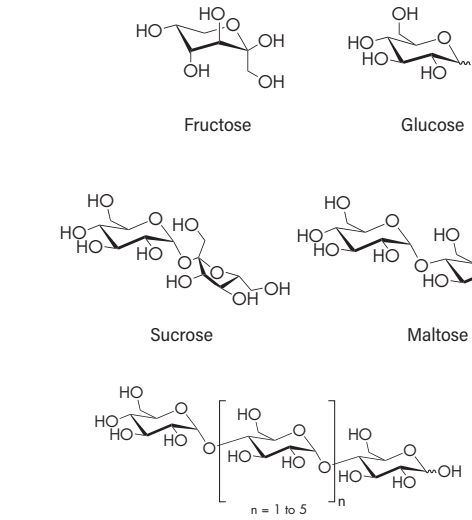
Gradient:

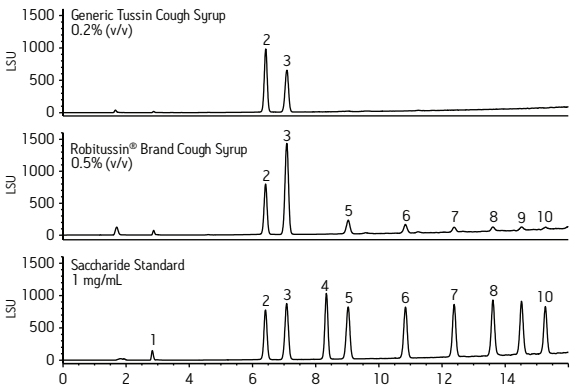
Time	%A	%B
0.00	90	10
16.00	30	70
16.01	90	10
30.00	90	10

Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	10.0 µL
ELSD pressure:	30 psi
Drift tube temp.:	50 °C

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.





1. p-Toluamide
2. Fructose
3. Glucose
4. Sucrose
5. Maltose
6. Maltotriose
7. Maltotetraose
8. Maltopentaose
9. Maltohexaose
10. Maltoheptaose

ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note WA64099 at waters.com

Analysis of Food Sugars/Saccharides in Honey

EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2424 ELSD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine

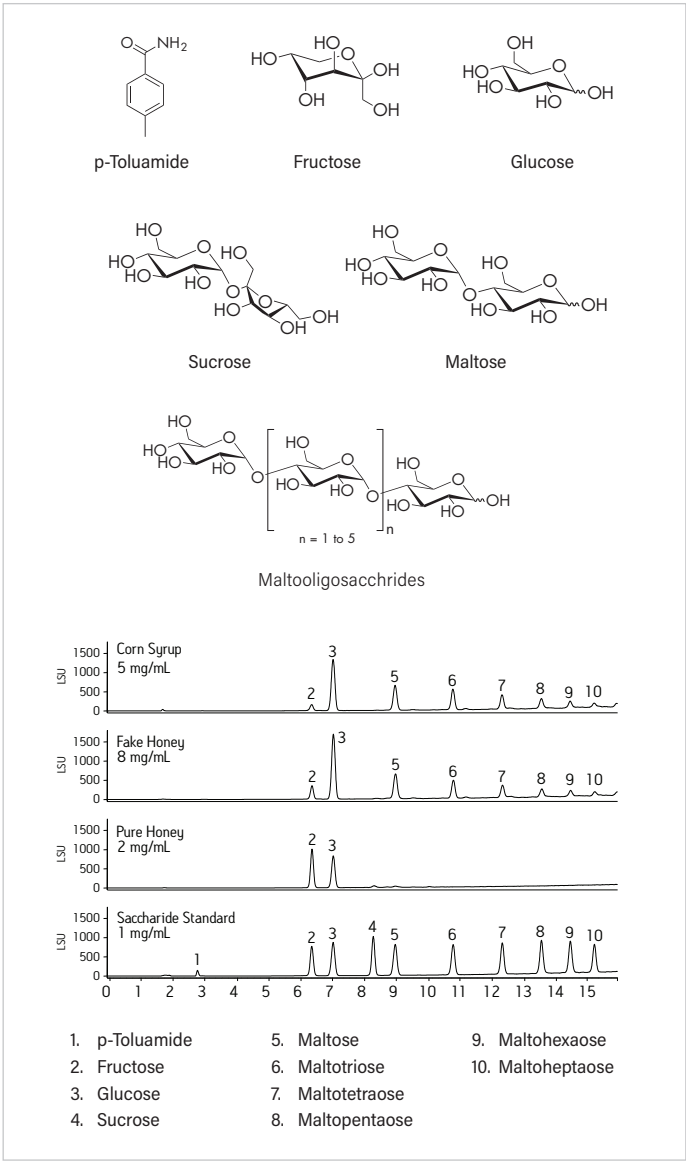
Gradient:

Time	%A	%B
0.00	90	10
16.00	30	70
16.01	90	10
30.00	90	10

Flow rate:	1.0 mL/min
Column temp.:	35 ˚C
Injection volume:	10.0 µL
ELSD pressure:	30 psi
Drift tube temp.:	50 ˚C

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

Waters Corporation
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For complete experimental details, refer to full application note WA64100 at waters.com

Analysis of Food Sugars/Saccharides in Maple Syrup

EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2424 ELSD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine

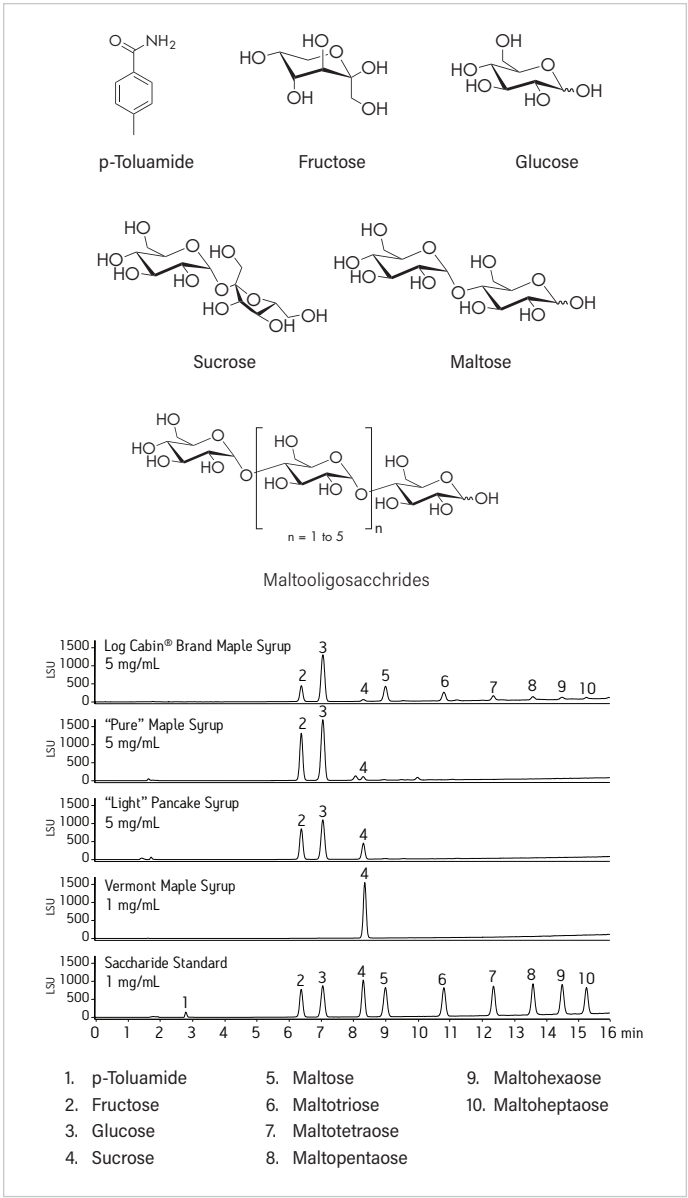
Gradient:

Time	%A	%B
0.00	90	10
16.00	30	70
16.01	90	10
30.00	90	10

Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	10.0 µL
ELSD pressure:	30 psi
Drift tube temp.:	50 °C

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.



1. p-Toluamide
2. Fructose
3. Glucose
4. Sucrose
5. Maltose
6. Maltotriose
7. Maltotetraose
8. Maltopentaose
9. Maltohexaose
10. Maltoheptaose

ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

For complete experimental details, refer to full application note WA64097 at waters.com

Analysis of Food Sugars/Saccharide Standards by ELSD

EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2424 ELSD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine

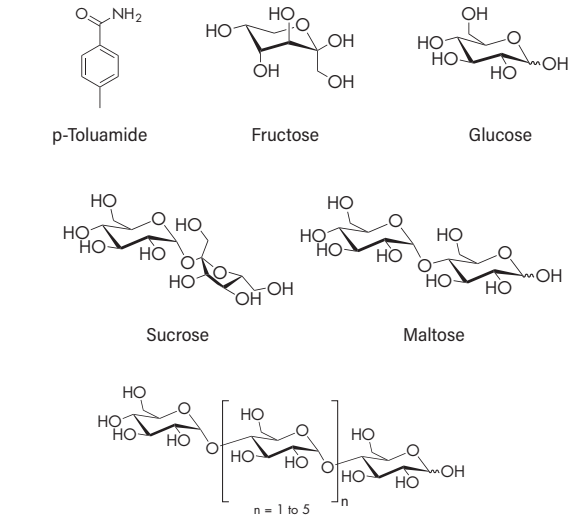
Gradient:

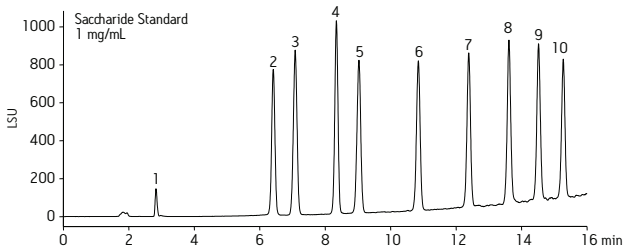
Time	%A	%B
0.00	90	10
16.00	30	70
16.01	90	10
30.00	90	10

Flow rate:	1.0 mL/min
Column temp.:	35 ˚C
Injection volume:	10.0 µL
ELSD pressure:	30 psi
Drift tube temp.:	50 ˚C

Sample preparation

Sample concentration: 1 mg/mL each





1. p-Toluamide
2. Fructose
3. Glucose
4. Sucrose
5. Maltose
6. Maltotriose
7. Maltotetraose
8. Maltopentaose
9. Maltohexaose
10. Maltoheptaose

ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note WA64104 at waters.com

Analysis of Food Sugars/Saccharide Standards by MS

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC with ACQUITY TQD

Column: XBridge BEH Amide, 3.5 µm, 4.6 x 150 mm

Mobile phase A: 80/20 acetonitrile/water with 0.1% ammonium hydroxide

Mobile phase B: 30/70 acetonitrile/water with 0.1% ammonium hydroxide

Gradient:

Time	%A	%B
0.00	100	0
30.60	40	60
30.61	100	0
52.00	100	0

Flow rate: 0.4 mL/min

Column temp.: 35 °C

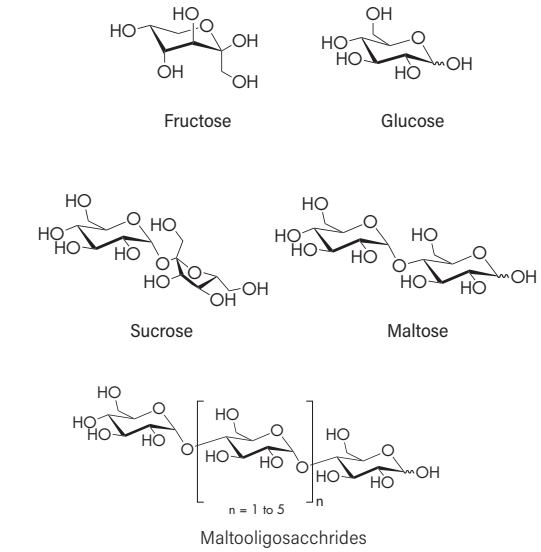
Injection volume: 10.0 µL

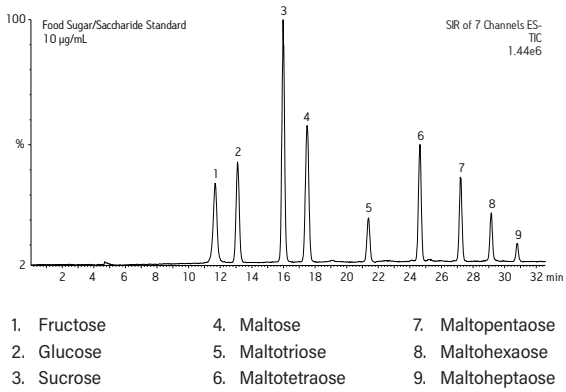
Ionization mode: ESI-

Acquisition mode: SIR (m/z): 178.96 (fructose, glucose); 341.04 (sucrose, maltose); 503.08 (maltotriose); 665.10 (maltotetraose); 827.22 (maltopentaose); 989.21 (maltohexaose); 1151.29 (maltoheptaose)

Sample preparation

Sample concentrations: 10 µg/mL each





1. Fructose
2. Glucose
3. Sucrose
4. Maltose
5. Maltotriose
6. Maltotetraose
7. Maltopentaose
8. Maltohexaose
9. Maltoheptaose

ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 150 mm Column	186004869
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial w/ Preslit Septa	600000668CV

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For complete experimental details, refer to full application note 720002738EN at waters.com

Analysis of Formaldehyde in Ambient Air

EXPERIMENTAL

LC conditions

System: Alliance HPLC with UV detection

Column: XBridge BEH Phenyl, 3.5 µm, 4.6 x 150 mm

Mobile phase A: 90% water, 10% tetrahydrofuran (THF)

Mobile phase B: Acetonitrile

Gradient: Eluent gradient for EPA methods 554 and 8315 Option 1.

Time	Flow	%A	%B	Curve
Initial	1.5	70	30	–
20.0	1.5	36	64	6
22.0	1.5	36	64	6
22.1	1.5	70	30	6

Eluent gradient for EPA Methods TO11 and 8315 Option 2.

Time	Flow	%A	%B	Curve
Initial	1.5	70	30	–
16.0	1.5	53	47	6
21.0	1.5	53	47	6
21.1	1.5	70	30	6

Flow rate: 1.5 mL/min

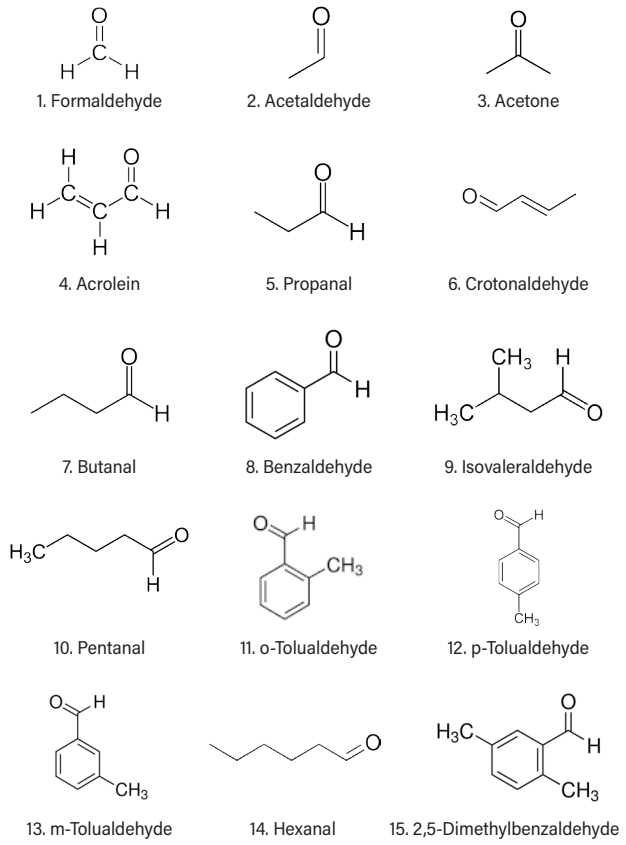
Column temp.: 35 °C

Injection: 20 µL each

UV detection: 360 nm

Sample preparation

AccuStandard mix (M- 8315-R1- DNPH and M- 8315-R2- DNPH) diluted 1:5 in 40:60 water/acetonitrile. Online derivatization carried out on Sep-Pak DNPH Silica Cartridge, backflush cartridge with acetonitrile.



ORDERING INFORMATION

Description	P/N
XBridge BEH Phenyl, 3.5 µm, 4.6 x 150 mm Column	186003335
Sep-Pak DNPH-Silica Cartridge	WAT037500
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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34 Maple Street, Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990

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For complete experimental details, refer to full application note WA60198 at waters.com

Analysis of Furanocoumarins in Fruit Juice

EXPERIMENTAL

LC conditions

System: Alliance 2695 with 996 PDA detector

Columns: XBridge BEH Shield RP18, 5 µm, 4.6 x 150 mm; XBridge BEH C_8, 5 µm, 4.6 x 150 mm

Mobile phase A: 2% acetic acid

Mobile phase B: Acetonitrile

Gradient:

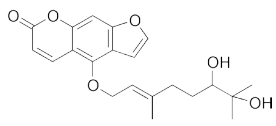
Time	%A	%B
0.0	90	10
15.0	80	20
20.0	75	25
30.0	60	40
55.0	30	70
67.0	5	95
80.0	5	95
85.0	90	10
95.0	90	10

Flow rate: 0.75 mL/min

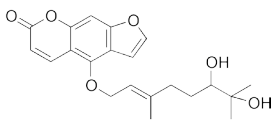
Column temp.: Ambient

Injection: 20 µL

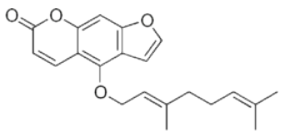
UV detection: 310 nm



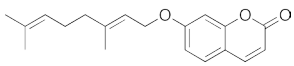
1. 6,7-Dihydroxybergomottin



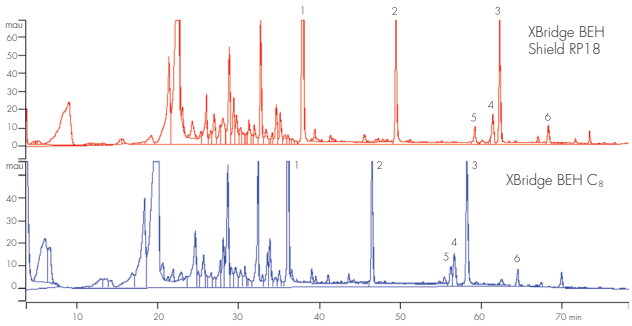
2. 6′,7′-Epoxybergamottin



3. Bergamottin



5. 7-Geranyloxycoumarin



1. 6,7-Dihydroxybergomottin
2. 6′,7′-Epoxybergamottin
3. Bergamottin
4. Furanocoumarin dimer
5. 7-Geranyloxycoumarin
6. Furanocoumarin dimer

Sample preparation

The juice samples were obtained from white grapefruit. Samples were centrifuged and the furanocoumarins then extracted into ethyl acetate.

ORDERING INFORMATION

Description	P/N
XBridge BEH Shield RP18, 5 µm, 4.6 x 150 mm Column	186003009
XBridge BEH C_8, 5 µm, 4.6 x 150 mm Column	186003017
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

Waters Corporation
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For complete experimental details, refer to full application note 720003721EN at waters.com

Analysis of Galantamine and Related Substances

EXPERIMENTAL

LC conditions

System: Alliance 2695

Column: XBridge BEH C_{18}, 3.5 µm, 4.6 x 100 mm

Mobile phase A: 95% phosphate buffer solution: 5% methanol

Buffer: Dissolve 0.79 g of dibasic sodium phosphate dihydrate and 2.46 g of monobasic sodium phosphate anhydrous in 1 L of water.

Mobile phase B: Acetonitrile

Gradient:

Time	%A	%B
0.00	100	0
6.00	100	0
20.00	95	5
35.00	85	15
50.00	80	20
51.00	40	60
55.00	40	60
56.00	100	0
60.00	100	0

Flow rate: 1.5 mL/min

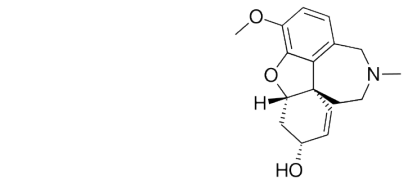
Column temp.: 55 °C

Injection volume: 20 µL

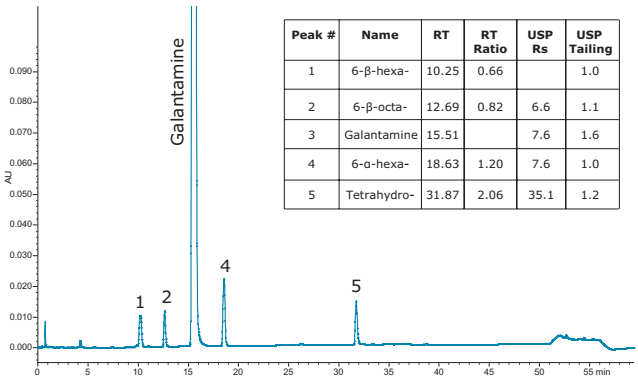
UV detection: 230 nm

Sample preparation

United States Pharmacopeia reference standards: USP Galantamine Hydrobromide RS and USP Galantamine Hydrobromide Related Compounds Mixture RS.



Galantamine



Peak #	Name	RT	RT Ratio	USP Rs	USP Tailing
1	6-β-hexa-	10.25	0.66		1.0
2	6-β-octa-	12.69	0.82	6.6	1.1
3	Galantamine	15.51		7.6	1.6
4	6-α-hexa-	18.63	1.20	7.6	1.0
5	Tetrahydro-	31.87	2.06	35.1	1.2

ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 3.5 µm, 4.6 x 100 mm Column	186003033
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note WA64086 at waters.com

Analysis of Ginsenoside Rb1 in Ginseng Root Powder Extract

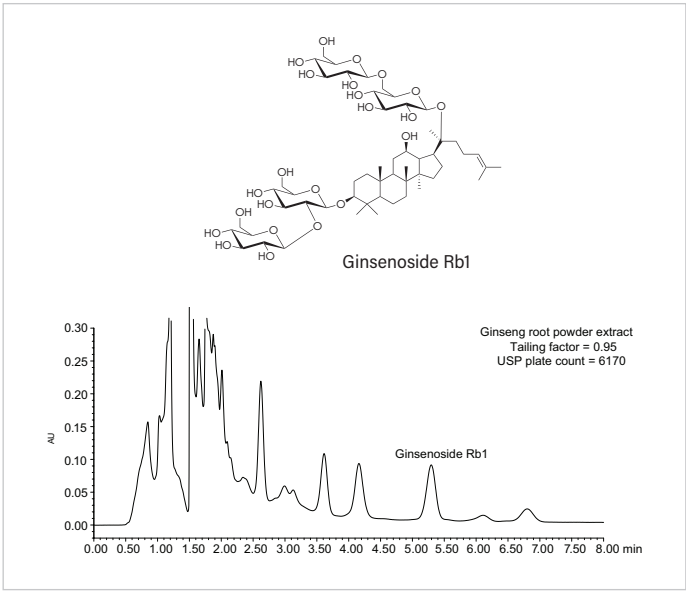
EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2998 PDA detector
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 150 mm
Mobile phase:	80:20 acetonitrile/water
Separation mode:	Isocratic
Flow rate:	1.4 mL/min
Column temp.:	60 °C
Injection volume:	11.5 µL
UV detection:	203 nm

Sample preparation

1. Weigh 200 mg ginseng root powder into an extraction vessel.
2. Add 1 mL 80% methanol, sonicate for 5 minutes.
3. Centrifuge at 10,000 rpm for 5 minutes.
4. Collect the supernatant.
5. Repeat steps 2-4 two more times.
6. Combine the extracts, mix well.
7. Filter through 13 mm nylon 0.2 µm filter for injection.



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 150 mm Column	186004869
Acrodisc, Syringe Filter, Nylon, 13 mm, 0.2 µm, 100/pk	WAT200524
Waters LCMS Certified Vial	600000751CV

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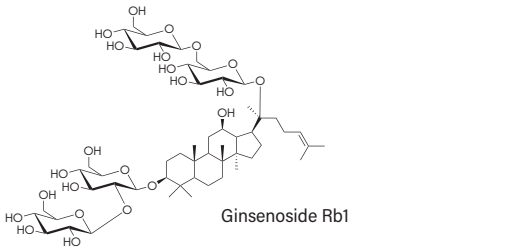
For complete experimental details, refer to full application note WA64087 at waters.com

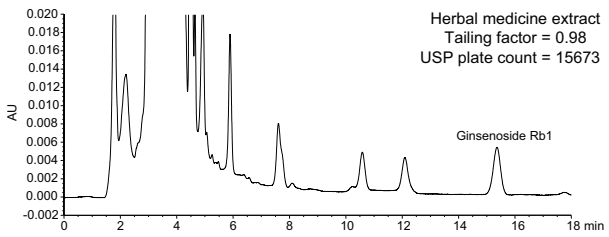
Analysis of Ginsenoside Rb1 in Herbal Medicine Extract

EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2998 PDA detector
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase:	80:20 acetonitrile/water
Separation mode:	Isocratic
Flow rate:	0.8 mL/min
Column temp.:	60 °C
Injection volume:	20 µL
UV detection:	203 nm





ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Sep-Pak C_{18} Plus Short Cartridge	WAT020515
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

Sample preparation

Pretreatment

1. Weigh 2 g of herbal medicine powder into a centrifuge tube.
2. Add 30 mL of 60% methanol/40% water.
3. Shake for 15 minutes.
4. Centrifuge at 4000 rpm for 10 minutes.
5. Obtain the supernatant.
6. Repeat steps 2-5 with the residue using 15 mL of 60% methanol/40% water.
7. Combine the supernatant, and make exactly 50 mL by adding 60% methanol/40% water.
8. Take 10 mL of this solution and add 3 mL of sodium hydroxide test solution (1 mol/L).
9. Let stand for 30 minutes.
10. Add 3 mL of HCl test solution (1 mol/L).
11. Add 60% methanol/40% water to make exactly 20 mL.

Solid-Phase Extraction Procedure

1. Condition Sep-Pak Plus C_{18} Cartridge, 360 mg (55-105 µm) with 2 mL methanol.
2. Equilibrate with 2 mL of 30% methanol/70% water just before loading.
3. Load 5 mL of the solution from step 11 in the pretreatment stage.
4. Wash with 2 mL of 30% methanol/70% water.
5. Wash with 1 mL of sodium carbonate test solution (1 mol/L).
6. Wash with 10 mL of 30% methanol/70% water.
7. Elute with 5 mL methanol (this is the injection solution).

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For complete experimental details, refer to full application note 720005229EN at waters.com

Analysis of Goldenseal

EXPERIMENTAL

LC conditions

System:	Alliance HPLC with ACQUITY QDa Detector
Column:	CORTECS C_{18}+, 2.7 µm, 3.0 x 50 mm
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile

Gradient:

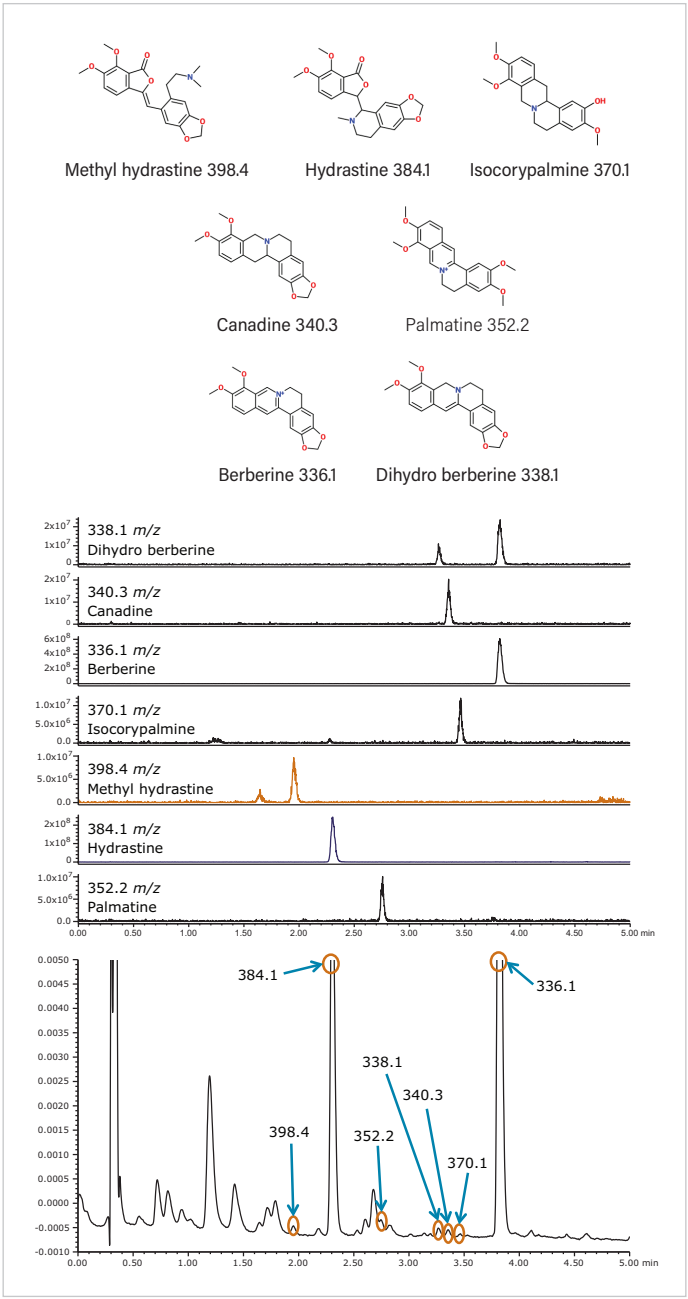
Time	%A	%B
0.00	93	7
5.00	70	30
5.01	93	7
6.01	93	7

Flow rate:	1.0 mL/min
Column temp.:	30 °C
Injection volume:	1.0 µL
UV detection:	300 nm
Ionization mode:	ESI+
Acquisition mode:	Full scan 150–1250 m/z

Sample preparation

Capsule samples: Add 2.5 mL of 90:10 methanol/water with 0.1% acetic acid to 20 mg sample, sonicate 15 minutes, centrifuge at 4000 rpm for 5 minutes. Remove supernatant and repeat extraction three additional times. Combine extracted liquid and filter through a 0.1 µm nylon syringe filter.

