macmod

SMARTER CHROMATOGRAPHY

FOOD & BEVERAGE GUIDE

INFORMATION & RESEARCH COLLECTED IN COLLABORATION WITH:

avantor™ | advancedmaterialstechnology

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Analysis of Whiskey via Direct Split Injection — GC

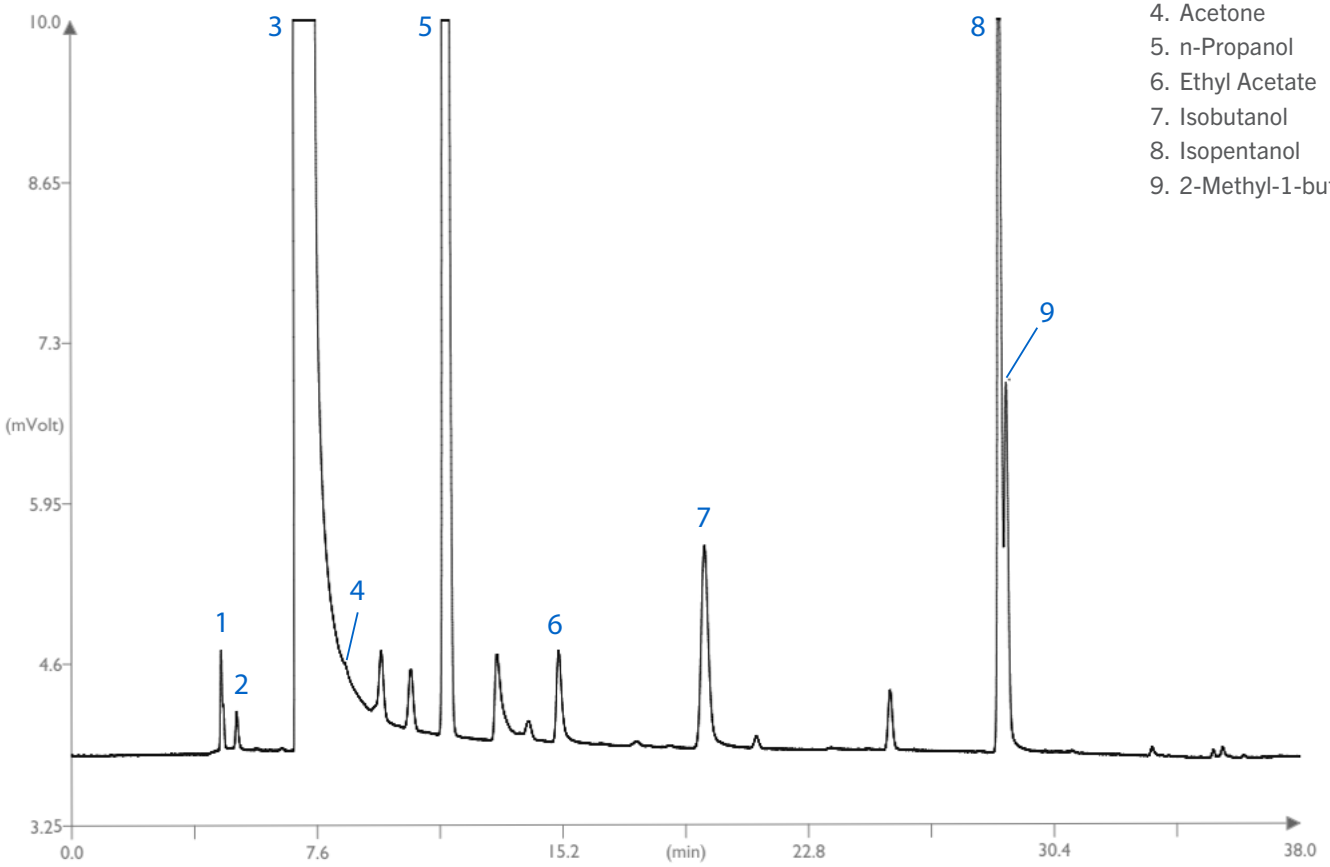
TEST CONDITIONS

Column:	Avantor® Hichrom HI-624, 1.4 µm, 0.25 mm x 60 m
Part Number:	HI082514060
Oven Program:	35 °C (5 min), 1 °C /min, 50 °C, 5 °C/min
Carrier Gas:	Hydrogen, 120 kPa
Injector:	Split, 200 °C, 120 mL/min split flow, 0.5 µL injected volume
Detector:	FID, 250 °C

The HI-624 is a versatile general-purpose column for a wide range of applications, including residual solvents and volatile organic compounds. It is a mid-polarity phase with a 6% cyanopropylphenyl, 94% methyl polysiloxane composition and is classified as USP G43.