Liquid sample: Two drops of liquid Goldenseal was added to 10 mL of 90:10 methanol/water with 0.1% acetic acid and filter through.



ORDERING INFORMATION

Description	P/N
CORTECS C_{18}+, 2.7 µm, 3.0 x 50 mm Column	186007400
Waters LCMS Certified Max Recovery Vial w/ Preslit Septa	600000670CV

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For complete experimental details, refer to full application note WA64109 at waters.com

Analysis of Histidine Dipeptides

EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC with 30-cm column cooler/heater and TQD detector
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	50/50 acetonitrile/water with 10 mM ammonium acetate and 0.04% ammonium hydroxide, pH 9.0
Mobile phase B:	95/5 acetonitrile/water with 10 mM ammonium acetate and 0.04% ammonium hydroxide, pH 9.0

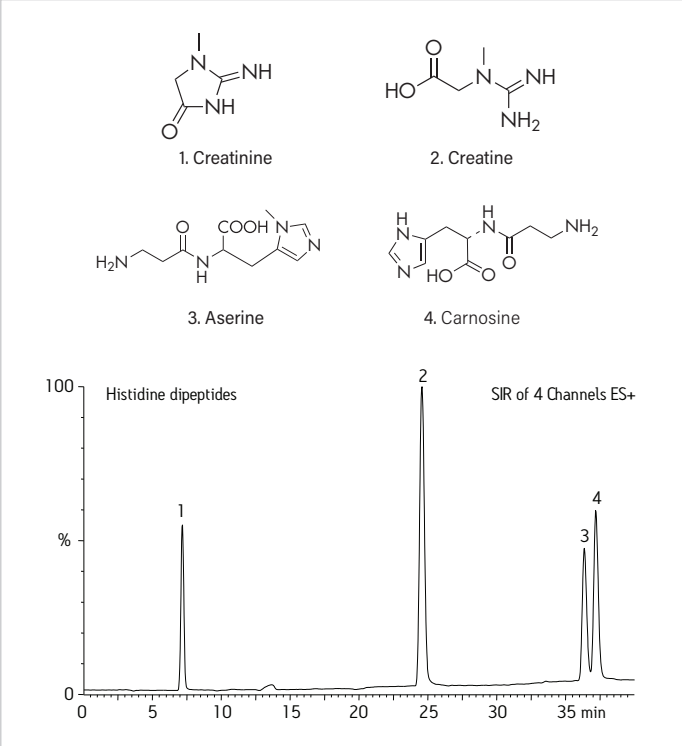
Gradient:

Time	%A	%B
0.00	90	10
16.00	30	70
16.01	90	10
30.00	90	10

Flow rate:	1.0 mL/min
Column temp.:	65 °C
Injection volume:	15.0 µL
Ionization mode:	ESI+
Acquisition mode:	SIR

Sample preparation

Add 25 mL of 50:50 acetonitrile/water to ~3 g sample, homogenize, centrifuge at 3200 rpm for 30 minutes, collect supernatant and filter using 0.45 µm PVDF syringe filter.



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial w/ Preslit Septa	600000668CV

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For complete experimental details, refer to full application note 720004130EN at waters.com

Analysis of Irbesartan Tablets

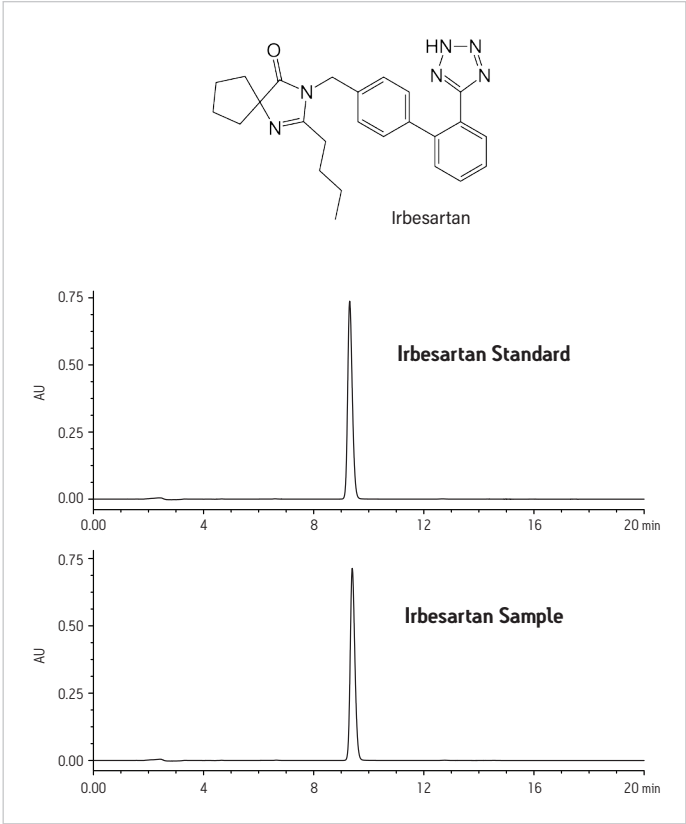
EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2489 UV/Visible detector
Column:	XSelect HSS T3, 5 µm, 4.6 x 250 mm
Mobile phase:	Acetonitrile and buffer solution (40:60); Buffer solution: 0.55% phosphoric acid in water adjusted to pH 3.0 with triethylamine
Separation mode:	Isocratic
Flow rate:	1.0 mL/min
Column temp.:	30 °C
Injection volume:	10 µL
UV detection:	220 nm

Sample preparation

The concentration of the working standard and sample specified in the USP monograph is 0.15 mg/mL. The sample was prepared from AVAPRO (irbesartan) tablets as specified in the USP Monograph for Irbesartan: USP34-NF29. Five tablets of irbesartan were weighed and finely powdered. An equivalent of 15 mg of this powder was weighed and transferred to a 100-mL volumetric flask. Seventy-five milliliters of methanol was added to the flask and this solution was sonicated for 15 minutes, with stirring at 5-minute intervals. Methanol was added to make up the volume to 100 mL and this solution was passed through a 0.45-µm porous glass microfiber membrane filter.



ORDERING INFORMATION

Description	P/N
XSelect HSS T3, 5 µm, 4.6 x 250 mm Column	186004793
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720004132EN at waters.com

Analysis of Lamotrigine and Related Compounds

EXPERIMENTAL

LC conditions

System: Alliance 2695

Column: XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm

Buffer: 20 mM potassium phosphate monobasic

Mobile phase A: 150:1 buffer:triethylamine, adjusted to pH 2.0 with phosphoric acid

Mobile phase B: Acetonitrile

Gradient:

Time	%A	%B	Column Volumes
Initial	76.5	23.5	-
4.00	76.5	23.5	2.43
14.00	20.0	80.0	6.08
15.00	76.5	23.5	0.61
19.00	76.5	23.5	2.43

Flow rate: 1.0 mL/min

Column temp.: 35 °C

Injection volume: 10 µL

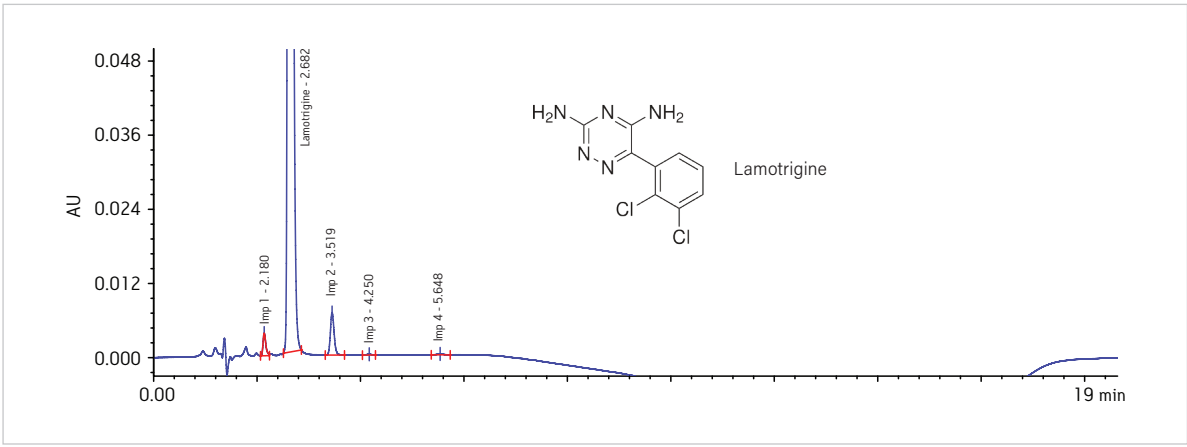
UV detection: 270 nm

Sample preparation

The samples were prepared by transferring an appropriate pooled number of tablets to a 1 L volumetric flask to obtain a concentration equivalent to 1.0 mg/mL lamotrigine. Tablets were dissolved in 200 mL water and 800 mL methanol. This solution was mechanically shaken for 20 minutes followed by centrifugation at 4000 rpm for 20 minutes. Aliquots from the dissolved tablet sample solution were diluted with diluent (0.1 M hydrochloric acid) to obtain a working sample concentration of 0.2 mg/mL.

ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm Column	186003116
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



For complete experimental details, refer to full application note 720005407EN at waters.com

Analysis of Levonorgestrel and Ethinyl Estradiol Tablets

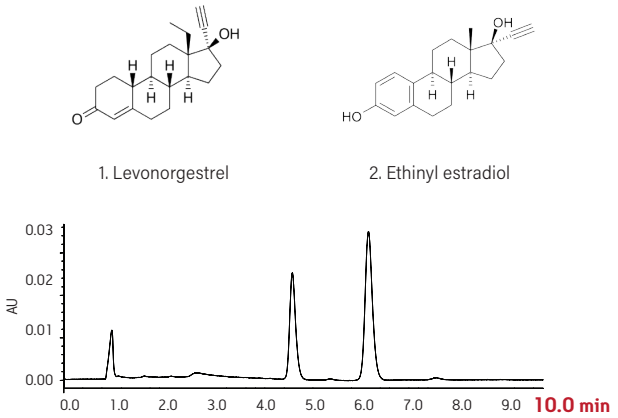
EXPERIMENTAL

LC conditions

System:	Alliance 2695
Column:	XBridge BEH C_8, 5 µm, 4.6 x 150 mm
Mobile phase:	7:3:9 acetonitrile:methanol:water
Separation mode:	Isocratic
Flow rate:	1 mL/min
Injection volume:	50 µL
UV detection:	215 nm

Sample preparation

Dissolve levonorgestrel and ethinyl estradiol commercially-available tablets in mobile phase to a final concentration of 15 µg/mL levonorgestrel and 3 µg/mL ethinyl estradiol. Sonicate for 5 minutes, shake mechanically for 20 minutes. Centrifuge at 4000 rpm for 10 minutes. Collect supernatant and re-centrifuge at 12,000 rpm for 30 minutes, pipet clear supernatant for injection.



ORDERING INFORMATION

Description	P/N
XBridge BEH C_8, 5 µm, 4.6 x 150 mm Column	186003017
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720005786EN at waters.com

Analysis of Lipid Soluble Antioxidants Using Atlantis T3, 3 µm Columns

EXPERIMENTAL

LC conditions

System:	ACQUITY Arc with 2998 PDA detector
Column:	Atlantis T3, 3 µm, 3.0 x 75 mm
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Mobile phase C:	Methanol
Mobile phase D:	2% formic acid in water (autoblended to 0.1% formic acid)

Gradient:

Time	%A	%B	%C	%D
0.00	60	17.5	17.5	5
2.00	40	27.5	27.5	5
3.00	14	40.0	41.0	5
7.50	14	40.0	41.0	5
8.00	60	17.5	17.5	5
10.00	60	17.5	17.5	5

Flow rate:	0.85 mL/min
Column temp.:	30 °C
Injection volume:	2.1 µL
UV detection:	280 nm

Sample preparation

Sample diluted to the described concentrations using 65:35 water:methanol.

Propyl gallate (0.05 mg/mL)

2,4,5-Trihydroxybutyrophenone (0.05 mg/mL)

Tert-butylhydroquinone (0.1 mg/mL)

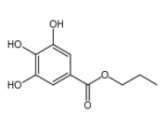
Butylated hydroxyanisole (0.1 mg/mL)

2,6-Di-tert-butyl-4-hydroxymethylphenol (0.1 mg/mL)

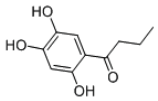
Octyl gallate (0.05 mg/mL)

ORDERING INFORMATION

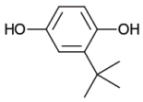
Description	P/N
Atlantis T3, 3 µm, 3.0 x 75 mm Column	186005653
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



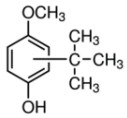
1. Propyl gallate



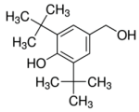
2. 2,4,5-Trihydroxybutyrophenone



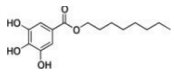
3. Tert-butylhydroquinone



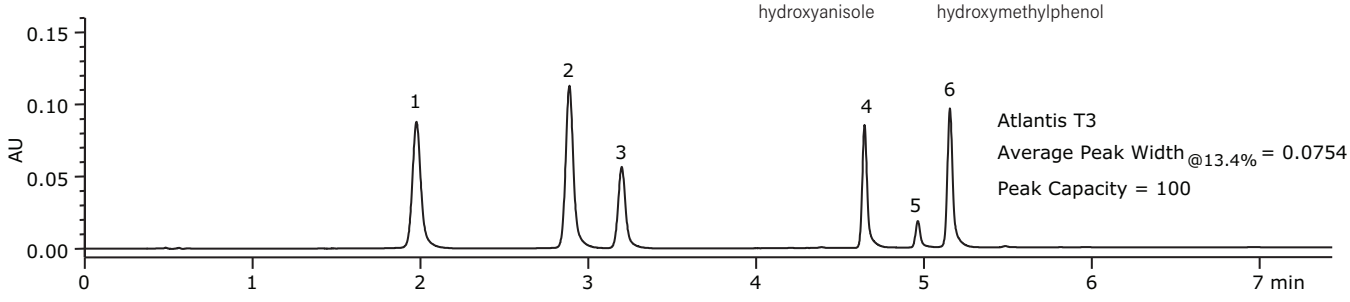
4. Butylated hydroxyanisole



5. 2,6-Di-tert-butyl-4-hydroxymethylphenol



6. Octyl gallate



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For complete experimental details, refer to full application note 720005786EN at waters.com

Analysis of Lipid Soluble Antioxidants Using CORTECS T3, 2.7 µm Columns

EXPERIMENTAL

LC conditions

System:	ACQUITY Arc with 2998 PDA detector
Column:	CORTECS T3, 2.7 µm, 3.0 x 75 mm
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Mobile phase C:	Methanol
Mobile phase D:	2% formic acid in water (autoblended to 0.1% formic acid)

Gradient:

Time	%A	%B	%C	%D
0.00	60	17.5	17.5	5
2.00	40	27.5	27.5	5
3.00	14	40.0	41.0	5
7.50	14	40.0	41.0	5
8.00	60	17.5	17.5	5
10.00	60	17.5	17.5	5

Flow rate:	0.85 mL/min
Column temp.:	30 °C
Injection volume:	2.1 µL
UV detection:	280 nm

Sample preparation

Sample diluted to the described concentrations using 65:35 water:methanol.

Propyl gallate (0.05 mg/mL)

2,4,5-Trihydroxybutyrophenone (0.05 mg/mL)

Tert-butylhydroquinone (0.1 mg/mL)

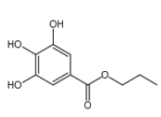
Butylated hydroxyanisole (0.1 mg/mL)

2,6-Di-tert-butyl-4-hydroxymethylphenol (0.1 mg/mL)

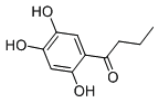
Octyl gallate (0.05 mg/mL)

ORDERING INFORMATION

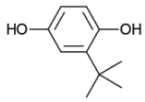
Description	P/N
CORTECS T3, 2.7 µm, 3.0 x 75 mm Column	186008488
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



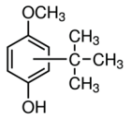
1. Propyl gallate



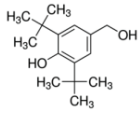
2. 2,4,5-Trihydroxybutyrophenone



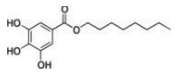
3. Tert-butylhydroquinone



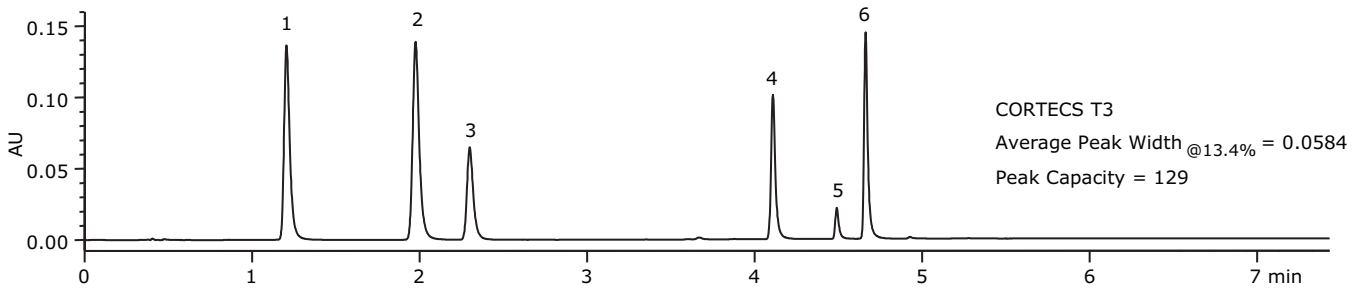
4. Butylated hydroxyanisole



5. 2,6-Di-tert-butyl-4-hydroxymethylphenol



6. Octyl gallate



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For complete experimental details, refer to full application note XBRIDGE5 at waters.com

Analysis of Local Anesthetics

EXPERIMENTAL

LC conditions

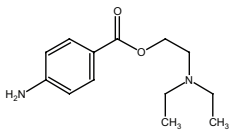
System:	Alliance 2695 with 2996 PDA detector
Column:	XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm
Mobile phase:	10 mM ammonium bicarbonate, pH 10.5/acetonitrile (50/5)
Separation mode:	Isocratic
Flow rate:	1.0 mL/min
Column temp.:	25 ˚C
Injection volume:	10 µL
UV detection:	210 nm

Sample preparation

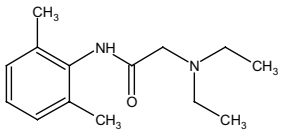
Sample concentration: 20 µg/mL in water/acetonitrile (50/50)

ORDERING INFORMATION

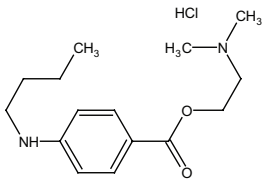
Description	P/N
XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm Column	186003116
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



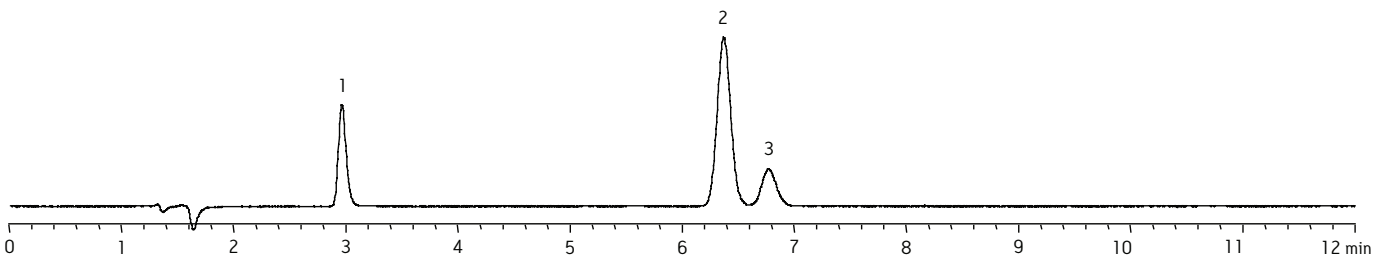
1. Procaine



2. Lidocaine



3. Tetracaine



For complete experimental details, refer to full application note 720003721EN at waters.com

Analysis of Loratadine Using XBridge BEH C_{18} Columns

EXPERIMENTAL

LC conditions

System: Alliance 2695

Column: XBridge BEH C_{18}, 5 µm, 4.6 x 250 mm

Mobile phase A: 0.96 g of 1-pentaesulfonic acid in 1 L water adjusted to pH 3.00 ± 0.05 with 10% phosphoric acid

Mobile phase B: Acetonitrile

Gradient:

Time	%A	%B
0.00	75	25
20.00	50	50
30.00	40	60
35.00	30	70
45.00	30	70
50.00	75	25

Flow rate: 1.2 mL/min

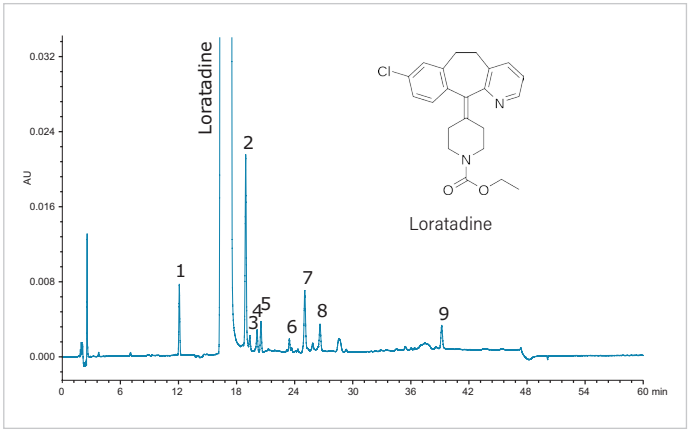
Column temp.: 35 °C

Injection volume: 20 µL

UV detection: 254 nm

Sample preparation

United States Pharmacopeia reference standards: USP Loratadine RS, USP Loratadine Related Compound A RS, and USP Loratadine Compound B RS, Claritin.



ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 3.5 µm, 4.6 x 100 mm Column	186003033
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720005579EN at waters.com

Analysis of Loratidine Using XBridge BEH C_8 Columns

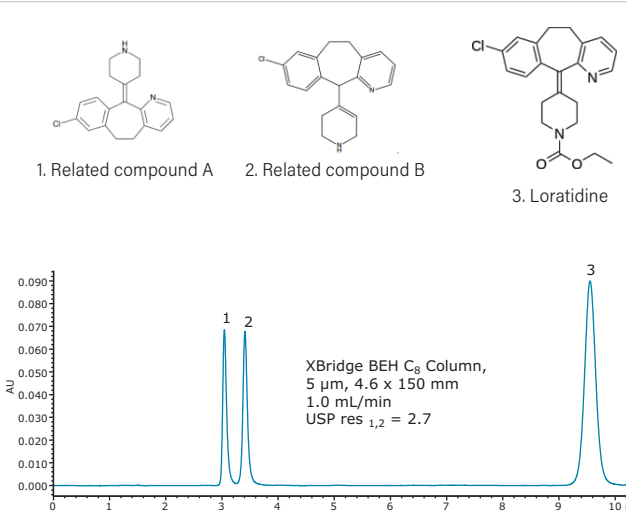
EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC H-Class
Column:	XBridge BEH C_8, 5 µm, 4.6 x 150 mm
Mobile phase:	Acetonitrile:methanol:0.01 M dibasic potassium phosphate (6:6:7) adjusted with phosphoric acid to an apparent pH of 7.2
Separation mode:	Isocratic
Flow rate:	1.0 mL/min
Column temp.:	30 °C
Injection volume:	15.0 µL
UV detection:	254 nm

Sample preparation

A sample containing loratidine (40 µg/mL), loratidine related compound A (10 µg/mL), and loratidine related compound B (10 µg/mL) was created using the 260:260:400:80 acetonitrile:methanol: 0.05 N HCl:0.6M dibasic potassium phosphate.



ORDERING INFORMATION

Description	P/N
XBridge BEH C_8, 5 µm, 4.6 x 150 mm Column	186003017
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note 720005579EN at waters.com

Analysis of Loratidine Using CORTECS C_8 Columns

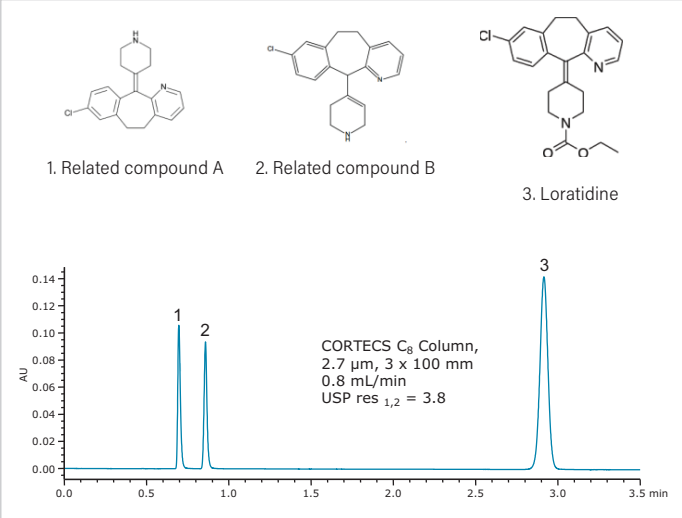
EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC H-Class
Column:	CORTECS C_8, 2.7 µm, 3 x 100 mm
Mobile phase:	Acetonitrile:methanol:0.01 M dibasic potassium phosphate (6:6:7) adjusted with phosphoric acid to an apparent pH of 7.2
Separation mode:	Isocratic
Flow rate:	0.8 mL/min
Column temp.:	30 °C
Injection volume:	4.3 µL
UV detection:	254 nm

Sample preparation

A sample containing loratidine (40 µg/mL), loratidine related compound A (10 µg/mL), and loratidine related compound B (10 µg/mL) was created using the 260:260:400:80 acetonitrile:methanol: 0.05 N HCl:0.6M dibasic potassium phosphate.



ORDERING INFORMATION

Description	P/N
CORTECS C_8, 2.7 µm, 3.0 x 100 mm Column	186008361
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720005200EN at waters.com

Analysis of 2- and 4-Methylimidazole in Beverages

EXPERIMENTAL

LC conditions

System: Alliance HPLC with ACQUITY QDa detector

Column: CORTECS HILIC, 2.7 µm, 2.1 x 100 mm

Mobile phase A: 10 mM ammonium formate pH-4 with formic acid

Mobile phase B: Acetonitrile (0.1% formic acid)

Gradient:

Time	%A	%B
0.00	7.5	92.5
8.50	7.5	92.5
9.00	60.0	40.0
11.00	60.0	40.0
11.50	7.5	92.5
20.50	7.5	92.5

Flow rate: 0.4 mL/min

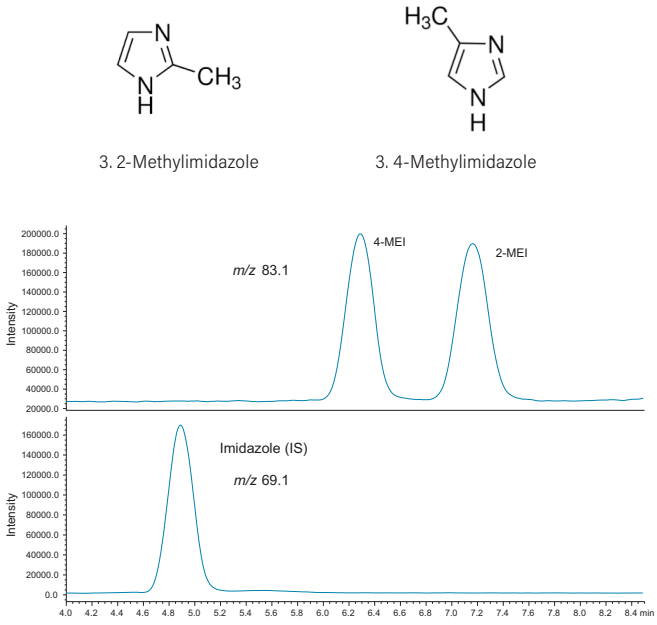
Injection volume: 5 µL

Ionization mode: ESI+

Acquisition mode: SIR (m/z) imidazole 69.1; 2 and 4-MEI 83.1

Sample preparation

Soft drink samples were sonicated to remove carbonation. Each of the six samples was fortified with 100 ppb imidazole as an internal standard. Separate portions of each sample were fortified with 100 ppb 2-MEI and 4-MEI to determine recovery.



ORDERING INFORMATION

Description	P/N
CORTECS HILIC, 2.7 µm, 2.1 x 100 mm Column	186007382
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720005558EN at waters.com

Analysis of Metoclopramide Using an HPLC 5 µm Column

EXPERIMENTAL

LC conditions

System: ACQUITY Arc with 2998 PDA and ACQUITY QDa detectors

Column: XSelect CSH C_{18}, 5 µm, 4.6 x 150 mm

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.1% formic acid in methanol

Gradient:

Time	%A	%B
0.00	95	5
15.00	40	60
16.50	40	60
16.80	95	5
21.00	95	5

Flow rate: 2.9 mL/min

Injection volume: 10.0 µL

UV detection: 270 nm

Ionization mode: ESI+, ESI-

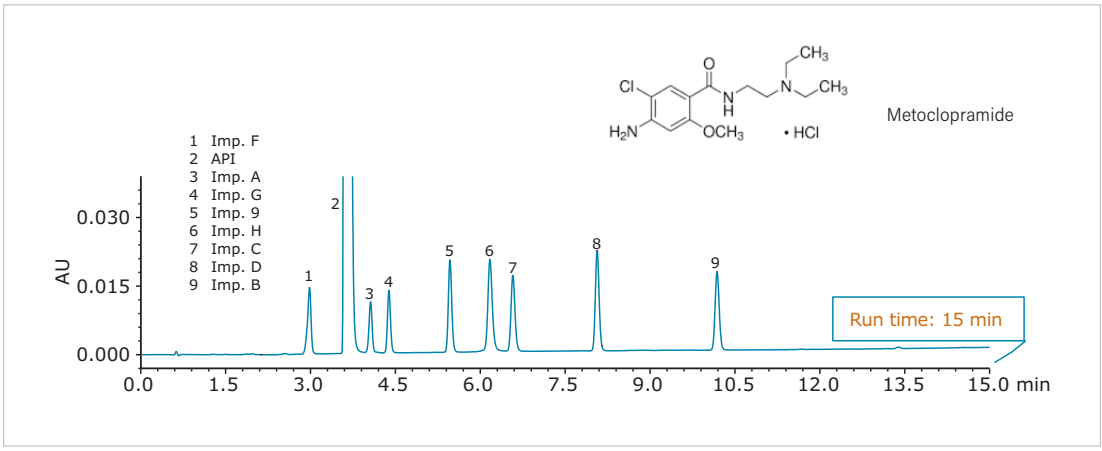
Acquisition mode: Full scan 100–440 m/z

Sample preparation

The related compounds of metoclopramide HCl used in this study are listed in Table 1 of the full application note (p/n 720005558EN). Separate stock solutions were prepared in methanol at 1.0 mg/mL. A metoclopramide stock solution was diluted with water to 0.5 mg/mL and spiked with related substances at 1.0% level.

ORDERING INFORMATION

Description	P/N
XSelect CSH C_{18}, 5 µm, 4.6 x 150 mm Column	186005290
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



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For complete experimental details, refer to full application note 720005558EN at waters.com

Analysis of Metoclopramide Using an HPLC 3.5 µm Column

EXPERIMENTAL

LC conditions

System:	ACQUITY Arc with 2998 PDA and ACQUITY QDa detectors
Column:	XSelect CSH C_{18}, 3.5 µm, 3.0 x 100 mm
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol

Gradient:

Time	%A	%B
0.00	95	5
10.00	40	60
11.00	40	60
11.20	95	5
14.00	95	5

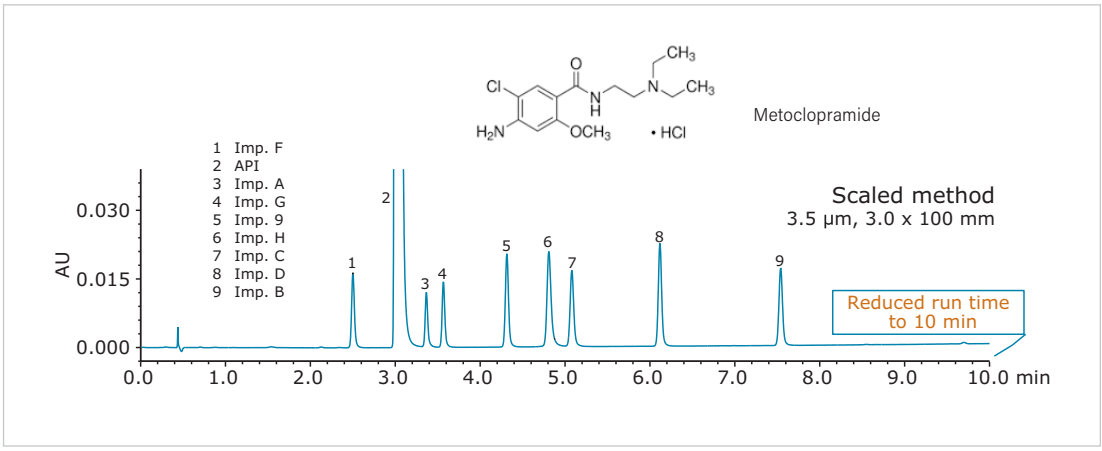
Flow rate:	1.2 mL/min
Injection volume:	2.8 µL
UV detection:	270 nm
Ionization mode:	ESI+, ESI-
Acquisition mode:	Full scan 100–440 m/z

Sample preparation

The related compounds of metoclopramide HCl used in this study are listed in Table 1 of the full application note (p/n 720005558EN). Separate stock solutions were prepared in methanol at 1.0 mg/mL. A metoclopramide stock solution was diluted with water to 0.5 mg/mL and spiked with related substances at 1.0% level.

ORDERING INFORMATION

Description	P/N
XSelect CSH C_{18}, 3.5 µm 3.0 x 100 mm Column	186005262
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



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For complete experimental details, refer to full application note 720005558EN at waters.com

Analysis of Metoclopramide Using an HPLC 2.5 µm Column

EXPERIMENTAL

LC conditions

System: ACQUITY Arc with 2998 PDA and ACQUITY QDa detectors

Column: XSelect CSH C_{18}, 2.5 µm, 3.0 x 75 mm

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.1% formic acid in methanol

Gradient:

Time	%A	%B
0.00	95	5
7.50	40	60
8.25	40	60
8.40	95	5
10.50	95	5

Flow rate: 1.2 mL/min

Injection volume: 2.1 µL

UV detection: 270 nm

Ionization mode: ESI+, ESI-

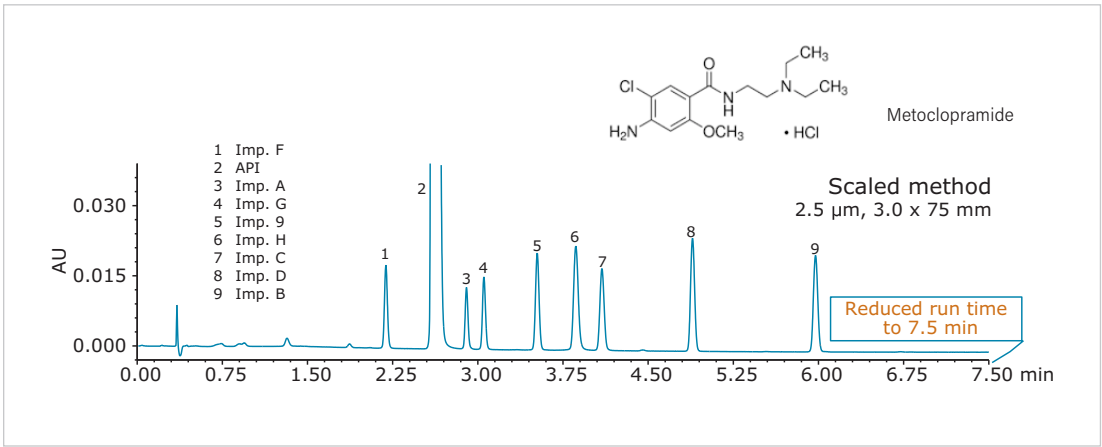
Acquisition mode: Full scan 100–440 m/z

Sample preparation

The related compounds of metoclopramide HCl used in this study are listed in Table 1 of the full application note (p/n 720005558EN). Separate stock solutions were prepared in methanol at 1.0 mg/mL. A metoclopramide stock solution was diluted with water to 0.5 mg/mL and spiked with related substances at 1.0% level.

ORDERING INFORMATION

Description	P/N
XSelect CSH C_{18}, 2.5 µm, 3.0 x 75 mm Column	186006106
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



For complete experimental details, refer to full application note 720005622EN at waters.com

Analysis of Miconazole Nitrate Cream Using XBridge BEH Phenyl Columns

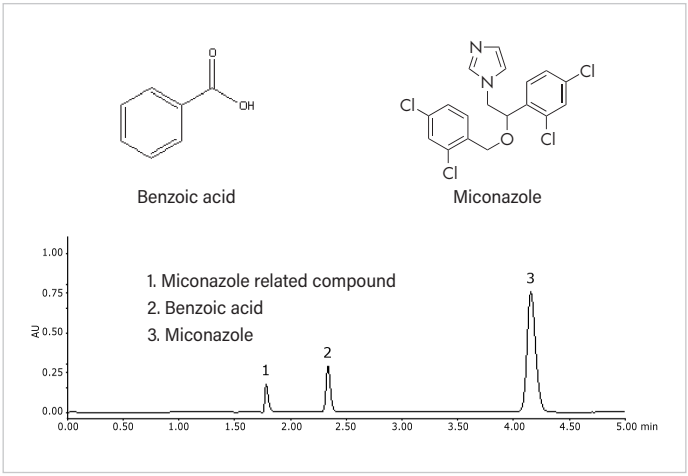
EXPERIMENTAL

LC conditions

System:	Alliance e2695 with 2489 UV/Visible detector
Column:	XBridge BEH Phenyl, 5 µm, 4.6 x 250 mm
Mobile phase:	Methanol/acetonitrile/tetrahydrofuran/ 1% triethylamine, pH 2.5 (25/20/15/40, v/v)
Separation mode:	Isocratic
Flow rate:	1.50 mL/min
Column temp.:	45 °C
Injection volume:	10 µL
UV detection:	225 nm

Sample preparation

A standard solution of 0.28 mg/mL of miconazole nitrate and 0.02 mg/mL of benzoic acid were prepared in mobile phase. A sample solution with a concentration of 0.28 mg/mL of miconazole nitrate and 0.02 mg/mL of benzoic acid was prepared by dispersing one applicator of an OTC (over-the-counter) cream preparation containing 100 mg of micronazole per dose in 357 mLs of mobile phase. The solution was heated for one hour at 40-45 °C in an ultrasonic bath, and then allowed to cool to room temperature. Twenty milliliters was filtered through a 0.45 µm PTFE filter, and a 2 mL aliquot was transferred to a TruView LCMS Clear Glass Vial for analysis.



ORDERING INFORMATION

Description	P/N
XBridge BEH Phenyl, 5 µm, 4.6 x 250 mm Column	186003353
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720005622EN at waters.com

Analysis of Miconazole Nitrate Cream Using CORTECS Phenyl Columns

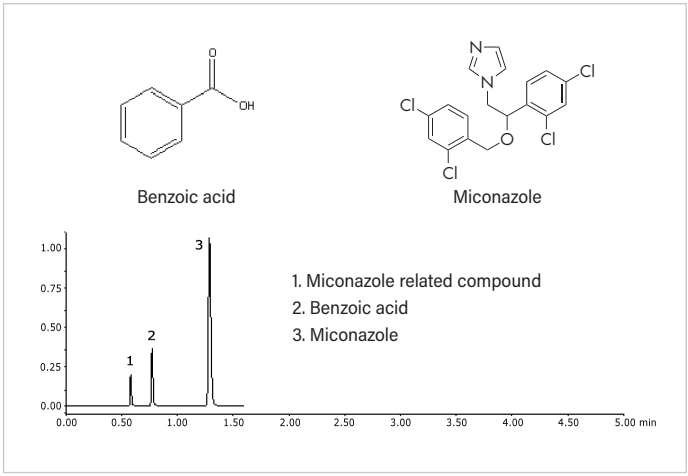
EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC with PDA detector
Column:	CORTECS Phenyl, 2.7 µm, 3.0 x 100 mm
Mobile phase:	Methanol/acetonitrile/tetrahydrofuran/ 1% triethylamine, pH2.5 (25/20/15/40, v/v)
Separation mode:	Isocratic
Flow rate:	0.64 mL/min
Column temp.:	45 °C
Injection volume:	1.7 µL
UV detection:	225 nm

Sample preparation

A standard solution of 0.28 mg/mL of miconazole nitrate and 0.02 mg/mL of benzoic acid were prepared in mobile phase. A sample solution with a concentration of 0.28 mg/mL of miconazole nitrate and 0.02 mg/mL of benzoic acid was prepared by dispersing one applicator of an OTC (over-the-counter) cream preparation containing 100 mg of micronazole per dose in 357 mLs of mobile phase. The solution was heated for one hour at 40-45 °C in an ultrasonic bath, and then allowed to cool to room temperature. Twenty milliliters was filtered through a 0.45 µm PTFE filter, and a 2 mL aliquot was transferred to a TruView LCMS Clear Glass Vial for analysis.



ORDERING INFORMATION

Description	P/N
CORTECS Phenyl, 2.7 µm, 3.0 x 100 mm Column	186008331
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720004147EN at waters.com

Analysis of Mometasone Furoate Ointment

EXPERIMENTAL

LC conditions

System: Alliance HPLC with 2489 UV/Visible detector

Column: XBridge Shield RP18, 5 µm, 4.6 x 250 mm

Mobile phase A: 100% water

Mobile phase B: 100% acetonitrile

Gradient:

Time	%A	%B
Initial	70	30
2	70	30
45	45	55
46	70	30
50	70	30

Flow rate: 2.0 mL/min

Column temp.: 25 °C

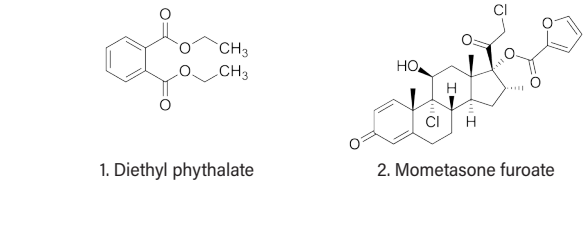
Injection volume: 20 µL

UV detection: 254 nm

Sample preparation

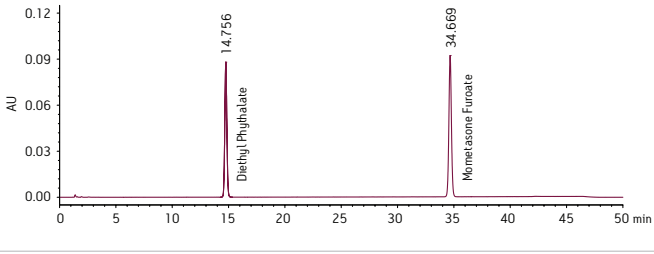
Mometasone furoate stock standard was prepared by dissolving an accurate amount of mometasone furoate reference material in Diluent A (100:1 tetrahydrofuran:acetic acid) to make a solution at approximately 0.2 mg/mL concentration. Internal standard and mometasone furoate stock were diluted with Diluent B (50:50:1 acetonitrile:water:acetic acid) to obtain a working standard of approximately 0.05 mg/mL of mometasone furoate and 0.35 mg/mL of diethyl phthalate, respectively.

Sample temp.: 15 °C



1. Diethyl phthalate

2. Mometasone furoate



ORDERING INFORMATION

Description	P/N
XBridge BEH Shield RP18, 5 µm, 4.6 x 250 mm Column	186003010
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note 720005609EN at waters.com

Analysis of Mono- and Disaccharides and Selected Alditols in Juice, Beer, Wine, and Whiskey

EXPERIMENTAL

LC conditions

System: ACQUITY Arc with ACQUITY QDa Detector

Column: XBridge BEH Amide ***XP***, 2.5 µm, 3.0 x 150 mm

Mobile phase A: 90% acetonitrile: 5% IPA:5% water*

Mobile phase B: 80% acetonitrile: 20% water*

*Both containing 500 ppb guanidine hydrochloride and 0.05% diethylamine.

Gradient:

Time	%A	%B
0.00	100	0
4.50	100	0
18.00	0	100
25.00	0	100
25.10	100	0
40.00	100	0

Flow rate: 0.8 mL/min

Column temp.: 85 °C

Injection volume: 1 µL

Ionization mode: ESI-

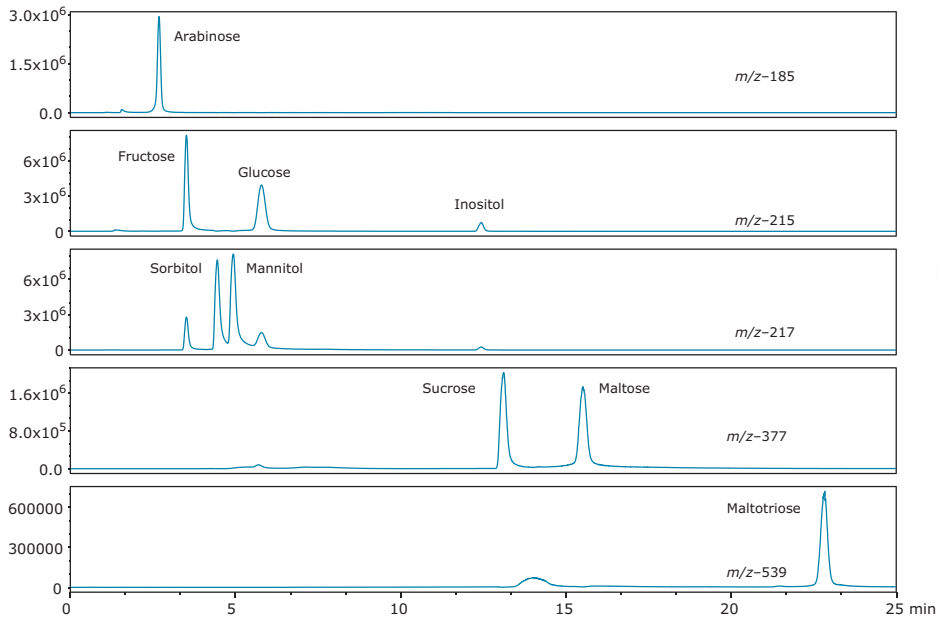
Acquisition mode: SIR (m/z) ([M+Cl]$^-$ ion): 185 (arabinose); 215 (fructose); 215 (glucose); 215 (inositol); 217 (sorbitol); 217 (mannitol); 377 (sucrose); 377 (maltose); 539 (maltotriose)

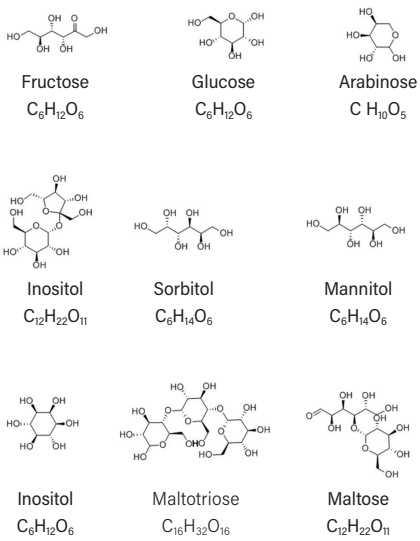
Sample preparation

A 100 mg/L stock of the nine saccharides listed below was prepared in 1:1 acetonitrile-water.

ORDERING INFORMATION

Description	P/N
XBridge BEH Amide ***XP***, 2.5 µm, 3.0 x 150 mm Column	186006725
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV





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For complete experimental details, refer to full application note 720004389EN at waters.com

Analysis of Monoamine Neurotransmitters

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC with Xevo TQ-S detector

Column: XBridge BEH Amide ***XP***, 2.5 µm, 2.1 x 75 mm

Mobile phase A: 95:5 water:acetonitrile containing 100 mM ammonium formate, pH 3.0

Mobile phase B: 85:15 acetonitrile:water containing 30 mM ammonium formate, pH 3.0

Gradient:

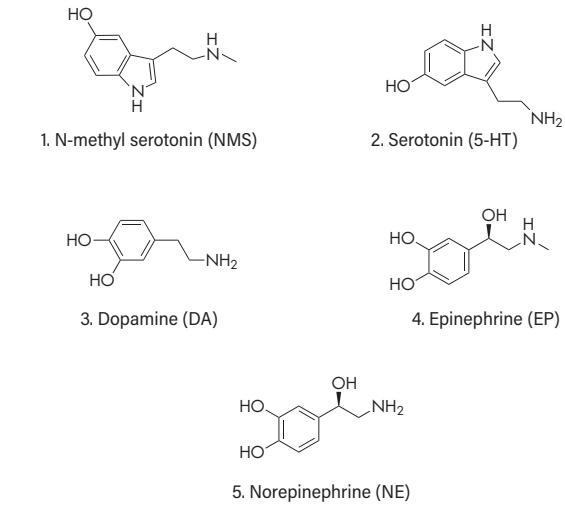
Time	%A	%B
0.00	0	100
2.50	30	70
2.60	0	100
4.00	0	100

Column temp.: 30 °C

Injection volume: 20 µL

Ionization mode: ESI+

Acquisition mode: MRM (m/z): NMS 191.1 > 160; 5-HT 177.0 > 160; DA 154 > 137; EP 184 > 166; NE 152 > 107



Sample preparation

Combined stock standards of dopamine, norepenephrine, epinephrine, serotonin, and N-methyl serotonin (NMS) were prepared in methanol containing 0.1% ascorbic acid and 2.5% 1N HCl to prevent oxidation. Working standards of 100 ng/mL DA, NE, EP, 5-HT, and 10 ng/mL NMS were prepared fresh each day in starting mobile-phase conditions.

Sample temp.: 5 °C

ORDERING INFORMATION

Description	P/N
XBridge BEH Amide ***XP***, 2.5 µm, 2.1 x 75 mm Column	186006090
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

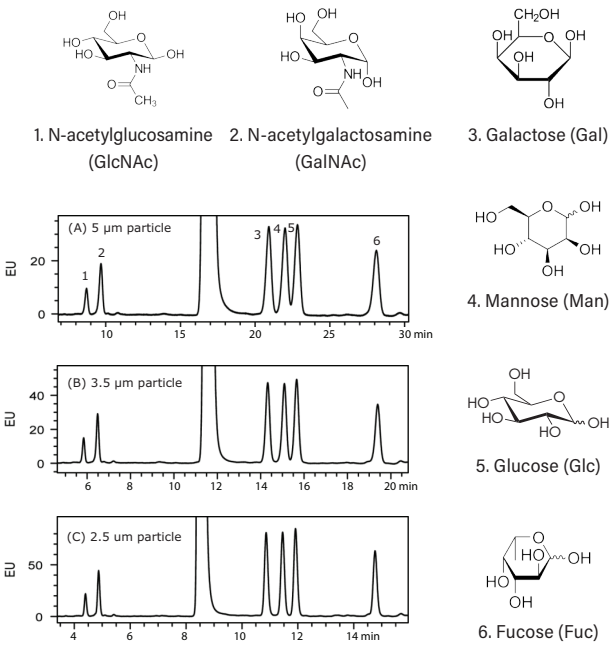
For complete experimental details, refer to full application note 720005255EN at waters.com

Analysis of Monosaccharides

EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC H-Class Bio with FLR detector
Columns:	XBridge BEH C_{18}, 5 µm, 4.6 x 100 mm XBridge BEH C_{18}, 3.5 µm, 2.1 x 100 mm XBridge BEH C_{18} ***XP***, 2.5 µm, 2.1 x 100 mm
Mobile phase A:	0.2% N-butylamine, 0.5% phosphoric acid, and 1% THF in water
Mobile phase B:	50% mobile phase A in acetonitrile
Gradient and flow rate:	See table
Column temp.:	30 °C
Injection volume:	4.6 x 100 mm format, 4.8 µL; 2.1 x 100 mm format, 1 µL
FLR detection:	Excitation wavelength = 360 nm; Emission wavelength = 425 nm



Sample preparation

Monosaccharides from bovine fetuin were released by acid hydrolysis using 2 M TFA with hydrolysis occurring for 3 h at 100 °C. Resulting hydrolysates were then dried by centrifugal evaporation followed by reconstitution in 5 µL of 80 mg/mL sodium acetate trihydrate. A 2AA labeling solution was prepared by dissolving 30 mg of 2AA in 1 mL of 2% (w/v) boric acid in methanol. This suspension was then used to dissolve 30 mg of sodium cyanoborohydride. Of this preparation, 10 µL was added to each of the monosaccharide mixtures. Monosaccharides were labeled at 80 °C for 60 minutes. Upon completion of labeling, serial dilutions were performed to generate a 1000-fold dilution of the labeled material. For preparation of monosaccharide standards, labeling was performed as outlined above with the omission of acid hydrolysis.

Step	%B	Method details (flow rate and time)					
		5 µm		3.5 µm		2.5 µm	
		Flow (mL min^{-1})	Time (min)	Flow (mL min^{-1})	Time (min)	Flow (mL min^{-1})	Time (min)
1	7	0.480	0.00	0.685	0.00	0.200	0.00
2	7	0.480	7.78	0.685	5.45	0.200	3.89
3	17	0.480	27.78	0.685	19.47	0.200	13.88
4	100	0.480	28.89	0.685	20.24	0.200	14.43
5	100	0.480	40.00	0.685	28.03	0.200	19.99
6	7	0.480	41.11	0.685	28.81	0.200	20.54
7	7	0.480	50.00	0.685	35.04	0.200	24.98

ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 5 µm, 4.6 x 100 mm Column	186003115
XBridge BEH C_{18}, 3.5 µm, 2.1 x 100 mm Column	186003033
XBridge BEH C_{18} ***XP***, 2.5 µm, 2.1 x 100 mm Column	186006031
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note WA64111 at waters.com

Analysis of Morphine and Related Compounds

EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC with 30-cm column cooler/heater and TQD detector
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	50/50 acetonitrile/water with 10 mM ammonium formate, pH 3.0
Mobile phase B:	90/10 acetonitrile/water with 10 mM ammonium formate, pH 3.0

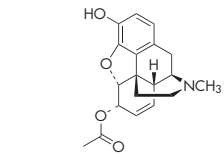
Gradient:

Time	%A	%B
0.00	0.1	99.9
15.00	0.1	99.9
62.50	99.9	0.1
62.60	0.1	99.9
75.00	0.1	99.9

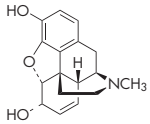
Flow rate:	1.0 mL/min
Column temp.:	30 °C
Injection volume:	15.0 µL
Ionization mode:	ESI-
Acquisition mode:	SIR (m/z): 287.5 (morphine); 329.5 (6-acetylmorphine); 463.6 (M3G)

Sample preparation

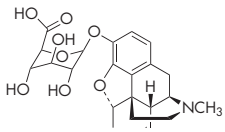
Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.