ANALYTES

1. Acetaldehyde
2. Methanol
3. Ethanol
4. Acetone
5. n-Propanol
6. Ethyl Acetate
7. Isobutanol
8. Isopentanol
9. 2-Methyl-1-butanol



Beer Fermentation Analysis — HPLC/UHPLC

TEST CONDITIONS

Column:	HALO® Penta-HILIC, 90 Å, 2.7 µm, 3.0 x 50 mm
Part Number:	92813-405
Mobile Phase A:	Water
Mobile Phase B:	Acetonitrile

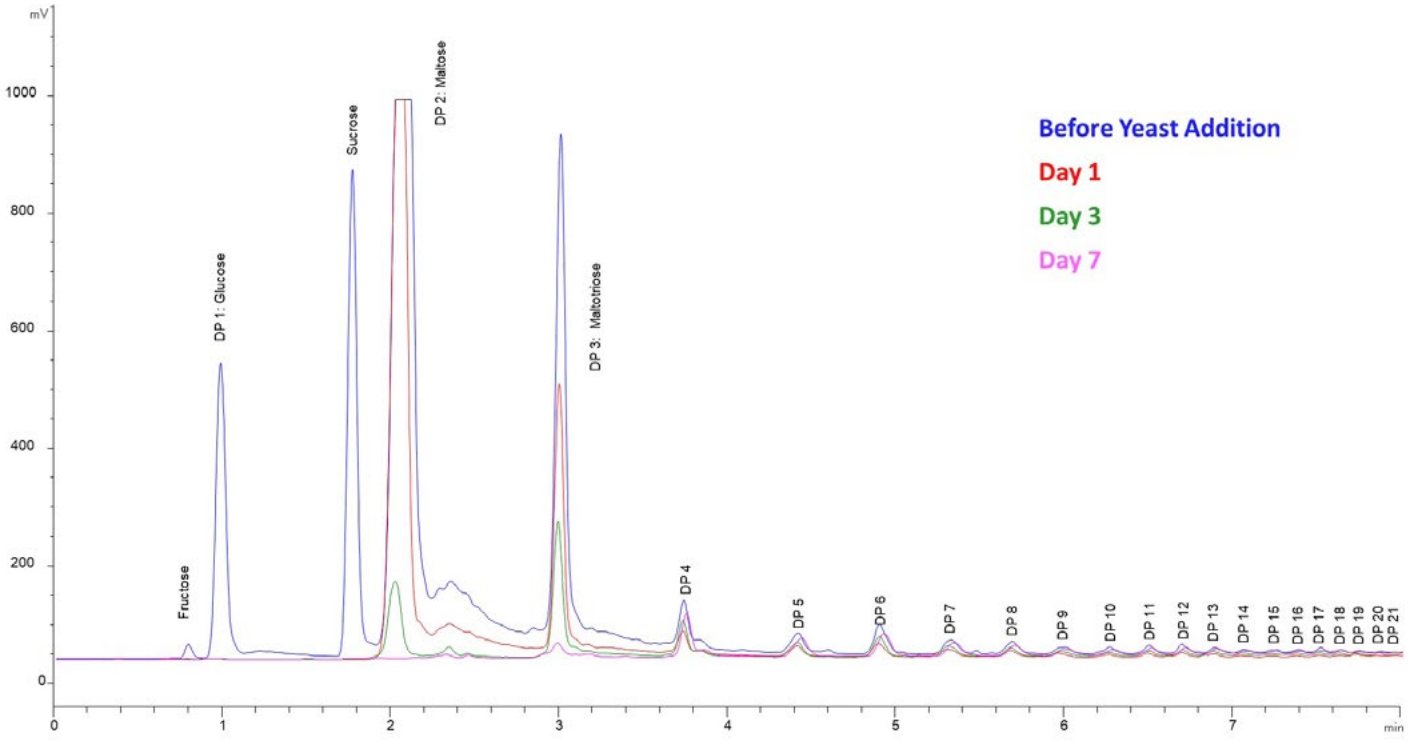
Gradient:

Time (min)	%B
0.0	92
8.0	52

Flow Rate:	0.75 mL/min
Temperature:	65 °C
Detection:	ELSD, 40 °C, 45 psi
Injection Volume:	2 µL
Data Rate:	10 Hz, 2 sec filter

Data Courtesy of Merlin K. L. Bicking, Ph. D. (ACCTA, Inc.)