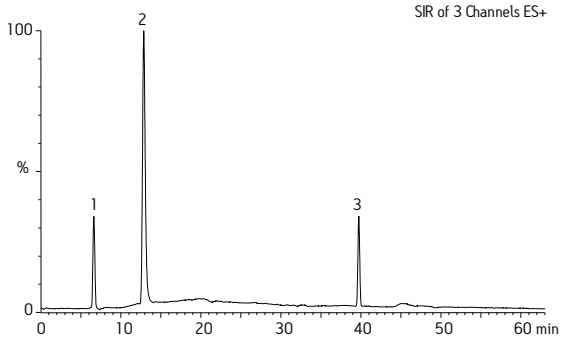
1. 6-Acetylmorphine (1 µg/mL)



2. Morphine (5 µg/mL)



3. Morphine-3β-D-glucuronide (M3G) (10 µg/mL)



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial w/ Preslit Septa	600000668CV

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For complete experimental details, refer to full application note WA64079 at waters.com

Analysis of Morphine and Metabolites Using XBridge BEH HILIC Columns

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC with PDA detector

Column: XBridge BEH HILIC, 3.5 µm, 2.1 x 50 mm

Mobile phase A: 10 mM ammonium formate in water, 0.125% formic acid in 50:50 acetonitrile/water

Mobile phase B: 10 mM ammonium formate in water, 0.125% formic acid in 90:10 acetonitrile/water

Gradient:

Time	%A	%B
0.00	0.1	99.9
1.05	0.1	99.9
4.35	99.9	0.1
4.50	0.1	99.9
6.00	0.1	99.9

Flow rate: 0.6 mL/min

Column temp.: 30 °C

Injection volume: 5 µL

UV detection: 280 nm

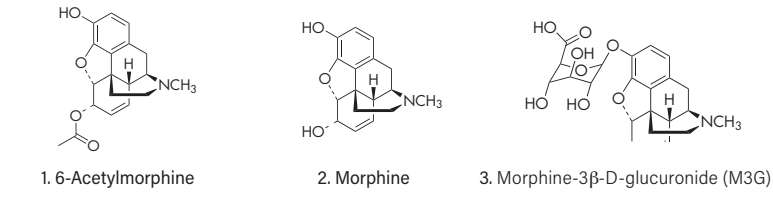
Sample preparation

Sample concentration: 25 µg/mL each

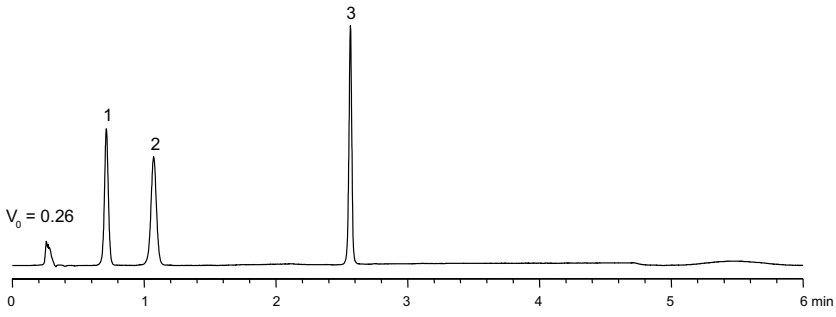
Sample diluent: 75:25 ACN:MeOH with 0.2% HCOOH

ORDERING INFORMATION

Description	P/N
XBridge BEH HILIC, 3.5 µm, 2.1 x 50 mm Column	186004432
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



1. 6-Acetylmorphine 2. Morphine 3. Morphine-3β-D-glucuronide (M3G)



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For complete experimental details, refer to full application note WA64701 at waters.com

Analysis of Morphine and Metabolites Using CORTECS 2.7 µm Columns

EXPERIMENTAL

LC conditions

System: Alliance HPLC with 2998 PDA detector

Column: CORTECS HILIC, 2.7 µm, 4.6 x 150 mm

Mobile phase A: 10 mM ammonium formate in 50% acetonitrile/49.875% water/0.125% formic acid

Mobile phase B: 10 mM ammonium formate in 90% acetonitrile/9.875% water/0.125% formic acid

Gradient:

Time	%A	%B	Curve
Initial	0.1	99.9	–
4.42	0.1	99.9	6
18.29	99.9	0.1	6
18.99	0.1	99.9	11
26.01	0.1	99.9	11

Flow rate: 1.99 mL/min

Column temp.: 30 ˚C

Injection volume: 14.4 µL

UV detection: 280 nm

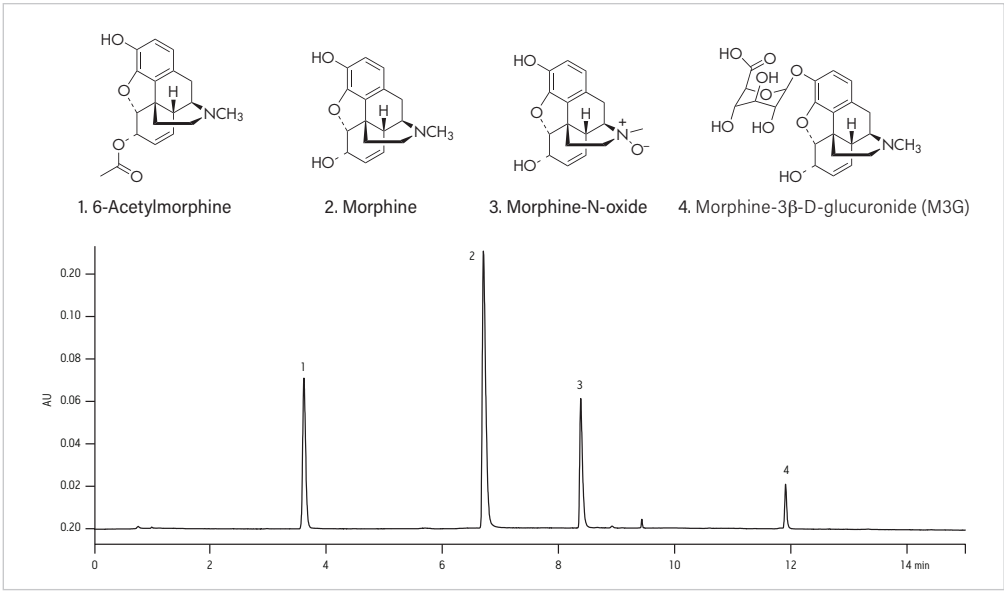
Sample preparation

Sample: Morphine metabolites

Sample diluent: Mobile phase B

ORDERING INFORMATION

Description	P/N
CORTECS HILIC, 2.7 µm, 4.6 x 150 mm Column	186007393
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



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34 Maple Street, Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990

For complete experimental details, refer to full application note WA60184 at waters.com

Analysis of Mouthwash

EXPERIMENTAL

LC conditions

System: Alliance 2695 with 2996 PDA detector

Column: XBridge BEH Shield RP18, 3.5 µm, 4.6 x 100 mm

Mobile phase A: 0.1 mM ammonium acetate, pH 5.4

Mobile phase B: Acetonitrile

Gradient:

Time	%A	%B
0.00	95	5
2.00	85	15
7.00	5	95
8.00	5	95
9.00	95	5
12.00	95	5

Flow rate: 1.2 mL/min

Column temp.: 30 °C

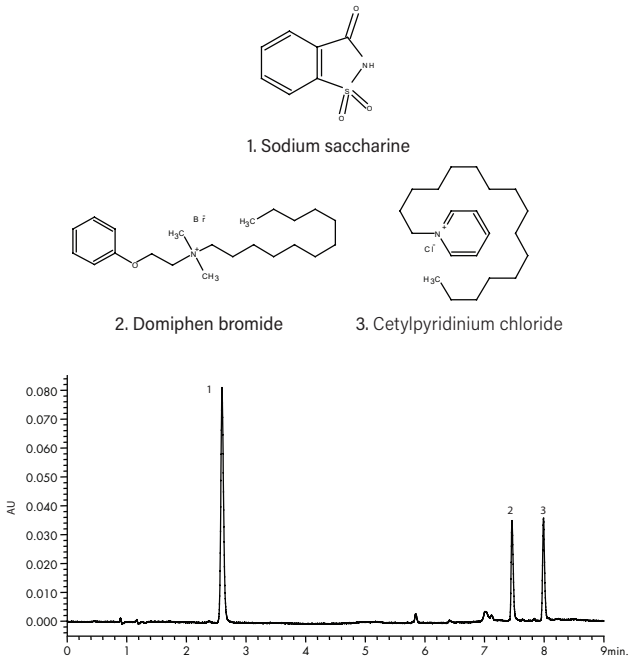
Injection volume: 20 µL

UV detection: 262 nm

Sample preparation

Sample: Sodium saccharin (50 µg/mL), Domiphen bromide (50 µg/mL), Cetylpyridinium chloride (10 µg/mL) in ACN/CH_3COONH_4 (5/95)

Sample temp.: 20 °C



ORDERING INFORMATION

Description	P/N
XBridge BEH Shield RP18, 3.5 µm, 4.6 x 100 mm Column	186003044
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note WA60199 at waters.com

Analysis of Nerve Agent Degradation Products in Drinking Water

EXPERIMENTAL

LC conditions

System:	Alliance 2690
Column:	XBridge BEH C_{18}, 3.5 µm, 2.1 x 150 mm
Mobile phase A:	10 mM ammonium formate in water
Mobile phase B:	10 mM ammonium formate in methanol

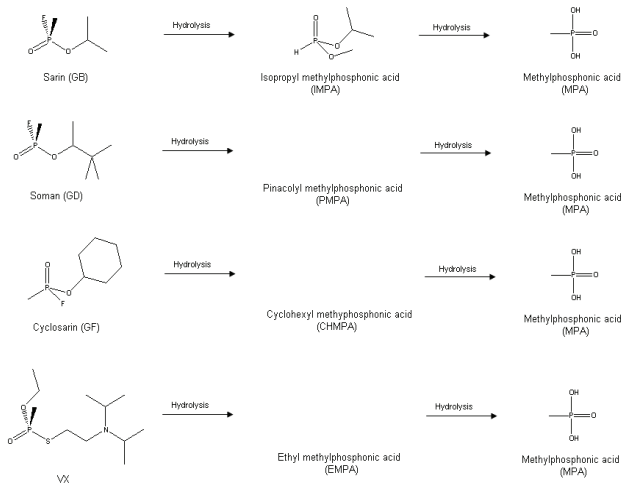
Gradient:

Time	%A	%B
0.00	99	1
2.00	99	1
17.00	30	70
25.00	30	70

Flow rate:	0.2 mL/min
Injection volume:	5 µL
Ionization mode:	ESI-

Sample preparation

Five milliliters of water sample was spiked with solutions of GB, GD, GF, VX and RVX in methanol to the appropriate concentration and then stored at room temperature for 5 days prior to analysis (ensuring that hydrolysis occurred). The water sample was filtered through a 0.2 µm membrane filter and then analyzed by LC-MS.



(1) Methylphosphonic acid
(2) Ethyl methylphosphonic acid
(3) Isopropyl methylphosphonic acid
(4) Isobutyl methylphosphonic acid
(5) Cyclohexyl methylphosphonic acid
(6) Pinacolyl methylphosphonic acid
(7) Benzilic acid

ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 3.5 µm, 2.1 x 150 mm Column	186003023
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note WA64076 at waters.com

Analysis of Neurotransmitters

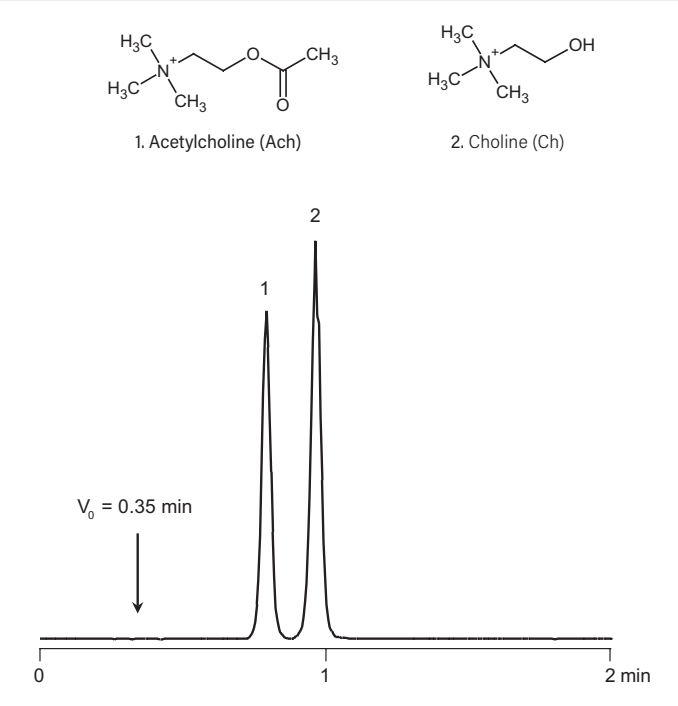
EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC with TQD detector
Column:	XBridge BEH HILIC, 3.5 µm, 2.1 x 50 mm
Mobile phase A:	10 mM ammonium formate with 0.125% formic acid in water
Mobile phase B:	10 mM ammonium formate with 0.125% formic acid in 90/5/5 acetonitrile/methanol/water
Isocratic conditions:	10% A; 90% B
Flow rate:	0.5 mL/min
Column temp.:	30 °C
Injection volume:	10.0 µL
Ionization mode:	ESI+
Acquisition mode:	SIR (m/z): 146.1 (acetylcholine); 104.0 (choline)

Sample preparation

Sample diluent:	75/25 acetonitrile/methanol with 0.2% formic acid
Sample concentration:	5 ng/mL each



ORDERING INFORMATION

Description	P/N
XBridge BEH HILIC, 3.5 µm, 2.1 x 50 mm Column	186004432
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note XBRIDGE8 at waters.com

Analysis of Nimodipine

EXPERIMENTAL

LC conditions

System:	Alliance 2695 with 2996 PDA detector
Column:	XBridge BEH Shield RP18, 3.5 µm, 4.6 x 100 mm
Mobile phase A:	200 mM ammonium formate pH 3
Mobile phase B:	Acetonitrile
Mobile phase C:	Water

Gradient:

Time	%A	%B	C%
0.00	10	30	60
50.00	10	60	30

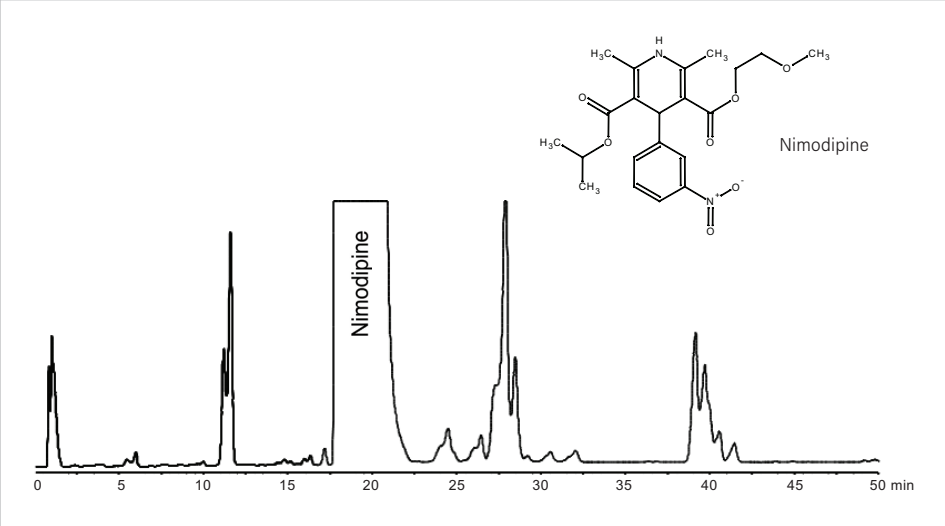
Flow rate:	1.4 mL/min
Column temp.:	30 ˚C
Injection:	100 µL
UV detection:	254 nm

Sample preparation

Sample concentration: 30 mg/mL in DMSO

ORDERING INFORMATION

Description	P/N
XBridge BEH Shield RP18, 3.5 µm, 4.6 x 100 mm Column	186003044
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



For complete experimental details, refer to full application note 720005580EN at waters.com

Analysis of Nitroaromatic Compounds

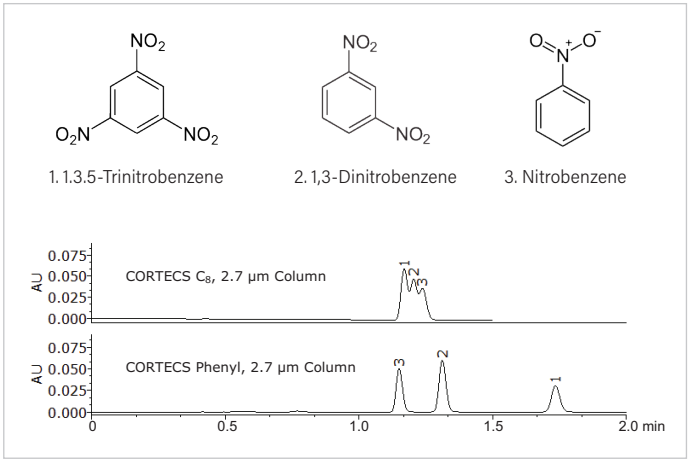
EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC
Column:	CORTECS Phenyl, 2.7 µm, 2.1 x 100 mm; CORTECS C_8, 2.7 µm, 2.1 x 100 mm
Mobile phase:	45:55 methanol:water
Separation mode:	Isocratic
Flow rate:	0.5 mL/min
Column temp.:	40 °C
Injection volume:	1.0 µL
UV detection:	254 nm

Sample preparation

The nitroaromatic compounds were prepared as 10 µg/mL solutions in 100% methanol.



ORDERING INFORMATION

Description	P/N
CORTECS Phenyl, 2.7 µm, 2.1 x 100 mm Column	186008321
CORTECS C_8, 2.7 µm, 2.1 x 100 mm Column	186008351
TruView LCMS Certified Max Recovery Vial	186005662CV
Neutrals QC Reference Material	186006360

For complete experimental details, refer to full application note XBRIDGE9 at waters.com

Analysis of Non-Steroidal Inflammatory Drugs (NSAIDS)

EXPERIMENTAL

LC conditions

System: Alliance 2695 with 2996 PDA detector

Column: XBridge BEH Phenyl, 3.5 µm, 4.6 x 100 mm

Mobile phase A: Water

Mobile phase B: Methanol

Mobile phase C: 0.2% formic acid in water

Gradient:

Time	%A	%B	C%
0.00	40	50	10
13.33	25	65	10
16.67	25	65	10
17.33	40	50	10
20.00	40	50	10

Flow rate: 1.0 mL/min

Column temp.: 30 ˚C

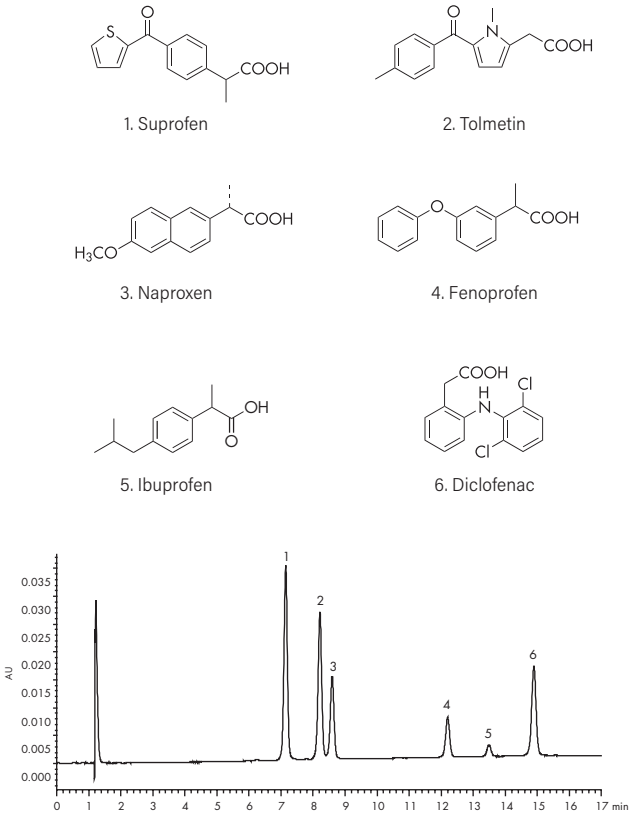
Injection volume: 15 µL

UV detection: 254 nm

Sample preparation

Sample: Fenoprofen (20 µg/mL), Ibuprofen (20 µg/mL), Diclofenac (10 µg/mL), Tolmetin (10 µg/mL), Suprofen (10 µg/mL), Naproxen (10 µg/mL) in H_2O/MeOH (60/40)

Sample temp.: 15 ˚C



ORDERING INFORMATION

Description	P/N
XBridge BEH Phenyl, 3.5 µm, 4.6 x 100 mm Column	186003334
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note WA64116 at waters.com

Analysis of Nucleobases

EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2998 PDA detector
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	50/50 acetonitrile/water with 10 mM ammonium formate, pH 3.0
Mobile phase B:	95/5 acetonitrile/water with 10 mM ammonium formate, pH 3.0

Gradient:

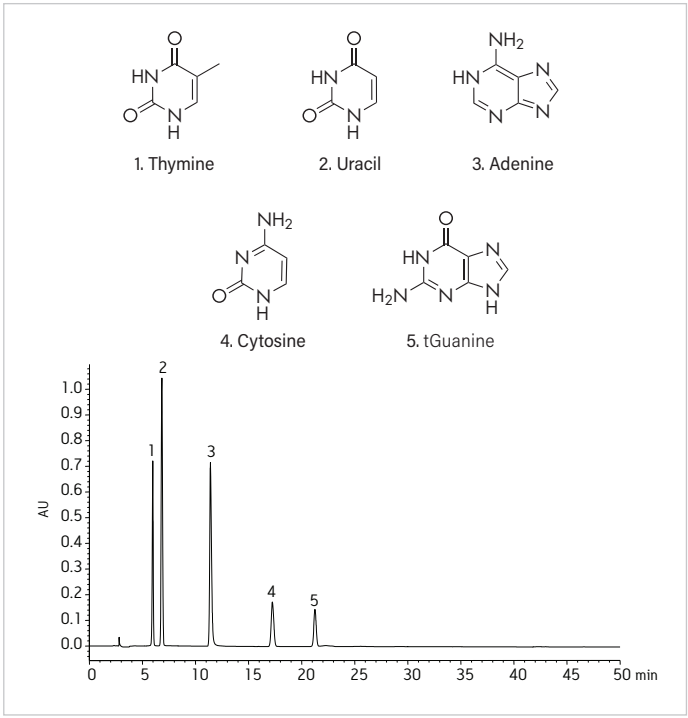
Time	%A	%B
0.00	1	99
50.00	50	50
50.10	1	99
60.00	1	99

Flow rate:	1.2 mL/min
Column temp.:	30 °C
Injection volume:	60.0 µL
UV detection:	260 nm

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.

Sample concentration: 20 µg/mL



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

For complete experimental details, refer to full application note WA64121 at waters.com

Analysis of Nucleotide Phosphates

EXPERIMENTAL

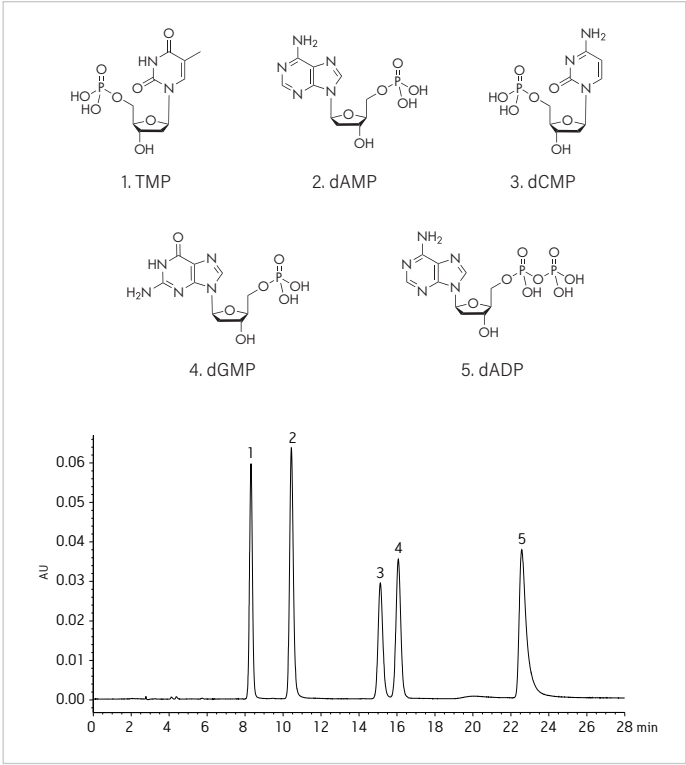
LC conditions

System:	Alliance HPLC with 2998 PDA detector
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase:	80/20 acetonitrile/water with 2 mM monopotassium phosphate
Separation mode:	Isocratic
Flow rate:	1.2 mL/min
Column temp.:	25 °C
Injection volume:	40.0 µL
UV detection:	254 nm

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.

Sample concentration: 10 µg/mL



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

For complete experimental details, refer to full application note WA64075 at waters.com

Analysis of Nutrients

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC with PDA detector

Column: XBridge BEH HILIC, 3.5 µm, 2.1 x 50 mm

Mobile phase A: 10 mM ammonium formate in water, 0.125% formic acid in 50/50 acetonitrile/water

Mobile phase B: 10 mM ammonium formate in water, 0.125% formic acid in 90/10 acetonitrile/water

Gradient:

Time	%A	%B
0.00	0.1	99.9
1.05	0.1	99.9
4.35	99.9	0.1
4.50	0.1	99.9
6.00	0.1	99.9

Flow rate: 0.6 mL/min

Column temp.: 30 °C

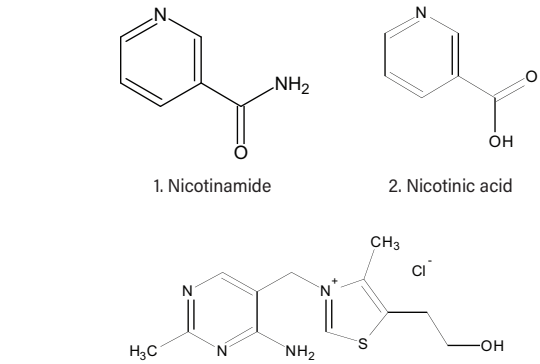
Injection volume: 5 µL

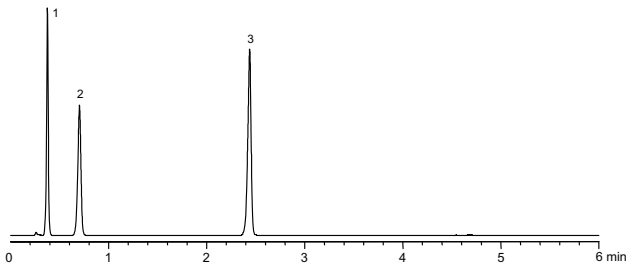
UV detection: 268 nm

Sample preparation

Sample diluent: 75/25 acetonitrile/methonal with 0.2% formic acid

Sample concentration: 25 µg/mL each





ORDERING INFORMATION

Description	P/N
XBridge BEH HILIC, 3.5 µm, 2.1 x 50 mm Column	186004432
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

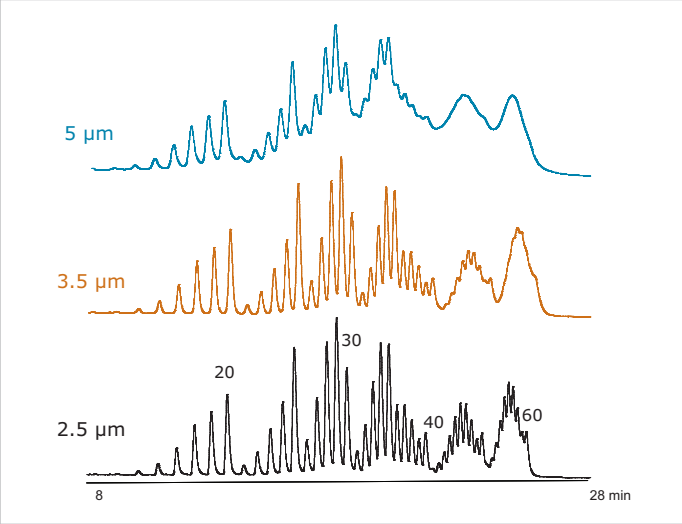
For complete experimental details, refer to full application note 720002376EN at waters.com

Analysis of Oligonucleotides

EXPERIMENTAL

LC conditions

System:	Alliance 2796 Bioseparations System with 2996 PDA detector
Column:	XBridge Oligonucleotide BEH C_{18}, 2.5 µm, 2.1 x 50 mm
Mobile phase A:	100 mM TEAA, pH 7
Mobile phase B:	80% A, 20% acetonitrile
Gradient:	40 to 62.5% B in 30 min
Flow rate:	0.2 mL/min
Column temp.:	60 °C
UV detection:	260 nm



ORDERING INFORMATION

Description	P/N
XBridge Oligonucleotide BEH C_{18}, 2.5 µm, 2.1 x 50 mm Column	186003952
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720005103EN at waters.com

Analysis of Omeprazole

EXPERIMENTAL

LC conditions

System:	Alliance 2695 with 2998 PDA and ACQUITY QDa detectors
Column:	CORTECS C_{18}+, 2.7 µm, 4.6 x 150 mm
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile

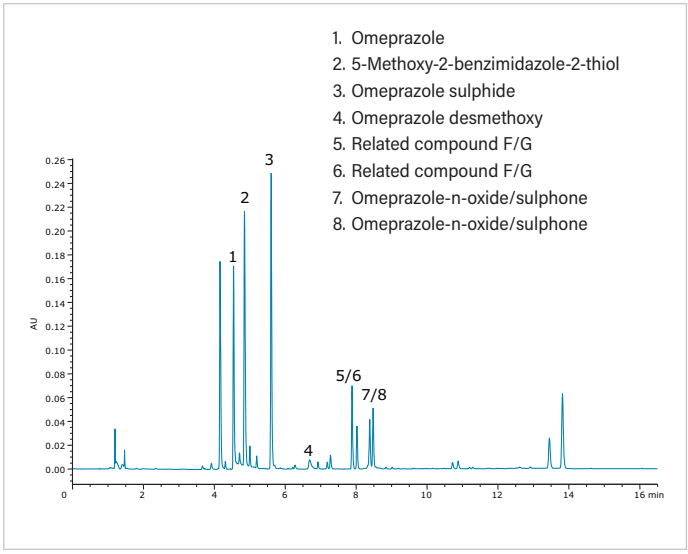
Gradient:

Time	%A	%B
0.00	90	10
16.50	22	78
16.60	90	10
20.00	90	10

Flow rate:	1.2 mL/min
Column temp.:	30 °C
Injection volume:	9.6 µL
UV detection:	280 nm
Ionization mode:	ESI+
Acquisition mode:	Full scan 120–420 m/z

Sample preparation

Two Omeprazole tablets (20 mg Omeprazole) were separately crushed with a mortar and pestle and transferred to two 100 mL volumetric flasks. To one flask (A), 25 mL 0.1 N HCl was added and the solution was left at room temperature for 1.5 hours. Twenty-five milliliters 0.1 N NaOH was added to neutralize the solution. Methanol was added to the flask to bring the sample up to 100 mL. The other solution (B) was diluted to 100 mL with 50:50 methanol:water. Both solutions were then filtered through a 0.2 µm filter. To create the sample for injection 0.66 mL of solution A and 0.34 mL of solution B were combined in an LCMS Certified Max Recovery Vial.



ORDERING INFORMATION

Description	P/N
CORTECS C_{18}+, 2.7 µm, 4.6 x 150 mm Column	186007408
Waters LCMS Certified Max Recovery Vial	600000749CV

For complete experimental details, refer to full application note WA64110 at waters.com

Analysis of Organic Acids

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC with 30-cm column cooler/heater and ACQUITY TQD Detector

Column: XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm

Mobile phase A: 50/50 acetonitrile/water with 10 mM ammonium acetate and 0.04% ammonium hydroxide, pH 9.0

Mobile phase B: 95/5 acetonitrile/water with 10 mM ammonium acetate and 0.04% ammonium hydroxide, pH 9.0

Gradient:

Time	%A	%B
0.00	0.1	99.9
3.00	0.1	99.9
3.10	40.0	60.0
14.00	70.0	30.0
14.10	0.1	99.9
26.00	0.1	99.9

Flow rate: 1.0 mL/min

Column temp.: 50 °C

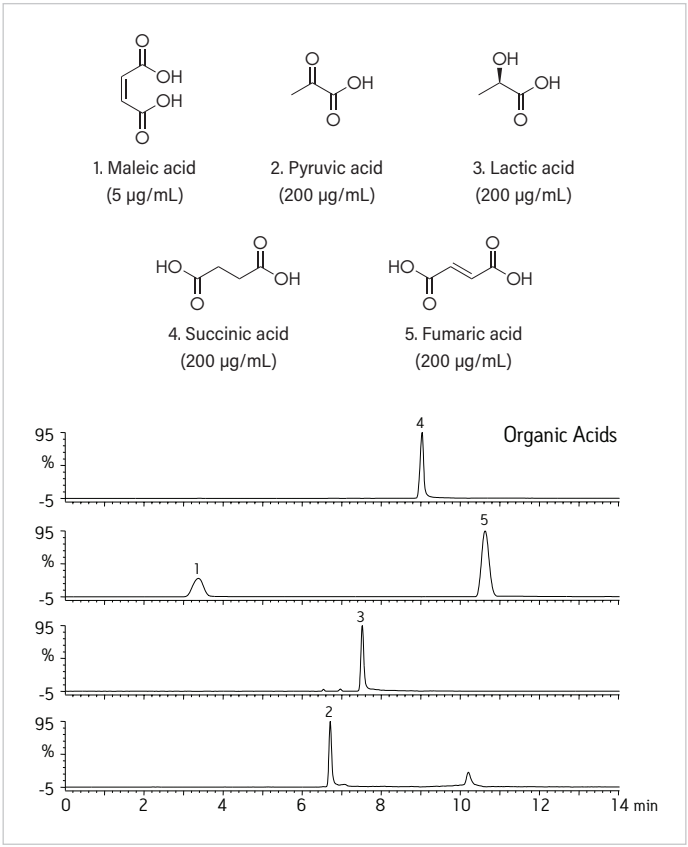
Injection volume: 15.0 µL

Ionization mode: ESI-

Acquisition mode: MRM (m/z): pyruvic acid 86.92> 42.9; lactic acid 88.92 > 42.9; succinic acid 116.93 > 72.9; maleic acid and fumaric acid 114.88 > 70.9

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial w/ Preslit Septa	600000668CV

For complete experimental details, refer to full application note WA43181 at waters.com

Analysis of Organic Acids and Bases

EXPERIMENTAL

LC conditions

System: Alliance 2695 with 2996 PDA detector

Stability Testing

Column: XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm

Mobile phase A: 200 mM potassium phosphate (pH 12):acetonitrile (80:20)

Mobile phase B: Water

Mobile phase C: Acetonitrile

Isocratic conditions: 10% A; 37% B; 53% C

Flow rate: 1.0 mL/min

Injection volume: 10 µL of 440 µg/mL (total concentration)

UV detection wavelength: 254 nm

Selectivity Study

Column: XBridge C_{18}, 3.5 µm, 4.6 x 100 mm

Mobile phase A1: 30 mM potassium phosphate, pH 2

Mobile phase A2: 30 mM potassium phosphate, pH 7

Mobile phase A2: 30 mM potassium phosphate, pH 12

Mobile phase B: Acetonitrile

Gradient:

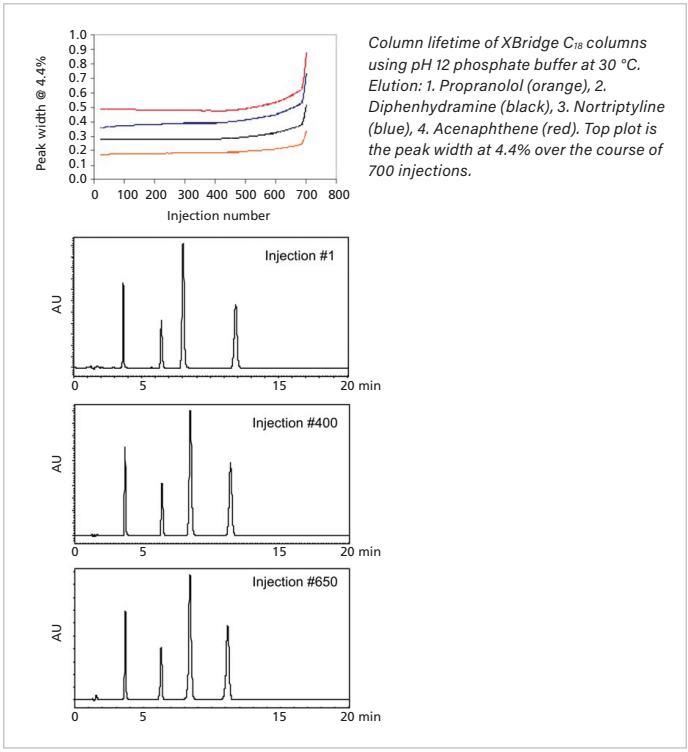
Time	%A	%B
0.0	90	10
7.0	20	80
8.0	20	80

Flow rate: 1.4 mL/min

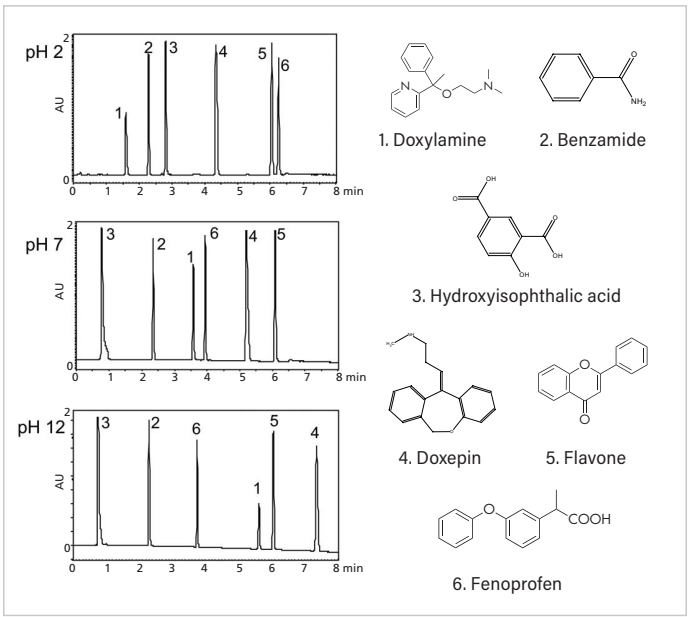
Column temp.: 30 °C

Injection volume: 20 µL of 5 µg/mL (each) standard

UV detection: 210 nm (pH 2, 7); 220 nm (pH 12)



Column lifetime of XBridge C_{18} columns using pH 12 phosphate buffer at 30 °C. Elution: 1. Propranolol (orange), 2. Diphenhydramine (black), 3. Nortriptyline (blue), 4. Acenaphthene (red). Top plot is the peak width at 4.4% over the course of 700 injections.



ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm Column	186003116
XBridge BEH C_{18}, 3.5 µm, 4.6 x 100 mm Column	186003033
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note WA64108 at waters.com

Analysis of Organophosphonic Acids by Isocratic MS

EXPERIMENTAL

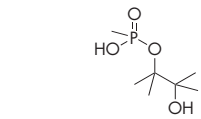
LC conditions

System:	ACQUITY UPLC with 30-cm column cooler/heater and ACQUITY TQD detector
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase:	90/10 acetonitrile/water with 10 mM ammonium acetate and 0.04% ammonium hydroxide, pH 9.0
Separation mode:	Isocratic
Flow rate:	1.0 mL/min
Column temp.:	65 °C
Injection volume:	15.0 µL
Ionization mode:	ESI-
Acquisition mode:	SIR (m/z): 122.9 (EMPA); 136.95 (IMPA); 150.95 (MMPA); 177.0 (CMPA); 179.0 (PMPA)

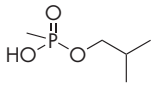
Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.

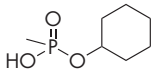
Sample concentration: 10 µg/mL each



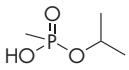
1. Pinacolyl methylphosphonic acid (PMPA)



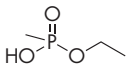
2. 2-(methyl)propyl methylphosphonic acid (MMPA)



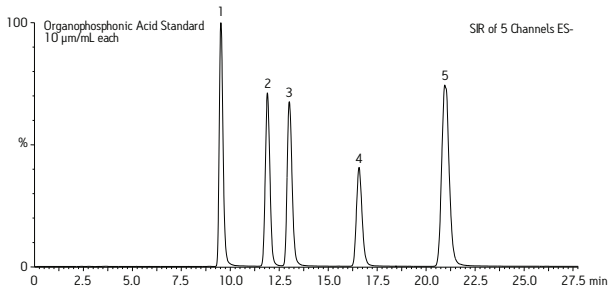
3. Cyclohexyl methylphosphonic acid (CMPA)



4. Isopropyl methylphosphonic acid (IMPA)



5. Ethyl methylphosphonic acid (EMPA)



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial w/ Preslit Septa	600000668CV

Waters Corporation
34 Maple Street, Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990

For complete experimental details, refer to full application note WA64107 at waters.com

Analysis of Organophosphonic Acids by MS

EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC with 30-cm column cooler/heater and ACQUITY TQD detector
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	50/50 acetonitrile/water with 10 mM ammonium acetate and 0.04% ammonium hydroxide, pH 9.0
Mobile phase B:	95/5 acetonitrile/water with 10 mM ammonium acetate and 0.04% ammonium hydroxide, pH 9.0

Gradient:

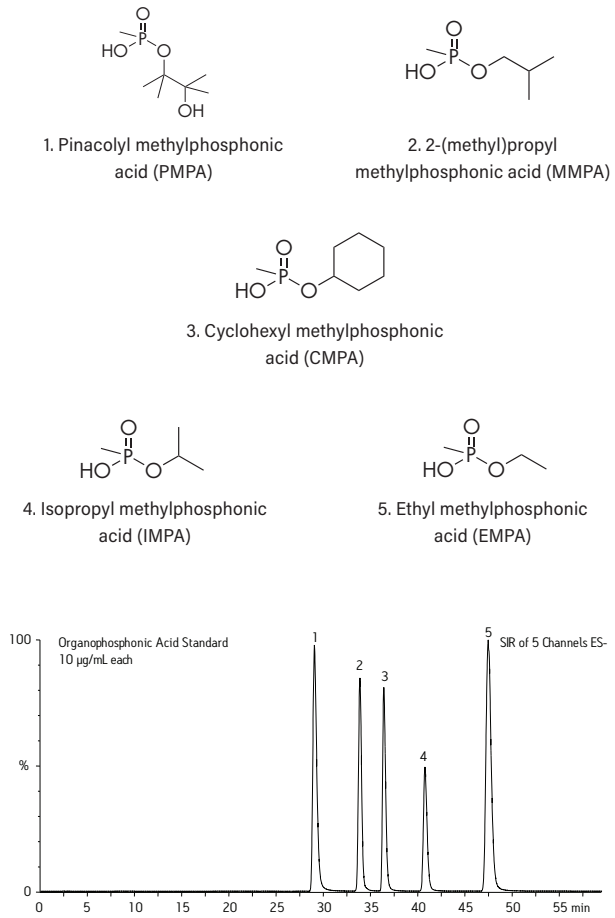
Time	%A	%B
0.00	0.1	99.9
60.00	10	90.0
60.01	0.1	99.9
72.00	0.1	99.9

Flow rate:	1.0 mL/min
Column temp.:	65 °C
Injection volume:	15.0 µL
Ionization mode:	ESI-
Acquisition mode:	SIR (m/z): 122.9 (EMPA); 136.95 (IMPA); 150.95 (MMPA); 177.0 (CMPA); 179.0 (PMPA)

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.

Sample concentration: 10 µg/mL each



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial w/ Preslit Septa	600000668CV

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For complete experimental details, refer to full application note 720005185EN at waters.com

Analysis of a Comprehensive Panel of Pain Management Drugs Using XBridge BEH Phenyl Columns

EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC I-Class, Fixed Loop (FL) with Column Manager (CMA), Xevo TQD Mass Spectrometer
Columns:	XBridge BEH Phenyl ***XP***, 2.5 µm, 3.0 x 50 mm
Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	Acetonitrile with 0.1% formic acid

Gradient:

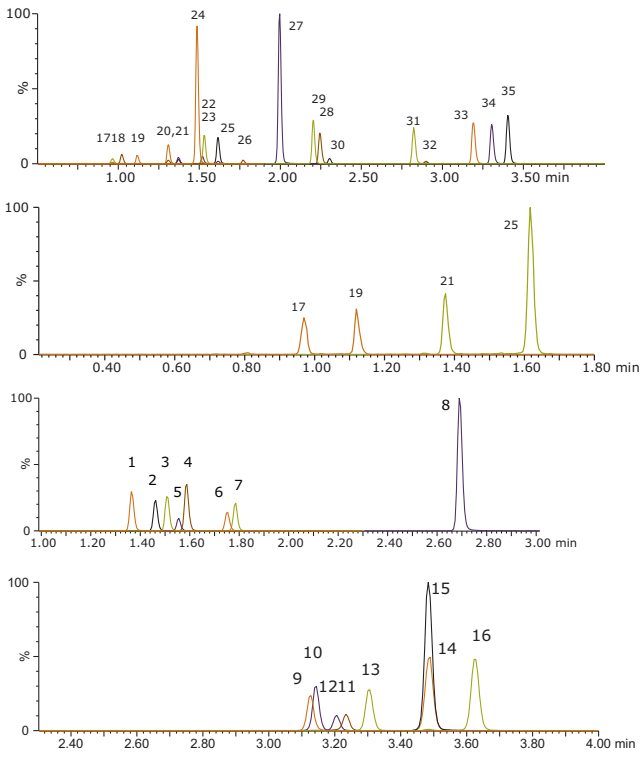
Time	%A	%B
0.0	95	5
4.0	40	60
4.1	95	5
5.0	95	5

Flow rate:	0.6 mL/min
Column temp.:	30 ˚C
Injection volume:	10 µL
Ionization mode:	ESI+
Acquisition mode:	MRM

Sample preparation

Panel of 35 common pain management compounds including opioids, benzodiazepines, stimulants, enzoylecgonine (BZE), and phencyclidine (PCP). Stock solutions were prepared in methanol. Working solutions were prepared in 5% acetonitrile containing 0.1% formic acid.

Sample temp.: 10 ˚C



1. Amphetamine
2. MDA
3. Methamphetamine
4. MDMA
5. Phentermine
6. MDEA
7. BZE
8. PCP
9. Nitrazepam
10. Oxazepam
11. Alprazolam
12. Lorazepam
13. Clonazepam
14. Flunitrazepam
15. Temazepam
16. Diazepam
17. Morphine
18. Oxymorphone
19. Hydromorphone
20. Dihydrocodeine
21. Codeine
22. Oxycodone
23. 6-AM
24. O-desmethyl tramadol
25. Hydrocodone
26. Norfentanyl
27. Tramadol
28. Normeperedine
29. Meperedine
30. Norbuprenorphine
31. Fentanyl
32. Buprenorphine
33. EDDP
34. Propoxyphene
35. Methadone

ORDERING INFORMATION

Description	P/N
XBridge BEH Phenyl ***XP***, 2.5 µm, 3.0 x 50 mm Column	186006069
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

Waters Corporation
34 Maple Street, Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990

For complete experimental details, refer to full application note 720005185EN at waters.com

Analysis of a Comprehensive Panel of Pain Management Drugs Using CORTECS C_{18} Columns

EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC I-Class, Fixed Loop (FL) with Column Manager (CMA), Xevo TQD Mass Spectrometer
Columns:	CORTECS C_{18}, 2.7 µm, 3.0 x 50 mm
Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	Acetonitrile with 0.1% formic acid

Gradient:

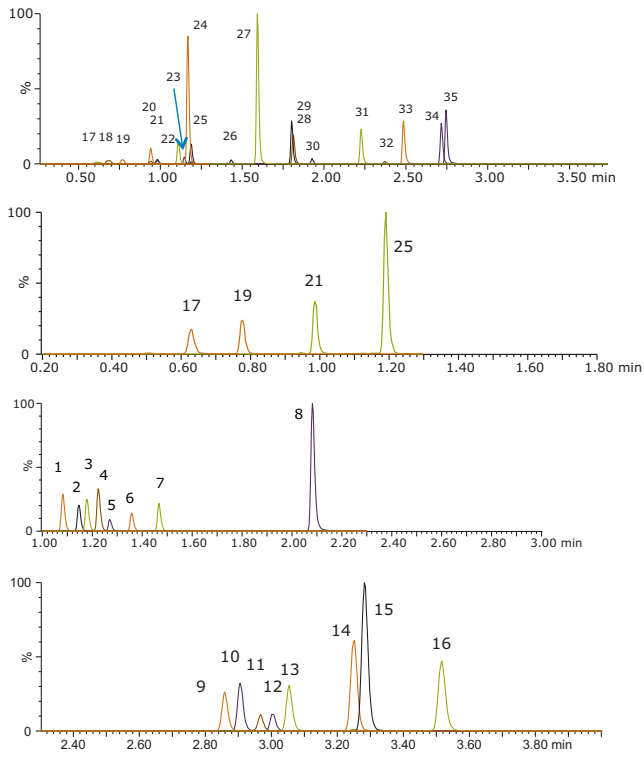
Time	%A	%B
0.0	95	5
4.0	40	60
4.1	95	5
5.0	95	5

Flow rate:	0.6 mL/min
Column temp.:	30 ˚C
Injection volume:	10 µL
Ionization mode:	ESI+
Acquisition mode:	MRM

Sample preparation

Panel of 35 common pain management compounds, including opioids, benzodiazepines, stimulants, enzoylecgonine (BZE), and phencyclidine (PCP). Stock solutions were prepared in methanol. Working solutions were prepared in 5% acetonitrile containing 0.1% formic acid.

Sample temp.: 10 ˚C



1. Amphetamine
2. MDA
3. Methamphetamine
4. MDMA
5. Phentermine
6. MDEA
7. BZE
8. PCP
9. Nitrazepam
10. Oxazepam
11. Alprazolam
12. Lorazepam
13. Clonazepam
14. Flunitrazepam
15. Temazepam
16. Diazepam
17. Morphine
18. Oxymorphone
19. Hydromorphone
20. Dihydrocodeine
21. Codeine
22. Oxycodone
23. 6-AM
24. O-desmethyl tramadol
25. Hydrocodone
26. Norfentanyl
27. Tramadol
28. Normeperedine
29. Meperedine
30. Norbuprenorphine
31. Fentanyl
32. Buprenorphine
33. EDDP
34. Propoxyphene
35. Methadone

ORDERING INFORMATION

Description	P/N
CORTECS C_{18}, 2.7 µm, 3.0 x 50 mm Column	186007370
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720004672EN at waters.com

Analysis of Peppermint Extract

EXPERIMENTAL

LC conditions

System:	AutoPurification
Columns:	XSelect CSH C_{18}, 5 µm, 4.6 x 100 mm; XSelect CSH Phenyl-Hexyl, 5 µm, 4.6 x 100 mm; XSelect CSH Fluoro-Phenyl, 5 µm, 4.6 x 100 mm
Mobile phase A:	0.1% trifluoroacetic acid (TFA) in water
Mobile phase B:	0.1% TFA in acetonitrile

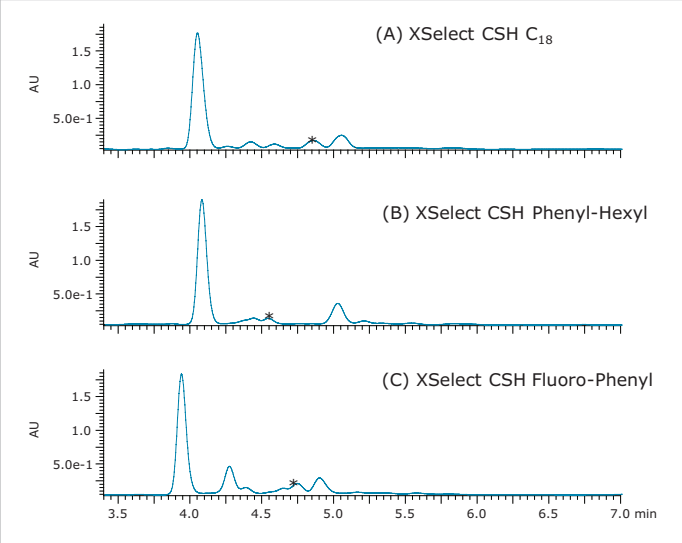
Gradient:

Time	%B
0.0	5.0
1.0	17.4
11.7	25.4
12.2	95.0
17.2	95.0
17.4	5.0
25.4	5.0

Flow rate:	1.46 mL/min
UV detection:	220 nm

Sample preparation

A total of 3.3 g dried peppermint was extracted with a 20 mL 80:20 methanol/water mixture for six hours at room temperature. The supernatant was filtered with an Acrodisc Syringe Filter with GHP Membrane, 25 mm, 0.45 µm.



ORDERING INFORMATION

Description	P/N
XSelect CSH C_{18}, 5 µm, 4.6 x 100 mm Column	186005289
XSelect CSH Phenyl-Hexyl, 5 µm, 4.6 x 100 mm Column	186005399
XSelect CSH Fluoro-Phenyl, 5 µm, 4.6 x 100 mm Column	186005344
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720005588EN at waters.com

Analysis of Peptides

EXPERIMENTAL

LC conditions

Systems: ACQUITY Arc with 2489 UV/Visible detector and ACQUITY QDa Mass Spectrometer, flow path 1

Agilent 1100 Series HPLC with quaternary pump and DAD detector

Columns: XBridge BEH C_{18}, 3.5 µm, 4.6 x 100 mm

Mobile phase A: Water with 0.1% (v/v) TFA

Mobile phase B: Acetonitrile with 0.1% (v/v) TFA

Gradient:

Time	%A	%B
Initial	95	5
5.00	95	5
45.00	50	50
47.50	5	95
52.50	5	95
52.60	95	5
60.00	95	5

Flow rate: 0.5 mL/min

Column temp.: 40 °C

Injection volume: 75 µL

UV detection: 214 nm

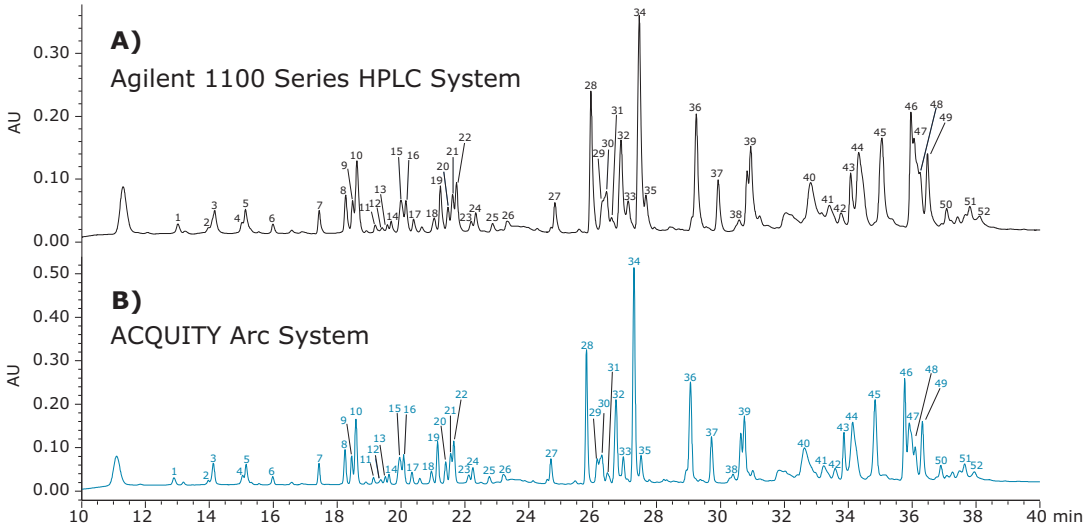
Sample preparation

A 90-µL aliquot of infliximab at 10 mg/mL was reduced with dithiothreitol and alkylated with iodoacetamide. Samples were then digested with trypsin at a 1:20 enzyme to substrate ratio and incubated at 37 °C for 18 hours. Neat TFA was added to deactivate the trypsin. Digested samples had an estimated final concentration of 0.4 mg/mL and were injected without any further dilution.

Sample temp.: 4 °C

ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 3.5 µm, 4.6 x 100 mm Column	186003033
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



For complete experimental details, refer to full application note 720005663EN at waters.com

Analysis of Pesticides (AI 1, Tebuconazole)

EXPERIMENTAL

LC conditions

System:	ACQUITY Arc with 2998 PDA detector and ACQUITY QDa Mass Spectrometer
Column:	CORTECS C_{18}+, 2.7 µm, 3.0 x 100 mm
Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	Acetonitrile

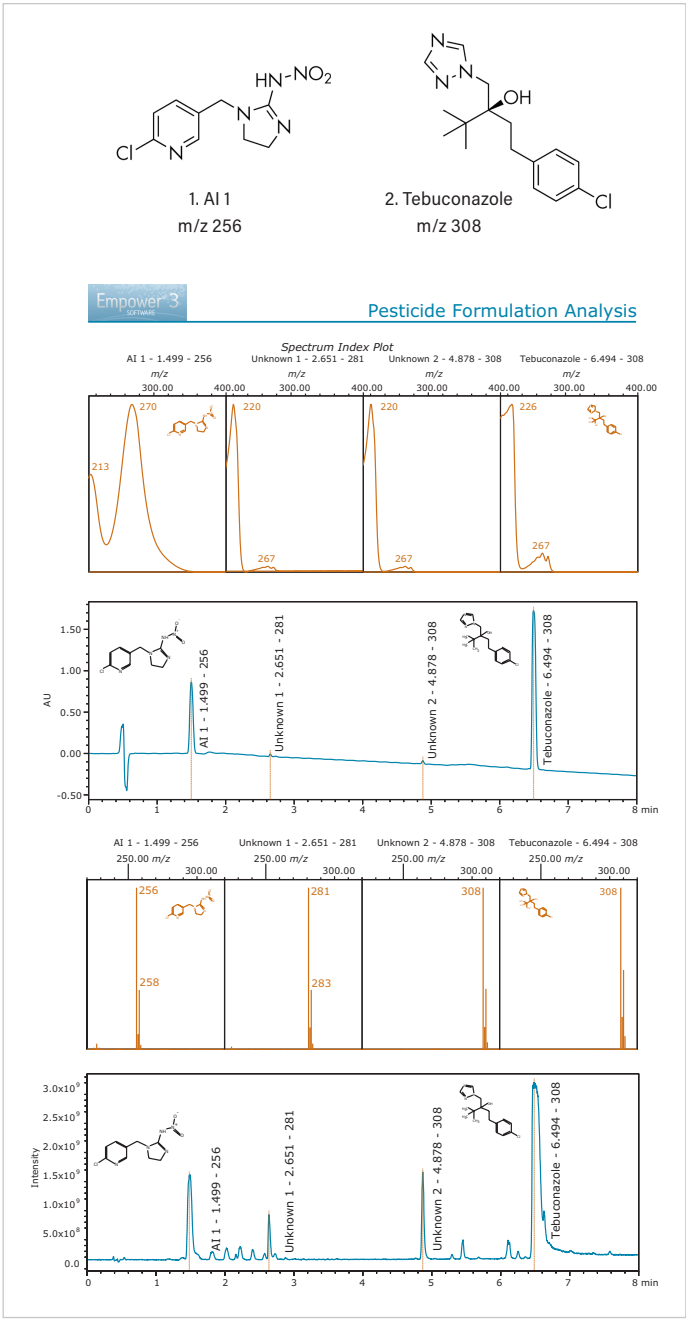
Gradient:

Time	%A	%B
0.00	80	20
10.00	20	80
11.00	10	90
12.00	10	90
12.10	80	20

Flow rate:	0.80 mL/min
Column temp.:	50 °C
Injection volume:	5 µL
UV detection:	220 nm
Ionization mode:	ESI+
Acquisition mode:	Full scan 100-1000 m/z

Sample preparation

Add 1 g commercially available pesticide formulation to 9 mL 50:50 (v/v) acetonitrile/water, sonicate for 20 minutes and filter using a 0.2 µm PVDF syringe filter.