A Belgian ale is analyzed with a HALO® Penta-HILIC column using an evaporative light scattering detector (ELSD). Sugars, oligosaccharides, and polysaccharide levels are monitored throughout the fermentation process in order to track yeast behavior. These levels will decrease over time as the yeast converts the sugars to ethanol.The Penta-HILIC/ELSD combination is a great way to perform rapid sugar analysis providing high resolution and good peak shape at elevated temperatures.



Amino Acids in Peas (Pisum sativum) by HPLC-HRAM-MS — LC-MS

TEST CONDITIONS

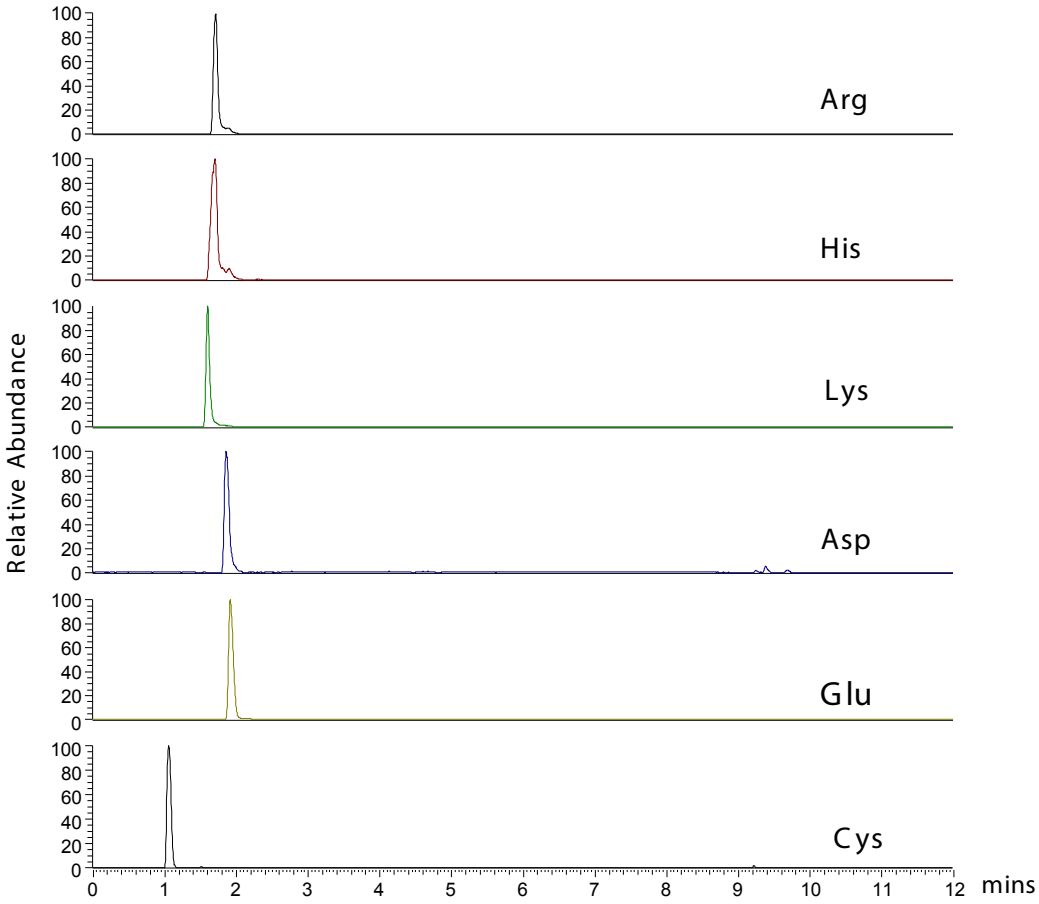
Column: Avantor® ACE® AQ, 100 Å, 3 µm, 3.0 x 150 mm
Part Number: ACE1161503
Mobile Phase: A: 0.1% Formic acid in H_2O
B: 0.1% Formic acid in MeCN