ORDERING INFORMATION

Description	P/N
CORTECS C_{18}+, 2.7 µm, 3.0 x 100 mm Column	186007402
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note WA60205 at waters.com

Analysis of Pharmaceuticals in Environmental Samples

EXPERIMENTAL

LC conditions

Column: XBridge BEH C_{18}, 5 µm, 2.1 x 50 mm

For ESI- method

Mobile phase A: 5 mM ammonium hydroxide

Mobile phase B: Acetonitrile

Gradient:

Time	%A	%B
0	95	5
1	95	5
12	40	60
13	95	5
25	95	5

For ESI+ method

Mobile phase A: 0.5% formic acid

Mobile phase B: Acetonitrile

Gradient:

Time	%A	%B
0	95	5
1	95	5
14	30	70
15	95	5
25	95	5

Flow rate: 0.2 mL/min

Column temp.: 30 °C

Injection volume: 20 µL

Ionization mode: ESI+ and ESI-

Acquisition mode: MRM

Sample preparation

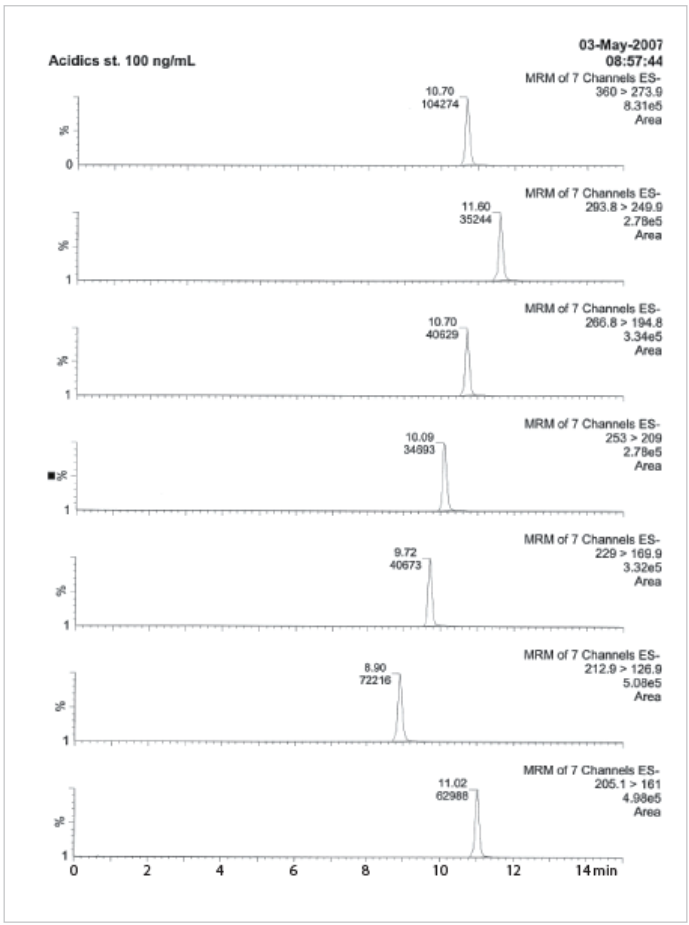
Solid-phase extraction was used to separate the pharmaceuticals from the water component of the sample. The samples were filtered through 0.45 µm filters which were pre-washed with hexane, acetone, methanol, and water. The pH of the samples was adjusted to 2.0 using concentrated HCL. Oasis MCX 3 cc was used as the solid-phase adsorbent. The adsorbent was pre-conditioned with 2 mL of hexane, 2 mL of acetone, 10 mL of methanol, and 10 mL of non-contaminated groundwater (pH adjusted to 2.0). The samples were added to the cartridges at a flow rate of 8 mL/min. The cartridges were dried with nitrogen for 1 hour and the pharmaceuticals eluted using 4 x 1 mL of acetone. The extracts were then evaporated to 100 µL with nitrogen and 100 µL of methanol was added. Evaporation continued until the volume was 50 µL. Four hundred fifty microliters of ammonium hydroxide was added and the extracts stored at -18 °C.

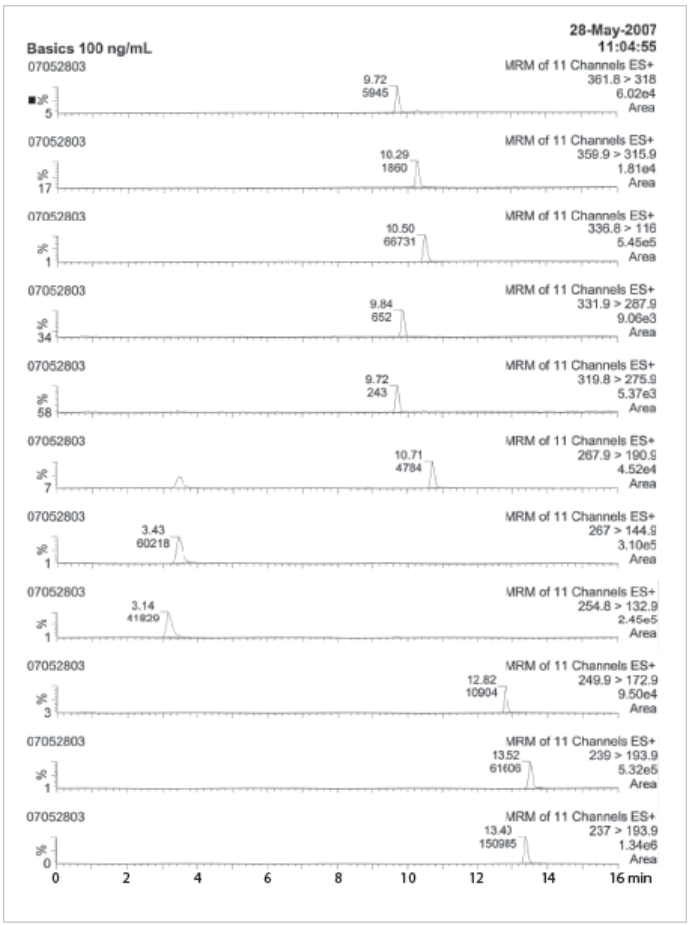
		Retention Time (min)	Ionization (ESI)	Precursor Ion (m/z)	Product Ion (m/z)
Ciprofloxacin	Antibiotic	9.8	Negative	331.9	287.9
Norfloxacin	Antibiotic	9.7	Negative	319.8	275.9
Ofloxacin	Antibiotic	9.7	Negative	361.8	317.9
Carbamazepine	Antiepileptic	13.4	Negative	237.0	193.9
Acebutolol	Beta blocker	10.5	Negative	336.8	116.0
Atenolol	Beta blocker	3.4	Negative	267.0	144.9
Metoprolol	Beta blocker	10.7	Negative	267.9	190.9
Sotalol	Beta blocker	3.1	Negative	254.8	132.9
Clofibric acid	Drug metabolite	8.9	Negative	212.9	126.9
Enrofloxacin (IS)	IS for the antibiotics	10.3	Negative	359.9	315.9
Dihydrocarba-mazepine (IS)	IS for carbamazepine	13.5	Negative	239.0	193.9
Alprenolol (IS)	IS for the beta blockers	12.8	Negative	249.9	172.9
Diclofenac	Anti-inflammatory	11.5	Positive	293.8	249.9
Ibuprofen	Anti-inflammatory	10.8	Positive	205.1	161.0
Ketoprofen	Anti-inflammatory	10.0	Positive	253.0	209.0
Naproxen	Anti-inflammatory	9.5	Positive	229.0	169.9
Bezafibrate	Lipid regulator	10.6	Positive	360.0	273.9
Fenoprop (IS)	IS for the anti-inflammatory, bezafibrate and clofibric acid	10.5	Positive	266.8	194.8

IS = internal standard

For complete experimental details, refer to full application note WA60205 at waters.com

Analysis of Pharmaceuticals in Environmental Samples *continued*





ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 5 µm, 2.1 x 50 mm Column	186003108
Oasis MCX 3 cc 60 mg Cartridge	186000253
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720005576EN at waters.com

Analysis of Polyethylene Glycols (PEGs)

EXPERIMENTAL

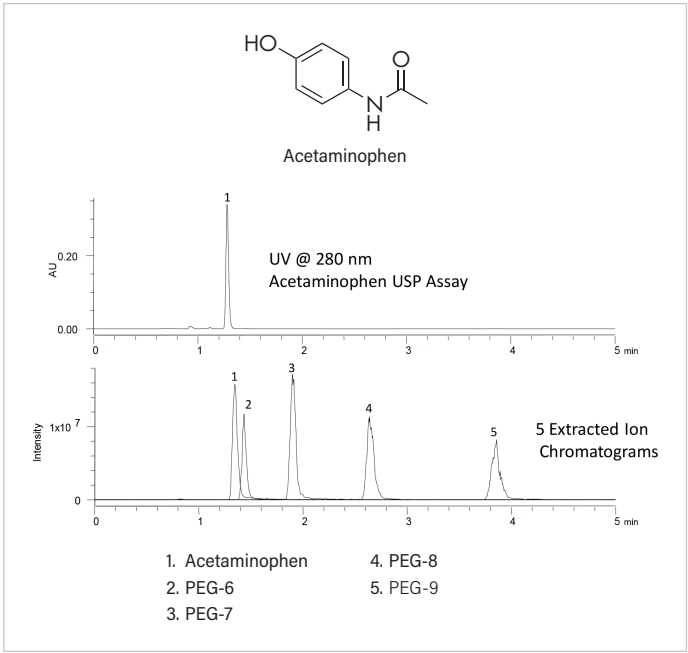
LC conditions

System:	ACQUITY UPLC H-Class with PDA dectector and ACQUITY QDa Mass Spectrometer
Column:	CORTECS C_8, 2.7 µm, 3 x 100 mm
Mobile phase:	80:20 water/methanol with 1% glacial acetic acid
Separation mode:	Isocratic
Flow rate:	0.6 mL/min
Column temp.:	30 °C
Injection volume:	1 µL
UV detection:	280 nm
Ionization mode:	ESI+
Acquisition mode:	Full scan 50-1000 m/z

Sample preparation

Acetaminophen in cough syrup.

Sample temp.:	Ambient



ORDERING INFORMATION

Description	P/N
CORTECS C_8, 2.7 µm, 3 x 100 mm Column	186008361
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note WA64704 at waters.com

Analysis of HILIC Quality Control Reference Material

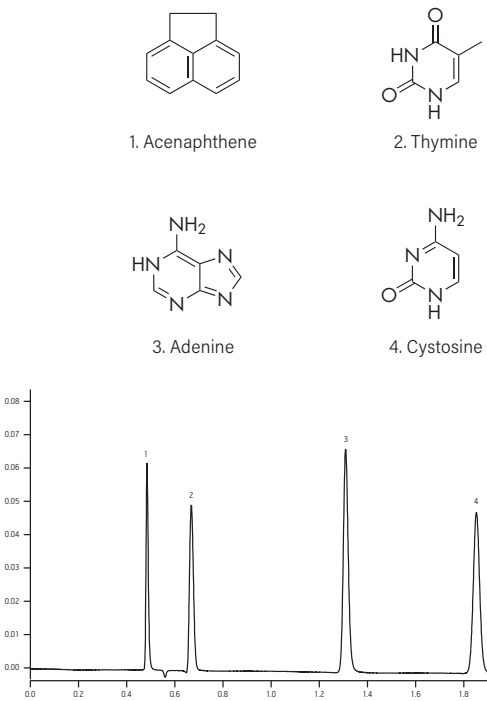
EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC with PDA detector
Column:	CORTECS HILIC, 2.7 µm, 4.6 x 100 mm
Mobile phase:	90:10 acetonitrile/100 mM ammonium formate (pH 3.0) (v/v)
Separation:	Isocratic
Flow rate:	2.0 mL/min
Column temp.:	30 ˚C
Injection volume:	9.6 µL

Sample preparation

Sample:	HILIC QC Reference Material



ORDERING INFORMATION

Description	P/N
CORTECS HILIC, 2.7 µm, 4.6 x 100 mm Column	186007392
HILIC QC Reference Material	186007226
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

Waters Corporation
34 Maple Street, Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990

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For complete experimental details, refer to full application note WA64705 at waters.com

Analysis of LCMS Quality Control Reference Material

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC with ACQUITY TQD Mass Spectrometer

Column: CORTECS C_{18}, 2.7 µm, 4.6 x 100 mm

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.1% formic acid in acetonitrile

Gradient:

Time	%A	%B	Curve
0.00	95	5	–
9.60	25	75	6
10.00	25	75	6
10.10	75	25	6
12.00	75	25	6

Flow rate: 2.0 mL/min

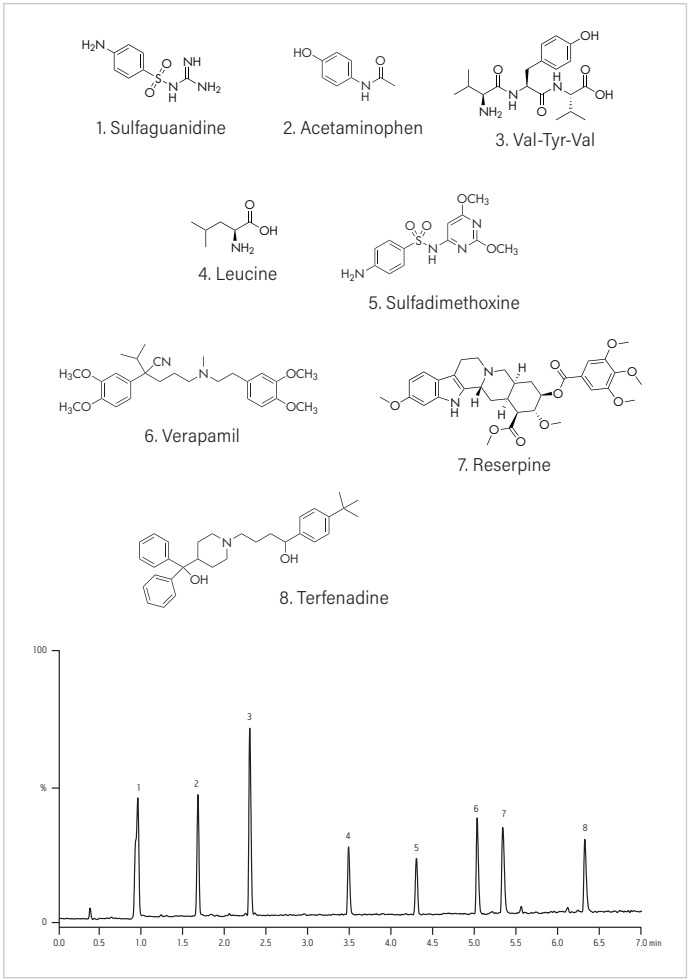
Column temp.: 40 ˚C

Injection volume: 9.6 µL

Ionization mode: ESI+

Sample preparation

Sample: LCMS QC Reference Material



ORDERING INFORMATION

Description	P/N
CORTECS C_{18}, 2.7 µm, 4.6 x 100 mm Column	186007377
LCMS QC Reference Material	186006963
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

Waters Corporation
34 Maple Street, Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990

For complete experimental details, refer to full application note WA60706 at waters.com

Analysis of Reversed-Phase Quality Control Reference Material

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC with PDA detector

Column: CORTECS C_{18}+, 2.7 µm, 2.1 x 100 mm

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.1% formic acid in acetonitrile

Gradient:

Time	%A	%B	Curve
Initial	95	5	–
2.70	5	95	6
3.00	5	95	6
3.12	95	5	6
4.00	95	5	6

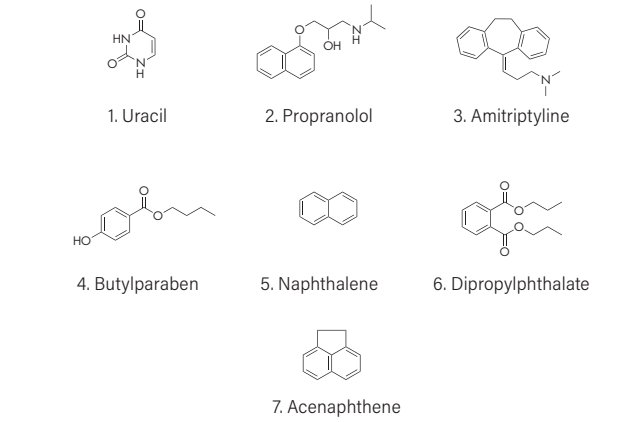
Flow rate: 1.0 mL/min

Column temp.: 30 ˚C

Injection volume: 2.0 µL

Sample preparation

Sample: Reversed-Phase QC Reference Material



ORDERING INFORMATION

Description	P/N
CORTECS C_{18}+, 2.7 µm, 2.1 x 100 mm Column	186007397
Reversed-Phase QC Reference Material	186006363
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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34 Maple Street, Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990

For complete experimental details, refer to full application note 720005550EN at waters.com

Analysis of Sialic Acid by HPLC

EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC with fluorescence detector
Column:	XBridge BEH C_{18}, 3.5 µm, 4.6 x 100 mm
Mobile phase:	Acetonitrile:methanol:water (9%:7%:84%)
Separation mode:	Isocratic
Flow rate:	0.43 mL/min
Injection volume:	6.7 µL
Column temp.:	30 °C
FLR detection:	Excitation wavelength = 373 nm; Emission wavelength = 448 nm

ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 3.5 µm, 4.6 x 100 mm Column	186003033
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

Sample preparation

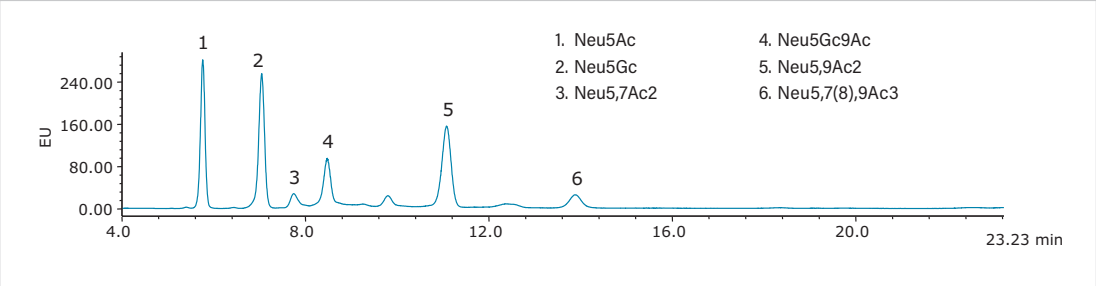
Preparation of 1,2-Diamino-4,5-methylenedioxybenzene dihydrochloride (DMB) labeling solution:

- Into a 2-mL glass vial, 436 µL of water and 38 µL of glacial acetic acid were mixed.
- 26 µL of 2-mercaptoethanol was added to the 2-mL vial and mixed.
- 440 µL of the above solution was added to a separate 2-mL glass vial containing 4 mg of sodium hydrosulfite and mixed.
- This solution was added to a 2-mL glass vial containing to 0.7 mg of DMB and mixed.
- Due to the light and moisture sensitivity this DMB-labeling solution was used immediately after preparation.

DMB Labeling of Sialic Acid Reference Panel:

- 20 µL of the DMB-labeling solution was added to the vial containing the purchased sialic acid reference panel.
- The sample was incubated in the dark using a heater block set to 50 °C.
- After three hours, the reaction was stopped by adding 480 µL of water to the reaction mixture.
- This DMB-labeled sample was injected onto the ACQUITY UPLC System with injection volumes scaled appropriately for column configurations.

Sample temp.: 4 °C



For complete experimental details, refer to full application note 720005550EN at waters.com

Analysis of Sialic Acid by UHPLC

EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC with fluorescence detector
Column:	XBridge BEH C_{18} ***XP***, 2.5 µm, 3.0 x 75 mm
Mobile phase:	Acetonitrile:methanol:water (9%:7%:84%)
Separation mode:	Isocratic
Flow rate:	0.26 mL/min
Injection volume:	2.0 µL
Column temp.:	30 °C
FLR detection:	Excitation wavelength = 373 nm; Emission wavelength = 448 nm

ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18} ***XP***, 2.5 µm, 3.0 x 75 mm Column	186006034
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

Sample preparation

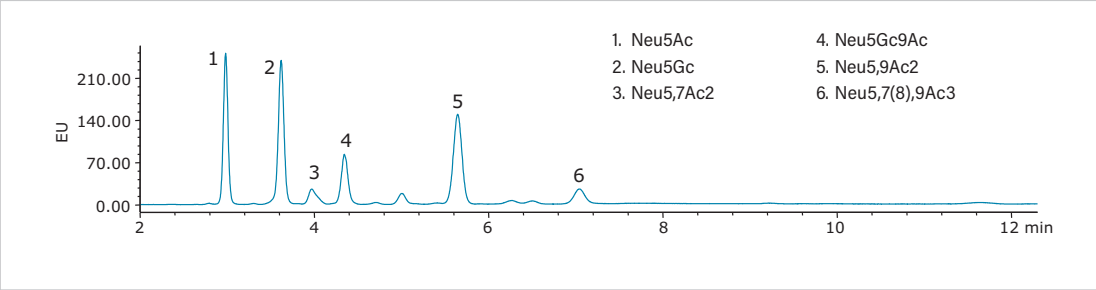
Preparation of 1,2-Diamino-4,5-methylenedioxybenzene dihydrochloride (DMB) labeling solution:

- Into a 2-mL glass vial, 436 µL of water and 38 µL of glacial acetic acid and were mixed.
- 26 µL of 2-mercaptoethanol was added to the 2-mL vial and mixed.
- 440 µL of the above solution was added to a separate 2-mL glass vial containing 4 mg of sodium hydrosulfite and mixed.
- This solution was added to a 2-mL glass vial containing to 0.7 mg of DMB and mixed.
- Due to the light and moisture sensitivity this DMB-labeling solution was used immediately after preparation.

DMB Labeling of Sialic Acid Reference Panel:

- 20 µL of the DMB-labeling solution was added to the vial containing the purchased sialic acid reference panel.
- The sample was incubated in the dark using a heater block set to 50 °C.
- After three hours, the reaction was stopped by adding 480 µL of water to the reaction mixture.
- This DMB-labeled sample was injected onto the ACQUITY UPLC System with injection volumes scaled appropriately for column configurations.

Sample temp.: 4 °C



For complete experimental details, refer to full application note 720001901EN at waters.com

Analysis of Simvastatin

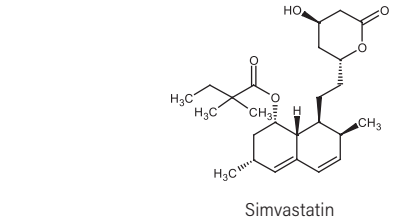
EXPERIMENTAL

LC conditions

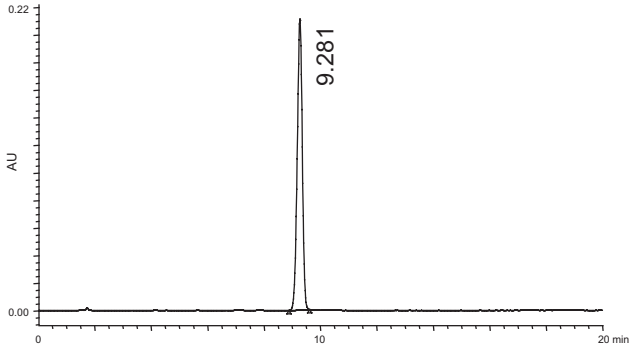
System:	ACQUITY UPLC with PDA detector
Column:	XBridge BEH C_{18}, 5 µm, 4.6 x 250 mm
Mobile phase:	65% acetonitrile/35% phosphate buffer, pH 4.5
Separation mode:	Isocratic
Flow rate:	1.5 mL/min
Column temp.:	45 °C
Injection volume:	10 µL
UV detection:	238 nm

Sample preparation

A standard solution of simvastatin was prepared according to the USP methodology, and then was diluted to 100 µg/mL.



Simvastatin



ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 5 µm, 4.6 x 250 mm Column	186003117
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note 720004589EN at waters.com

Analysis of Soft Drink

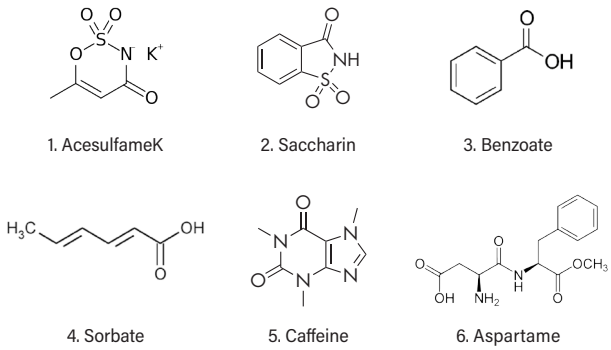
EXPERIMENTAL

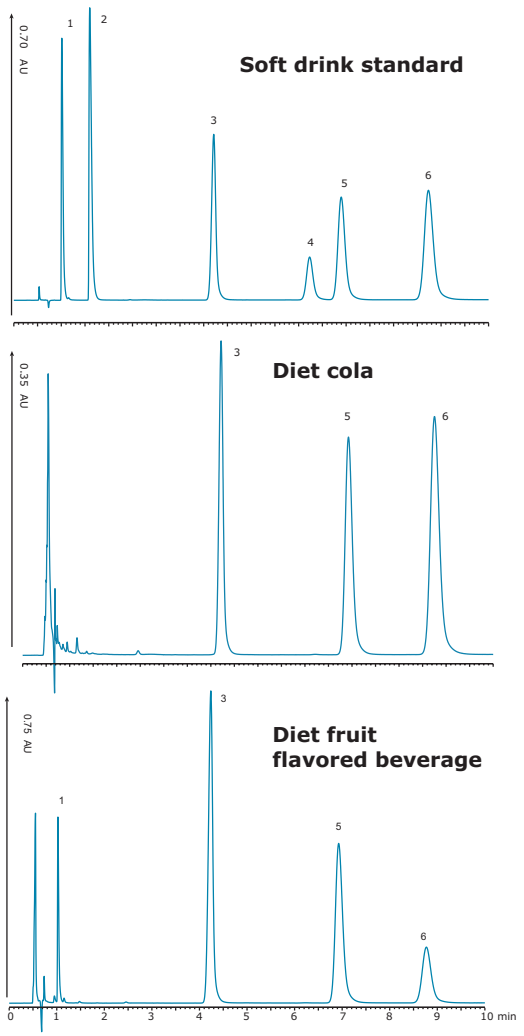
LC conditions

System:	ACQUITY UPLC H-Class
Column:	XBridge BEH Phenyl ***XP***, 2.5 µm, 4.6 x 50 mm
Mobile phase:	Waters Beverage Analysis Mobile-Phase Reagent
Separation mode:	Isocratic
Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	5 µL
UV detection:	214 nm

Sample preparation

One bottle of Waters Beverage Analysis Standards was poured into one bottle of Waters Beverage Analysis Standards Solid. The bottle containing this mixture was capped tightly, and shaken vigorously until the aspartame was completely dissolved.





ORDERING INFORMATION

Description	P/N
XBridge BEH Phenyl ***XP***, 2.5 µm, 4.6 x 50 mm Column	186006073
LCGC Certified Clear Glass Recovery Vial	186003270
Waters Beverage Analysis Kit	176002534

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For complete experimental details, refer to full application note 720004253EN at waters.com

Analysis of Soy Isoflavones in Foods and Dietary Supplements by HPLC

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC H-Class with ACQUITY SQD detector and 2998 PDA detector

Column: XSelect HSS Cyano, 5 µm, 4.6 x 150 mm

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.1% formic acid in acetonitrile

Gradient:

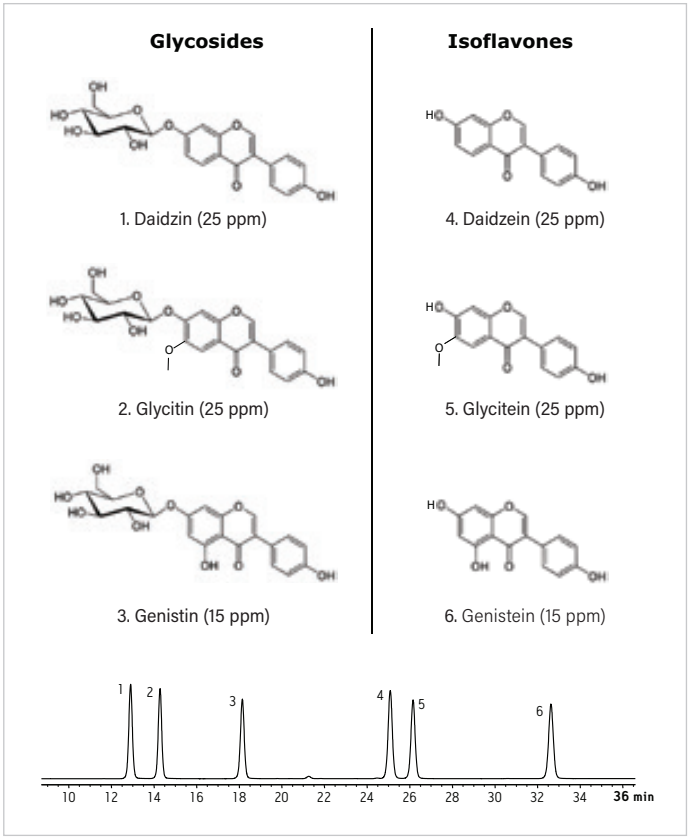
Time	%A	%B
0.00	90	10
3.00	90	10
33.00	70	30
36.00	70	30
36.10	90	10
51.10	90	10

Flow rate: 1.0 mL/min

Column temp.: 30 °C

Injection volume: 43 µL

Ionization mode: ESI+



Sample preparation

Standard solution: Prepared from daidzin (25 ppm), glycitin (25 ppm), genistin (15 ppm), daidzein (25 ppm), glycitein (25 ppm), and genistein (15 ppm) using 10/90 acetonitrile/water diluent.

ORDERING INFORMATION

Description	P/N
XSelect HSS Cyano, 5 µm, 4.6 x 150 mm Column	186005945
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

Waters Corporation
34 Maple Street, Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990

For complete experimental details, refer to full application note 720004253EN at waters.com

Analysis of Soy Isoflavones in Foods and Dietary Supplements by UHPLC

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC H-Class with ACQUITY SQD detector and 2998 PDA detector

Column: XSelect HSS Cyano, 2.5 µm, 4.6 x 75 mm

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.1% formic acid in acetonitrile

Gradient:

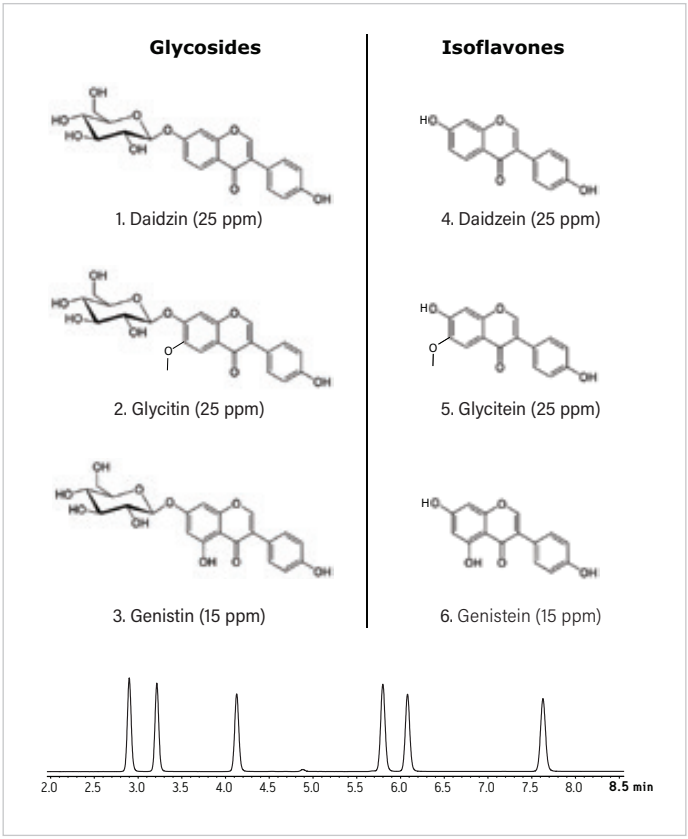
Time	%A	%B
0.00	90	10
0.75	90	10
8.25	70	30
9.00	70	30
9.03	90	10
12.78	90	10

Flow rate: 2.0 mL/min

Column temp.: 30 °C

Injection volume: 22 µL

Ionization mode: ESI+



Sample preparation

Standard solution: Prepared from daidzin (25 ppm), glycitin (25 ppm), genistin (15 ppm), daidzein (25 ppm), glycitein (25 ppm), and genistein (15 ppm) using 10/90 acetonitrile/water diluent.

ORDERING INFORMATION

Description	P/N
XSelect HSS Cyano *XP*, 2.5 µm, 4.6 x 75 mm Column	186006194
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note XBRIDGE13 at waters.com

Analysis of Statins

EXPERIMENTAL

LC conditions

System: Alliance 2695 with 2996 PDA detector

Column: XBridge BEH Phenyl, 3.5 µm 4.6 x 100 mm

Mobile phase A: Water

Mobile phase B: Acetonitrile

Mobile phase C: 100 mM ammonium bicarbonate

Gradient:

Time	%A	%B	%C
0.00	65	15	20
20.00	30	50	20
21.00	65	15	20
25.00	65	15	20

Flow rate: 1.2 mL /min

Column temp.: 25 ˚C

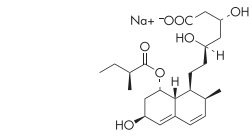
Injection volume: 20 µL

UV detection: 248 nm

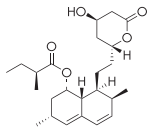
Sample preparation

Sample: Atorvastatin (10 µg/mL), Simvastatin (10 µg/mL), Lovastatin (10 µg/mL), Pravastatin sodium (10 µg/mL) in H_2O/NH_4HCO_3 (90/10)

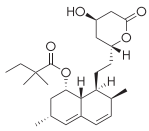
Sample temp.: 15 ˚C



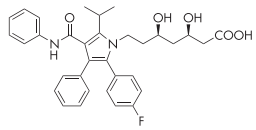
1. Pravastatin sodium



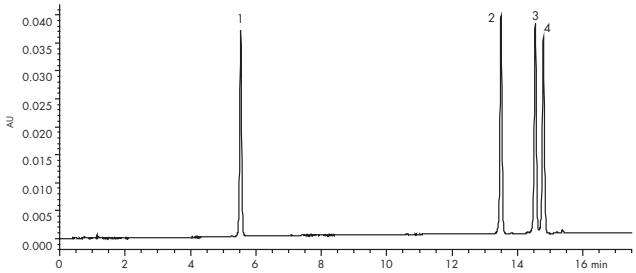
2. Lovastatin



3. Simvastatin



4. Atorvastatin



ORDERING INFORMATION

Description	P/N
XBridge BEH Phenyl, 3.5 µm, 4.6 x 100 mm Column	186003334
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note WA64103 at waters.com

Analysis of Stevia Related Compounds by ELSD

EXPERIMENTAL

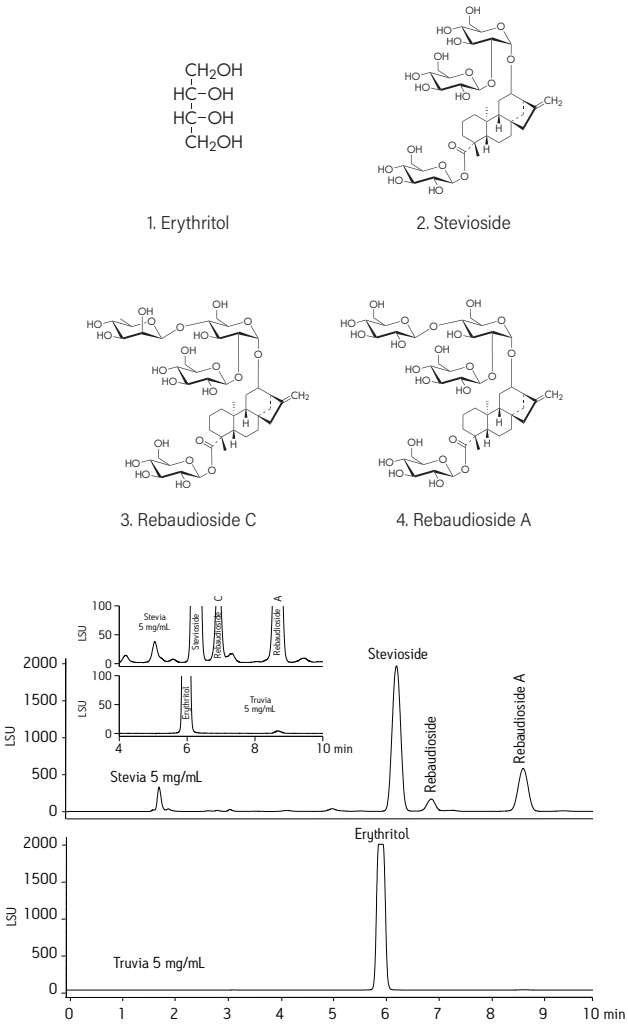
LC conditions

System:	Alliance HPLC with 2424 ELSD
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.2% triethylamine
Mobile phase B:	30/70 acetonitrile/water with 0.2% triethylamine
Isocratic conditions:	95% A/5% B (77.5% acetonitrile with 0.2% triethylamine)
Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	10.0 µL
ELSD pressure:	30 psi
Drift tube temp.:	50 °C

Sample preparation

Add 25 mL of 50:50 acetonitrile/water to ~3 g sample, homogenize, centrifuge at 3200 rpm for 30 minutes, collect supernatant and filter using 0.45 µm PVDF syringe filter.

Sample concentration: 5 mg/mL each



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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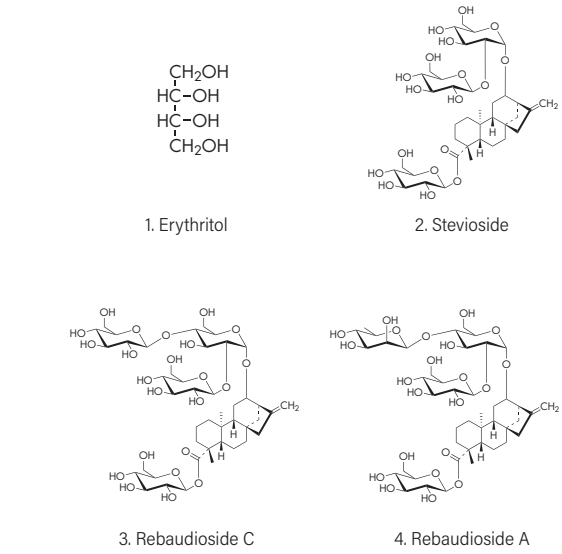
For complete experimental details, refer to full application note WA64106 at waters.com

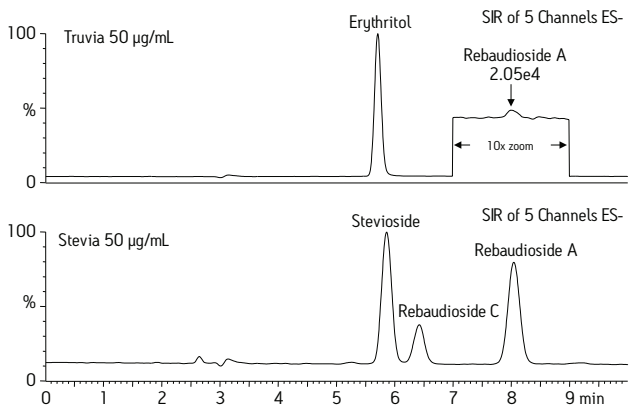
Analysis of Stevia Related Compounds by MS

EXPERIMENTAL

LC conditions

System:	ACQUITY UPLC with 30-cm column cooler/heater and Xevo TQD Mass Spectrometer
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	80/20 acetonitrile/water with 0.1 % ammonium hydroxide
Mobile phase B:	30/70 acetonitrile/water with 0.1 % ammonium hydroxide
Isocratic conditions:	95% A/5% B
Flow rate:	1.0 mL/min
Column temp.:	35 ˚C
Injection volume:	15.0 µL
Ionization mode:	ESI-
Acquisition mode:	SIR (m/z): 121.1 (erythritol); 803.8 (stevioside); 950.1 (rebaudioside C); 966.1 (rebaudioside A)





Sample preparation

Add 25 mL of 50:50 acetonitrile/water to ~3 g sample, homogenize, centrifuge at 3200 rpm for 30 minutes, collect supernatant and filter using 0.45 µm PVDF syringe filter.

Sample concentration: 50 µg/mL each

ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial w/ Preslit Septa	600000668CV

Waters Corporation
34 Maple Street, Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990

For complete experimental details, refer to full application note 720005669EN at waters.com

Analysis of Sucralose

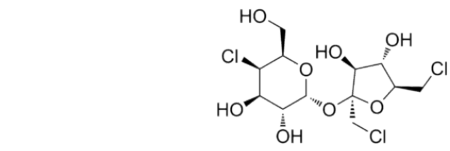
EXPERIMENTAL

LC conditions

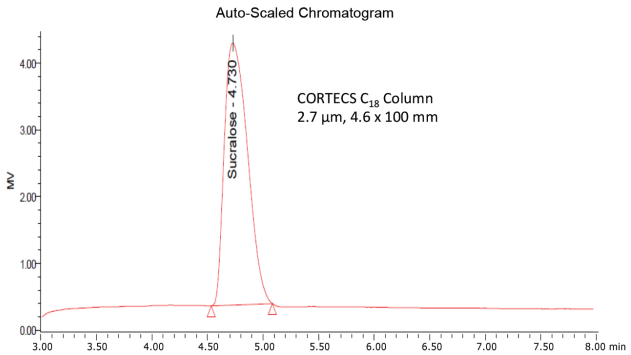
System:	Alliance HPLC with 2414 Refractive Index (RI) detector
Column:	CORTECS C_{18}, 2.7 µm, 4.6 x 100 mm
Mobile phase:	80:20 water/methanol
Separation mode:	Isocratic
Flow rate:	1 mL/min
Column temp.:	30 °C
Injection volume:	50 µL
Refractive Index (RI) Detector temp.:	30 °C

Sample preparation

Sucralose reference standard 100 µg/mL.



Sucralose



ORDERING INFORMATION

Description	P/N
CORTECS C_{18}, 2.7 µm, 4.6 x 100 mm Column	186007377
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note WA60708 at waters.com

Analysis of Sudan Dyes

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC H-Class with Xevo TQD Mass Spectrometer

Column: CORTECS C_{18}, 2.7 µm, 2.1 x 100 mm

Mobile phase A: Water + 0.1% formic acid

Mobile phase B: Methanol + 0.1% formic acid

Mobile phase C: Acetonitrile + 0.1% formic acid

Gradient:

Time	%A	%B	%C	Curve
Initial	80	10	10	–
0.5	40	30	30	6
5.0	0	50	50	6
9.0	0	50	50	6
9.1	80	10	10	6
12.0	80	10	10	6

Flow rate: 0.4 mL/min

Column temp.: 45 ˚C

Injection volume: 5 µL

Ionization mode: ESI+

Acquisition mode: MRM

Sample preparation

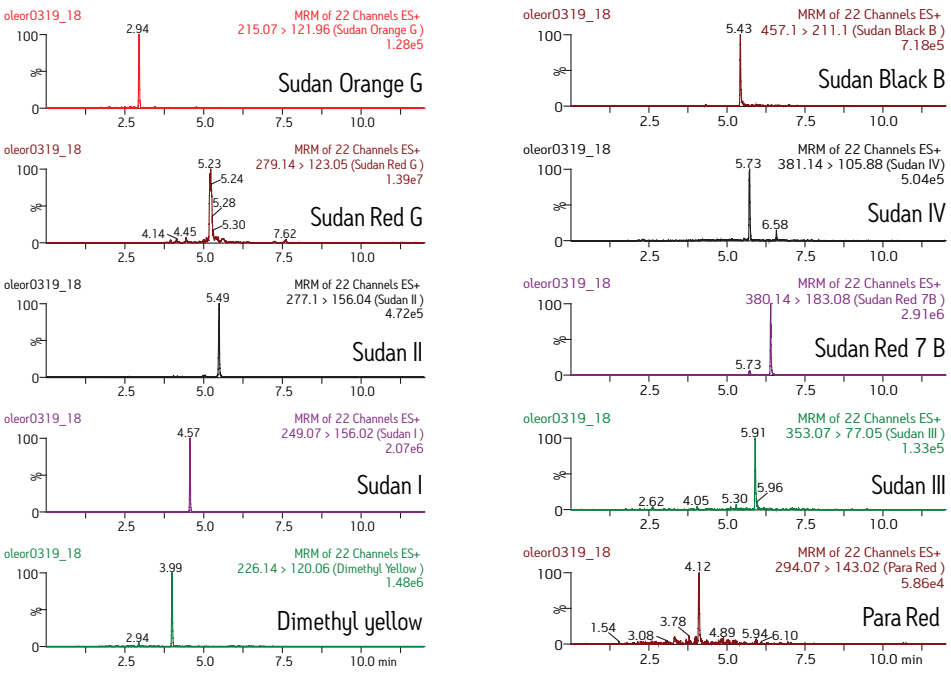
Sample: Sudan Dyes in Oleoresin

Oleoresin prepared using Certified Sep-Pak Silica 3 cc Vac Cartridge, 500 mg sorbent per cartridge, 55-105 µm particle size.

ORDERING INFORMATION

Description	P/N
CORTECS C_{18}, 2.7 µm, 2.1 x 100 mm Column	186007367
Certified Sep-Pak Silica 3 cc Vac Cartridge	186004615
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

100ppb Spiked Sudan Dyes in Oleoresin



1. Sudan orange G
2. Sudan black B
3. Sudan red G
4. Sudan IV
5. Sudan II
6. Sudan red 7 B
7. Sudan I
8. Sudan III
9. Dimethyl yellow
10. Para red

For complete experimental details, refer to full application note WA64698 at waters.com

Analysis of Synthetic Cannabinoids

EXPERIMENTAL

LC conditions

System: ACQUITY UPLC I-Class System (SM-FL), Column Manager (CMA) with Xevo TQD Mass Spectrometer

Column: CORTECS C_{18}, 2.7 µm, 2.1 x 100 mm

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.1% formic acid in acetonitrile

Gradient:

Time	%A	%B	Curve
Initial	70	30	–
3.0	50	50	6
4.5	50	50	6
10.5	10	90	6
13.0	10	90	6
13.1	70	30	11
15.0	70	30	11

Column temp.: 30 ˚C

Injection volume: 5 µL

Ionization mode: ESI+

Acquisition mode: MRM

Sample preparation

Sample: Synthetic cannabinoid mix

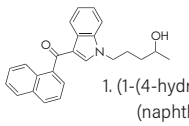
Challenge Sample: Prepared plasma using Ostro Sample Preparation Plate

ORDERING INFORMATION

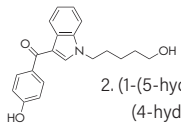
Description	P/N
CORTECS C_{18}, 2.7 µm, 2.1 x 100 mm Column	186007367
Ostro Sample Preparation Plate	186005518
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

Synthetic Cannabinoids

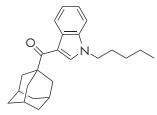
		Retention Time	
Peak	Compound	Initial Injection	Injection 1000
2	RCS-4, M10	1.91	1.90
10	JWH-018, 4-OH met.	4.18	4.18
21	AB 001	10.49	10.48
Pressure		3753	3749



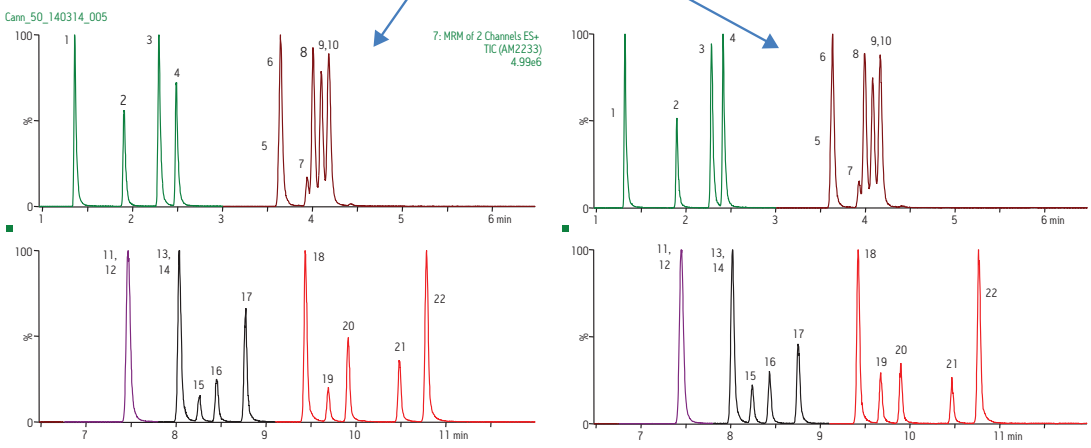
1. (1-(4-hydroxypentyl)-1 H-indol-3-yl) (naphthalen-1-yl)methanone



2. (1-(5-hydroxypentyl)-1 H-indol-3-yl) (4-hydroxyphenyl)methanone



3. AB 001



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For complete experimental details, refer to full application note 720004582EN at waters.com

Analysis of Tetracyclines in Milk

EXPERIMENTAL

LC conditions

System: Alliance e2690/5 with 2998 PDA detector

Column: XBridge BEH C_{18} ***XP***, 2.5 µm, 100 x 4.6 mm

Mobile phase A: 10 mM oxalic acid in water

Mobile phase B: 10 mM oxalic acid in acetonitrile

Gradient:

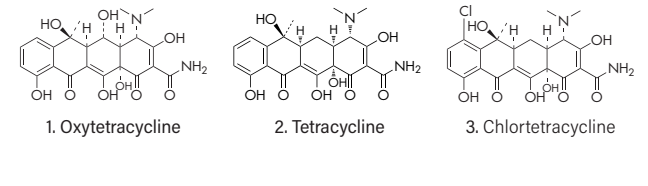
Time	%A	%B
0.00	85	15
8.00	50	50
11.25	50	50
11.60	85	15
12.85	85	15

Flow rate: 1.20 mL/min

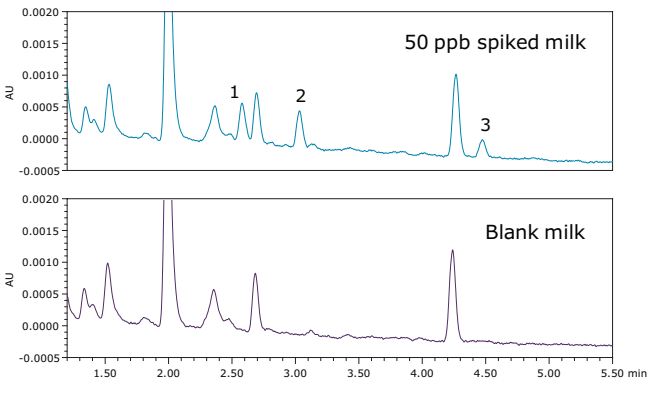
Column temp.: 30 °C

Injection volume: 35 µL

UV detection: 355 nm



1. Oxytetracycline 2. Tetracycline 3. Chlortetracycline



Sample preparation

Sample and Buffer Preparation

1. EDTA/McIlvaine Buffer: In a 1-L volumetric flask, dissolve 28.41 g anhydrous dibasic sodium phosphate in approximately 900 mL water, dilute to volume, and mix. In a separate 1-L volumetric flask, dissolve 21.01 g citric acid monohydrate in approximately 900 mL water, dilute to volume, and mix. Combine 1-L citric acid solution with 625 mL of phosphate solution. Add 60.5 g disodium EDTA, and mix well until dissolved. Prepare fresh weekly.
2. Initial Extraction/Precipitation: Transfer 1.5 mL of milk to a 15-mL centrifuge tube. Add 6 mL of EDTA/McIlvaine buffer, and vortex for 30 seconds. Centrifuge at 4000 rpm for 5 minutes Collect the supernantant, and adjust to pH 10 with 0.75 mL 1 M NaOH.
3. SPE Cleanup: SPE cleanup is performed using an Oasis MAX Cartridge (1 cc, 30 mg). Condition the cartridge with 2 mL of methanol, followed by 2 mL of water. Set flow rate to approximately 1 mL/min. Load pH adjusted supernatant obtained from the initial extraction. Wash with 0.5 mL of 5% ammonium hydroxide, and then with 0.5 mL of methanol. Elute with 0.5 mL 45:55 acetonitrile/75 mM aqueous oxalic acid. Dilute to 1.5 mL with reagent water prior to LC analysis.

ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18} ***XP***, 2.5 µm, 100 x 4.6 mm Column	186006039
Oasis MAX 1 cc Vac Cartridge	186000366
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note WA64114 at waters.com

Analysis of Thiourea

EXPERIMENTAL

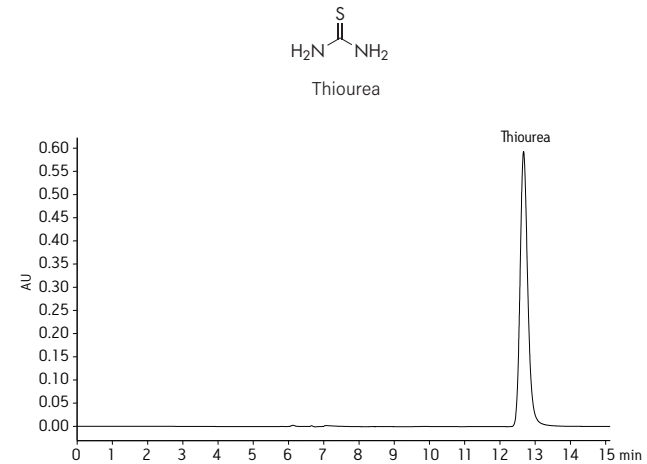
LC conditions

System:	Alliance HPLC with 2998 PDA detector
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase:	95/2.5/2.5 acetonitrile/isopropyl alcohol/water with 10 mM ammonium acetate, pH 9.0
Separation mode:	Isocratic
Flow rate:	0.5 mL/min
Column temp.:	25 ˚C
Injection volume:	40.0 µL
UV detection:	245 nm

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.

Sample concentration: 10 µg/mL



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note 720004554EN at waters.com

Analysis of Tioconazole and Related Compounds

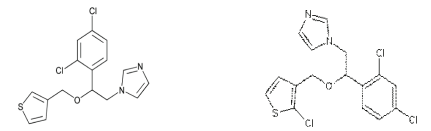
EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2998 PDA detector
Column:	XSelect CSH C_{18}, 5 µm, 4.6 x 250 mm; XSelect CSH C_{18} ***XP***, 2.5 µm, 4.6 x 150 mm; XSelect CSH C_{18} ***XP***, 2.5 µm, 4.6 x 100 mm
Mobile phase:	44:40:28 acetonitrile/methanol/water with 2 mL ammonium hydroxide
Separation mode:	Isocratic
Flow rate:	1.0 mL/min
Column temp.:	25 °C
Injection volume:	25 µL (250-mm column), 12 µL (150-mm column), 8 µL (100-mm column)
UV detection:	219 nm

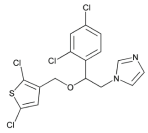
Sample preparation

The tioconazole sample was prepared in 100% methanol to the concentrations described in the table.

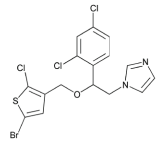


1. Related compound A

2. Tioconazole

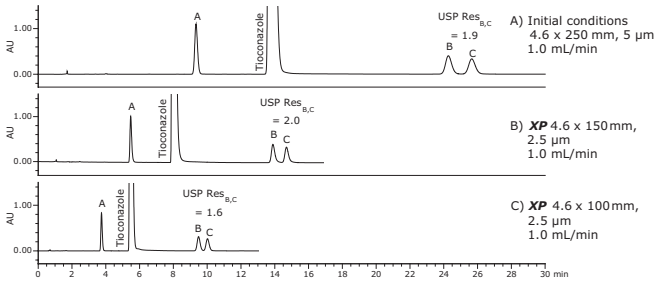


3. Related compound B



4. Related compound C

	Tioconazole	Related compound A	Related compound B	Related compound C
Concentration (mg/mL)	6.6	0.2	0.2	0.2



ORDERING INFORMATION

Description	P/N
XSelect CSH C_{18}, 5 µm, 4.6 x 250 mm Column	186005291
XSelect CSH C_{18} ***XP***, 2.5 µm, 4.6 x 150 mm Column	186006729
XSelect CSH C_{18} ***XP***, 2.5 µm, 4.6 x 100 mm Column	186006111
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note 720004643EN at waters.com

Analysis of Topiramate

EXPERIMENTAL

LC conditions

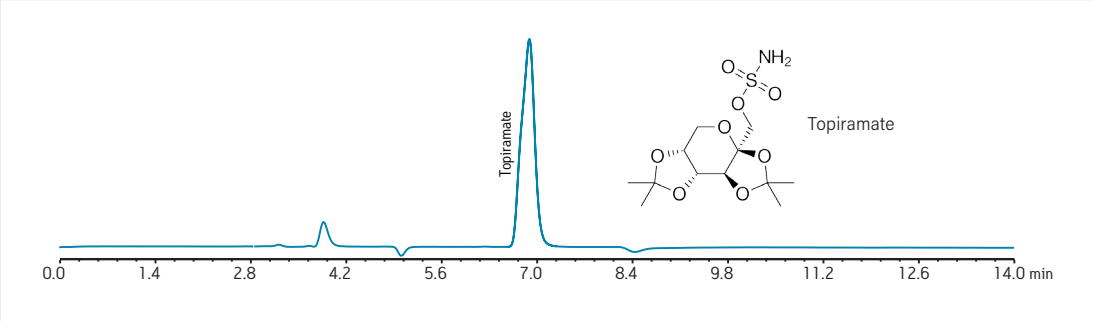
System:	Alliance HPLC with 2414 RI detector
Column:	XSelect HSS C_{18}, 5.0 µm, 4.6 x 250 mm
Mobile phase:	1:1 (v/v) acetonitrile/water
Separation mode:	Isocratic
Flow	0.6 mL/min
Colum temp.:	50 °C
Injection volume:	20 µL

Sample preparation

The standard solution used in this study was prepared according to the assay method defined in the USP Monograph for Topiramate Drug Substance.

ORDERING INFORMATION

Description	P/N
XSelect HSS C_{18}, 5.0 µm, 4.6 x 250 mm Columns	186004775
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



For complete experimental details, refer to full application note XBRIDGE12 at waters.com

Analysis of Tricyclic Antidepressants

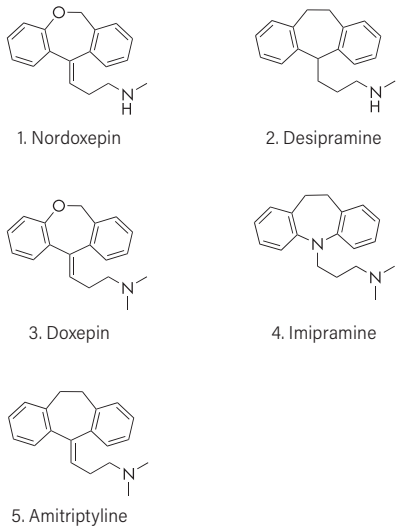
EXPERIMENTAL

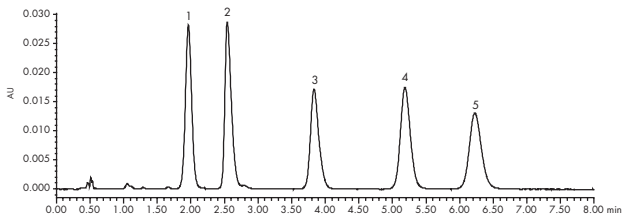
LC conditions

System:	Alliance 2695 with 2996 PDA detector
Column:	XBridge BEH Phenyl, 3.5 µm, 4.6 x 50 mm
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Mobile phase C:	100 mM ammonium bicarbonate
Isocratic conditions:	48% A; 42% B; 10% C
Flow rate:	1.2 mL/min
Column temp.:	30 ˚C
Injection volume:	10 µL
UV detection:	254 nm

Sample preparation

Sample:	Nordoxepin (10 µg/mL), Desipramine (10 µg/mL), Doxepin (10 µg/mL), Imipramine (10 µg/mL), Amitriptyline (10 µg/mL) in H_2O/ACN (50/50)
Sample temp.:	15 ˚C





ORDERING INFORMATION

Description	P/N
XBridge BEH Phenyl, 3.5 µm, 4.6 x 50 mm Column	186003332
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note XBRIDGE4 at waters.com

Analysis of Tricyclic Antidepressants at pH 7.0

EXPERIMENTAL

LC conditions

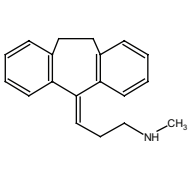
System:	Alliance 2695 with 2996 PDA detector
Column:	XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm
Mobile phase:	20 mM sodium phosphate buffer, pH 7.0/ acetonitrile/methanol (30/35/35)
Separation mode:	Isocratic
Flow rate:	1.5 mL/min
Column temp.:	25 ˚C
Injection volume:	10 µL
UV detection:	254 nm

Sample preparation

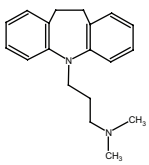
Sample concentration: 20 µg/mL in water

ORDERING INFORMATION

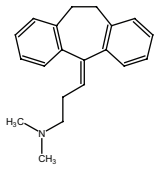
Description	P/N
XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm Column	186003116
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



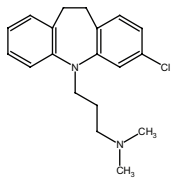
1. Nortriptyline



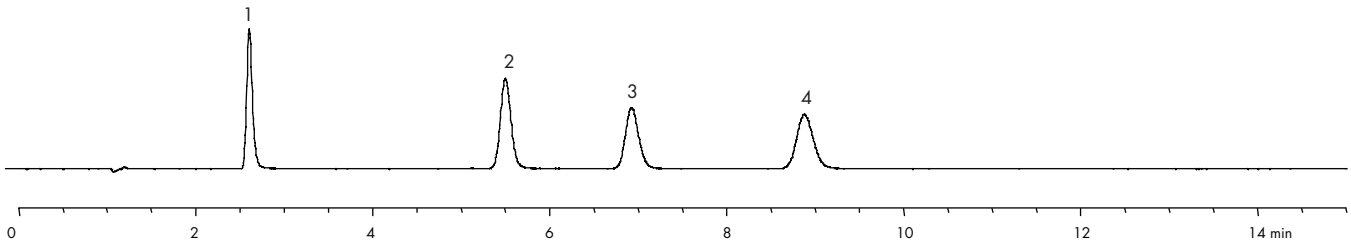
2. Imipramine



3. Amitriptyline



4. Clomipramine



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For complete experimental details, refer to full application note XBRIDGE3 at waters.com

Analysis of Tricyclic Antidepressants at pH 10.5

EXPERIMENTAL

LC conditions

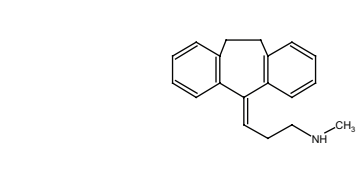
System:	Alliance 2695 with 2996 PDA detector
Column:	XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm
Mobile phase:	10 mM ammonium bicarbonate buffer, pH 10.5/acetonitrile (25:75)
Separation mode:	Isocratic
Flow rate:	1.5 mL/min
Column temp.:	25 ˚C
Injection volume:	10 µL
UV detection:	254 nm

Sample preparation

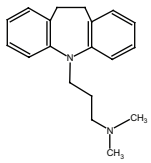
Sample concentration: 20 µg/mL in water

ORDERING INFORMATION

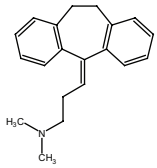
Description	P/N
XBridge BEH C_{18}, 5 µm, 4.6 x 150 mm Column	186003116
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



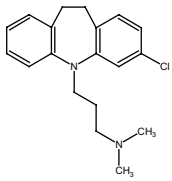
1. Nortriptyline



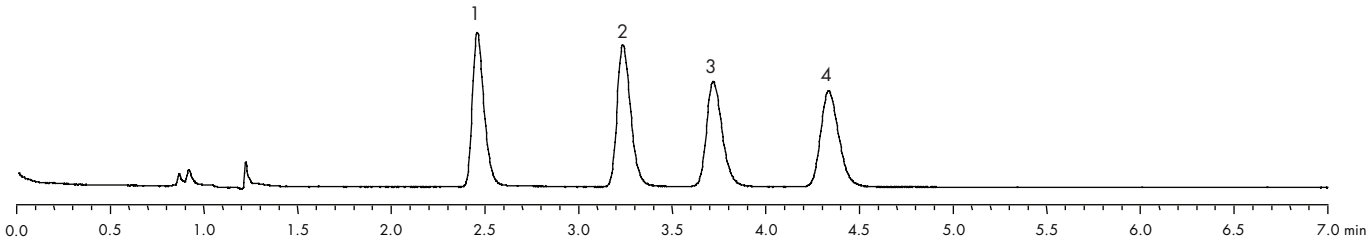
2. Imipramine



3. Amitriptyline



4. Clomipramine



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For complete experimental details, refer to full application note 720005567EN at waters.com

Analysis of UPC² Gradient Standard by HPLC

EXPERIMENTAL

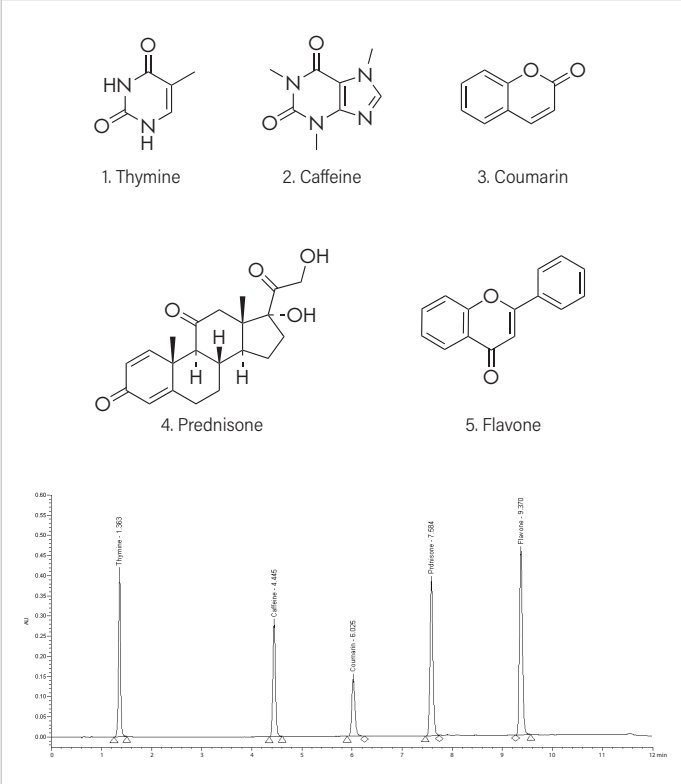
LC conditions

System:	ACQUITY Arc with 2998 PDA detector
Column:	XBridge BEH C_{18}, 5 µm, 4.6 x 50 mm
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol
Gradient:	5 to 80% B over 9 min
Flow rate:	1.0 mL/min
Column temp.:	30 °C
Injection volume:	5.0 µL
UV detection:	254 nm

Sample preparation

Combine 100 µL of the UPC² Gradient Standard with 900 µL of the 50/50 water/methanol solution.

Sample temp.:	10 °C



ORDERING INFORMATION

Description	P/N
XBridge BEH C_{18}, 5 µm, 4.6 x 50 mm Column	186003113
UPC² Gradient Standard	186006551
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

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For complete experimental details, refer to full application note 720005567EN at waters.com

Analysis of UPC² Gradient Standard by UHPLC

EXPERIMENTAL

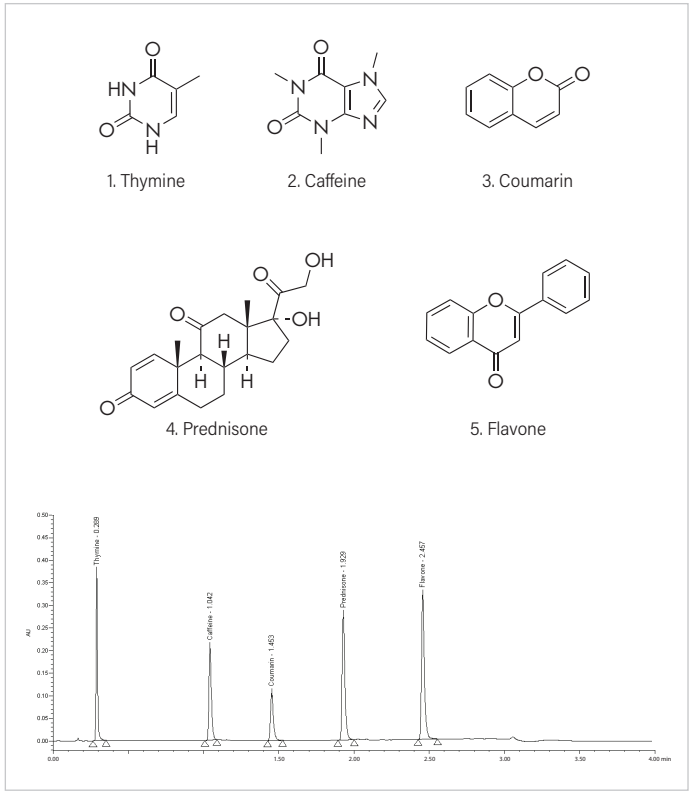
LC conditions

System:	ACQUITY Arc with 2998 PDA detector
Column:	CORTECS C_{18}, 2.7 µm, 3 x 50 mm
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol
Gradient:	5 to 80% B over 2.5 min
Flow rate:	1.5 mL/min
Column temp.:	30 °C
Injection volume:	2.1 µL
UV detection:	254 nm

Sample preparation

Combine 100 µL of the UPC² Gradient Standard with 900 µL of the 50/50 water/methanol solution.

Sample temp.:	10 °C



ORDERING INFORMATION

Description	P/N
CORTECS C_{18}, 2.7 µm, 3.0 x 50 mm Column	186007370
UPC² Gradient Standard	186006551
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV

For complete experimental details, refer to full application note WA64117 at waters.com

Analysis of Uric Acids

EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2998 PDA detector
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	50/50 acetonitrile/water with 10 mM ammonium acetate, pH 5.0
Mobile phase B:	90/10 acetonitrile/water with 10 mM ammonium acetate, pH 5.0

Gradient:

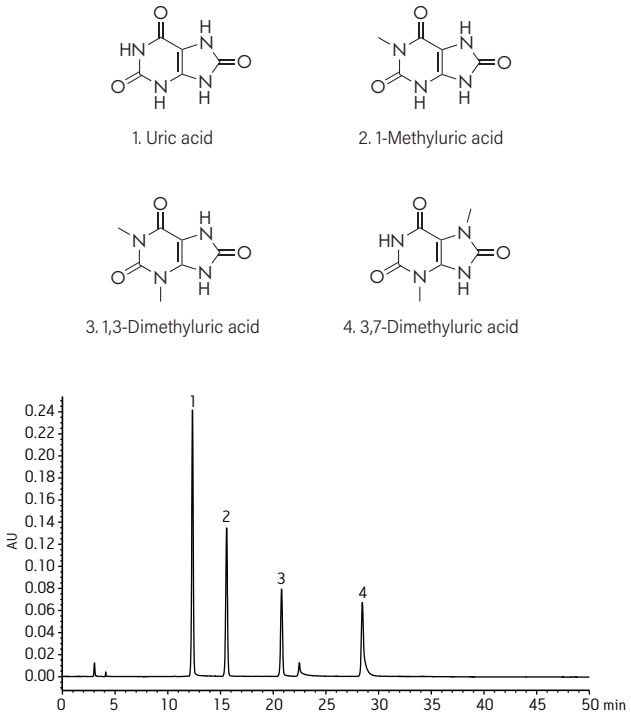
Time	%A	%B
0.00	0.1	99.9
50.00	50	50.0
50.10	0.1	99.9
60.00	0.1	99.9

Flow rate:	1.2 mL/min
Column temp.:	25 °C
Injection volume:	60.0 µL
UV detection:	285 nm

Sample preparation

Sample was diluted with 50:50 acetonitrile/water and filtered using 0.45 µm PVDF syringe filter.

Sample concentration: 10 µg/mL



ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

For complete experimental details, refer to full application note WA64120 at waters.com

Analysis of Water-Soluble Vitamins

EXPERIMENTAL

LC conditions

System:	Alliance HPLC with 2998 PDA detector
Column:	XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm
Mobile phase A:	50/50 acetonitrile/water with 10 mM ammonium acetate, pH 9.0
Mobile phase B:	90/10 acetonitrile/water with 10 mM ammonium acetate, pH 9.0

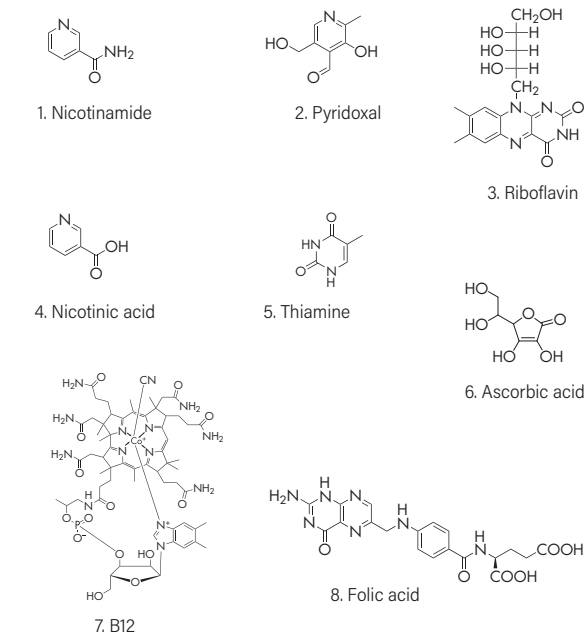
Gradient:

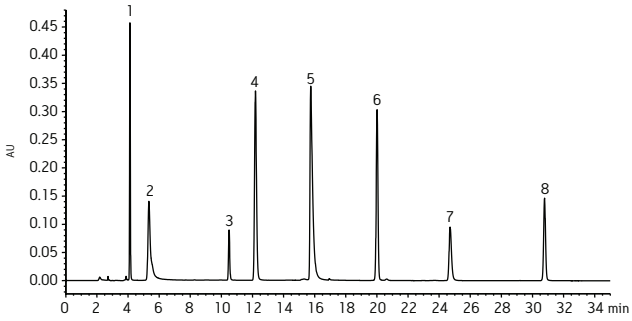
Time	%A	%B
0.00	0.1	99.9
35.00	70	30.0
35.10	0.1	99.9
45.00	0.1	99.9

Flow rate:	1.2 mL/min
Column temp.:	30 °C
Injection volume:	60.0 µL
UV detection:	265 nm

Sample preparation

Add 25 mL of 50:50 acetonitrile/water to ~3 g sample, homogenize, centrifuge at 3200 rpm for 30 minutes, collect supernatant and filter using 0.45 µm PVDF syringe filter.





ORDERING INFORMATION

Description	P/N
XBridge BEH Amide, 3.5 µm, 4.6 x 250 mm Column	186004870
Acrodisc, Syringe Filter, PVDF, 13 mm, 0.45 µm, 100/pk	WAT200512
Waters LCMS Certified Vial	600000751CV

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For complete experimental details, refer to full application note 720005157EN at waters.com

Analysis of Zidovudine

EXPERIMENTAL

LC conditions

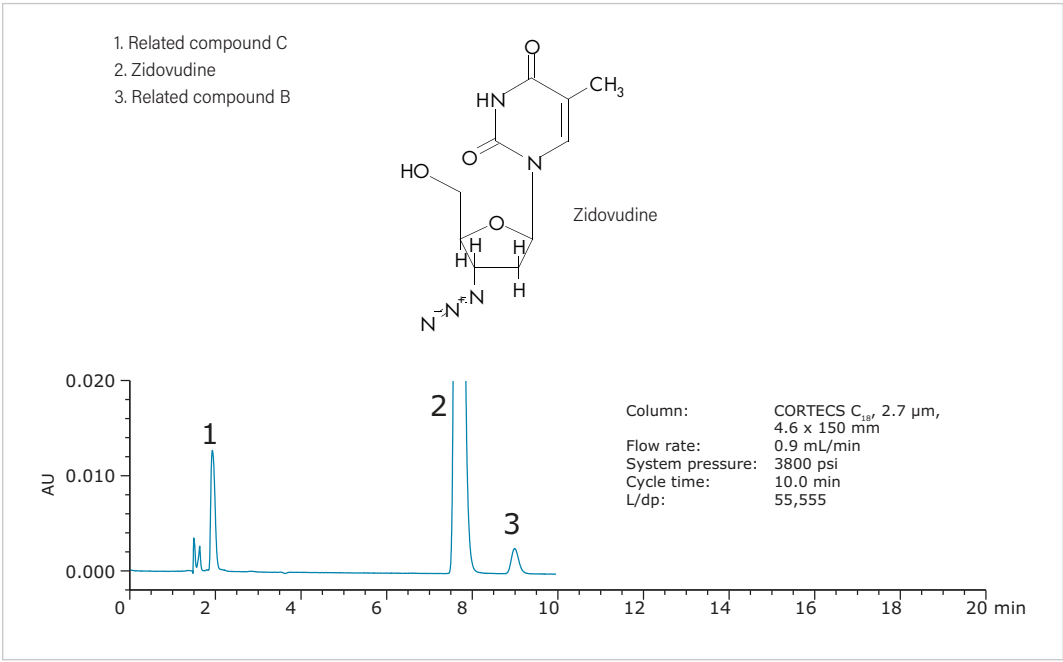
System:	Alliance HPLC
Column:	CORTECS C_{18}, 2.7 µm, 4.6 x 150 mm
Mobile phase:	80:20 water:methanol
Separation mode:	Isocratic
Flow rate:	0.9 mL/min
Column temp.:	30 °C
Injection volume:	6.0 µL
UV detection:	265 nm

Sample preparation

Zidovudine and its related compounds B and C reference standards were purchased from the USP. A sample containing 1.0 mg/mL zidovudine, 1.0 µg/mL related compound B, and 2.0 µg/mL related compound C was created in methanol and placed into a Waters LCMS Certified Max Recovery Vial injection.

ORDERING INFORMATION

Description	P/N
CORTECS C_{18}, 2.7 µm, 4.6 x 150 mm Column	186007378
Neutrals QC Reference Material	186006360
Waters LCMS Certified Max Recovery Vial	600000749CV



For complete experimental details, refer to full application note 720004079EN at waters.com

Analysis of Ziprasidone HCl

EXPERIMENTAL

LC conditions

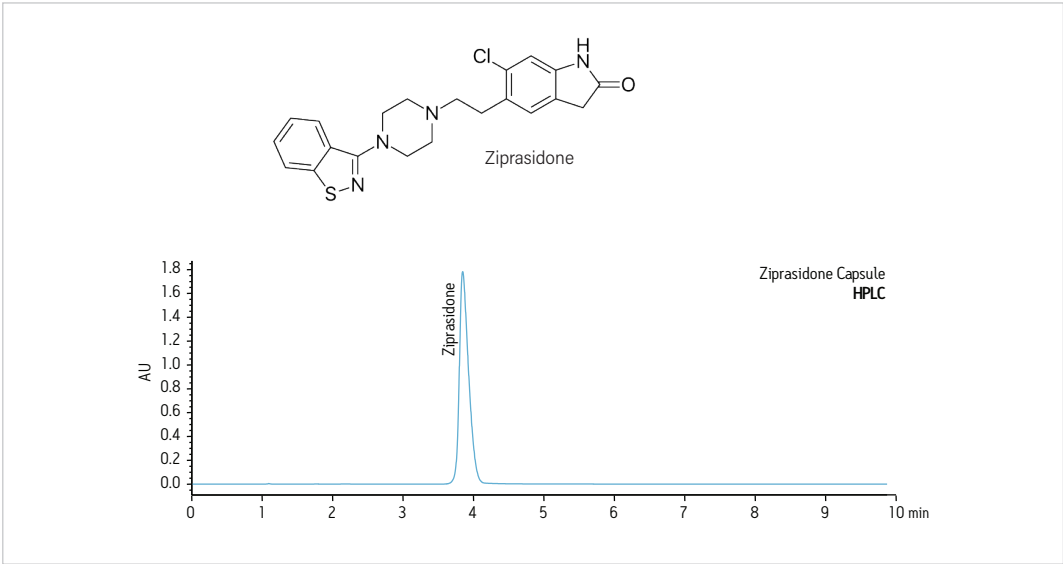
System:	Alliance 2695
Column:	XBridge BEH C_8, 5 µm, 4.6 x 150 mm
Mobile phase:	60:40 buffer:methanol
Buffer:	25 mM potassium phosphate, monobasic in water, pH 3.0 with potassium hydroxide
Separation mode:	Isocratic
Flow rate:	1.5 mL/min
Column temp.:	40 °C
Injection volume:	20 µL
UV detection:	229 nm

Sample preparation

Ziprasidone capsules, contents made up to 0.23 mg/mL in diluent (60:40 methanol:water). Ziprasidone standard, made up to 0.23 mg/mL in diluent. Samples were filtered through a 0.2 µm PTFE membrane prior to analysis.

ORDERING INFORMATION

Description	P/N
XBridge BEH C_8, 5 µm, 4.6 x 150 mm Column	186003017
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



For complete experimental details, refer to full application note 720004697EN at waters.com

Analysis of Ziprasidone HCl - Early-Eluting Impurities

EXPERIMENTAL

LC conditions

System:	Alliance 2695 with 2489 UV/Visible detector
Column:	XBridge BEH C_8, 5 µm, 4.6 x 150 mm
Mobile phase:	2:3 methanol/buffer
Buffer:	50 mM potassium phosphate monobasic, pH 3.0 adjusted with phosphoric acid
Separation mode:	Isocratic
Flow rate:	1.5 mL/min
Column temp.:	40 °C
Injection volume:	20 µL
UV detection:	229 nm

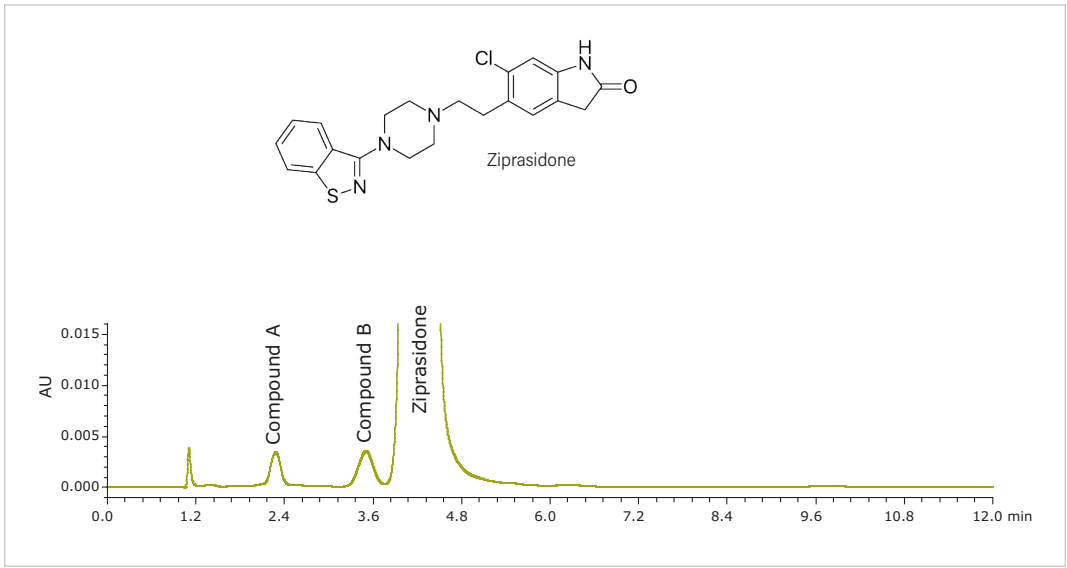
Sample preparation

All solutions were prepared in methanol/water/concentrated HCl at a composition of 20:5:0.01 to comply with the impurities methods defined in the USP Monograph for Ziprasidone HCl.

Sample temp.: 10 °C

ORDERING INFORMATION

Description	P/N
XBridge BEH C_8, 5 µm, 4.6 x 150 mm Column	186003017
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



For complete experimental details, refer to full application note 720004697EN at waters.com

Analysis of Ziprasidone HCl - Late-Eluting Impurities

EXPERIMENTAL

LC conditions

System:	Alliance 2695 with 2489 UV/Visible detector
Column:	XBridge BEH C_8, 5 µm, 4.6 x 150 mm
Mobile phase:	11:1:8 acetonitrile/methanol/buffer
Buffer:	50 mM potassium phosphate monobasic, pH 6.0 adjusted with 5N potassium hydroxide
Separation mode:	Isocratic
Flow rate:	1.0 mL/min
Column temp.:	35 °C
Injection volume:	20 µL
UV detection:	229 nm

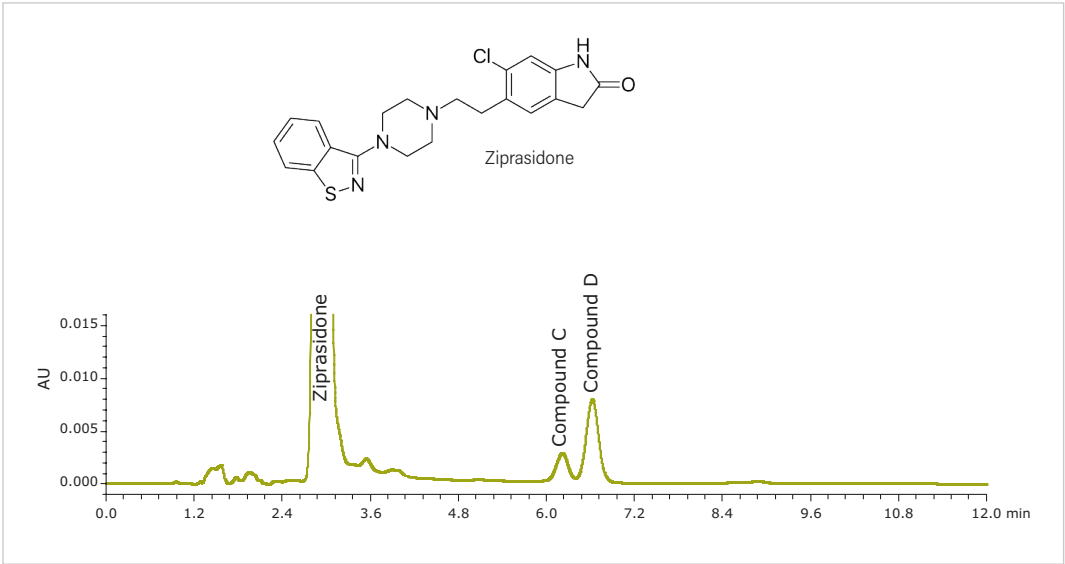
Sample preparation

All solutions were prepared in methanol/water/concentrated HCl at a composition of 20:5:0.01 to comply with the impurities methods defined in the USP Monograph for Ziprasidone HCl.

Sample temp.: 10 °C

ORDERING INFORMATION

Description	P/N
XBridge BEH C_8, 5 µm, 4.6 x 150 mm Column	186003017
TruView LCMS Certified Vial w/ Preslit Septa	186005666CV



Compound Index

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