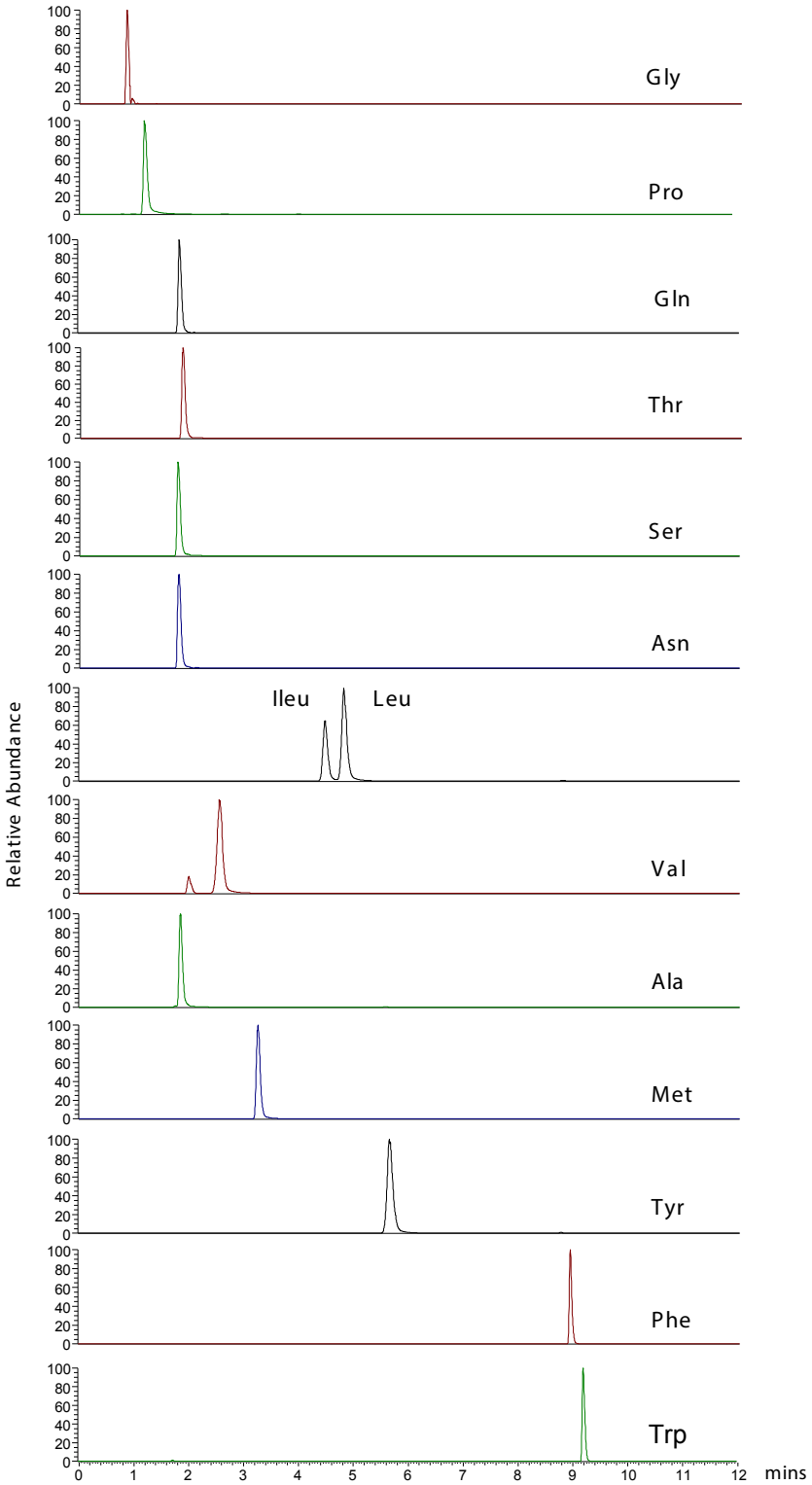
Gradient:

Time (min)	%B
0	0
10	100

Flow Rate: 0.4 mL/min
Temperature: 30 °C
Injection: 5 µL
Detection: Exactive Orbitrap high resolution MS
ESI positive ion mode
Capillary temperature: 350 °C

Reproduced with permission of Fera Science Ltd, York, UK




Relative Abundance
Gly
Pro
Gln
Thr
Ser
Asn
Ileu
Leu
Val
Ala
Met
Tyr
Phe
Trp
mins

Free Acids and Lactic Acid — GC

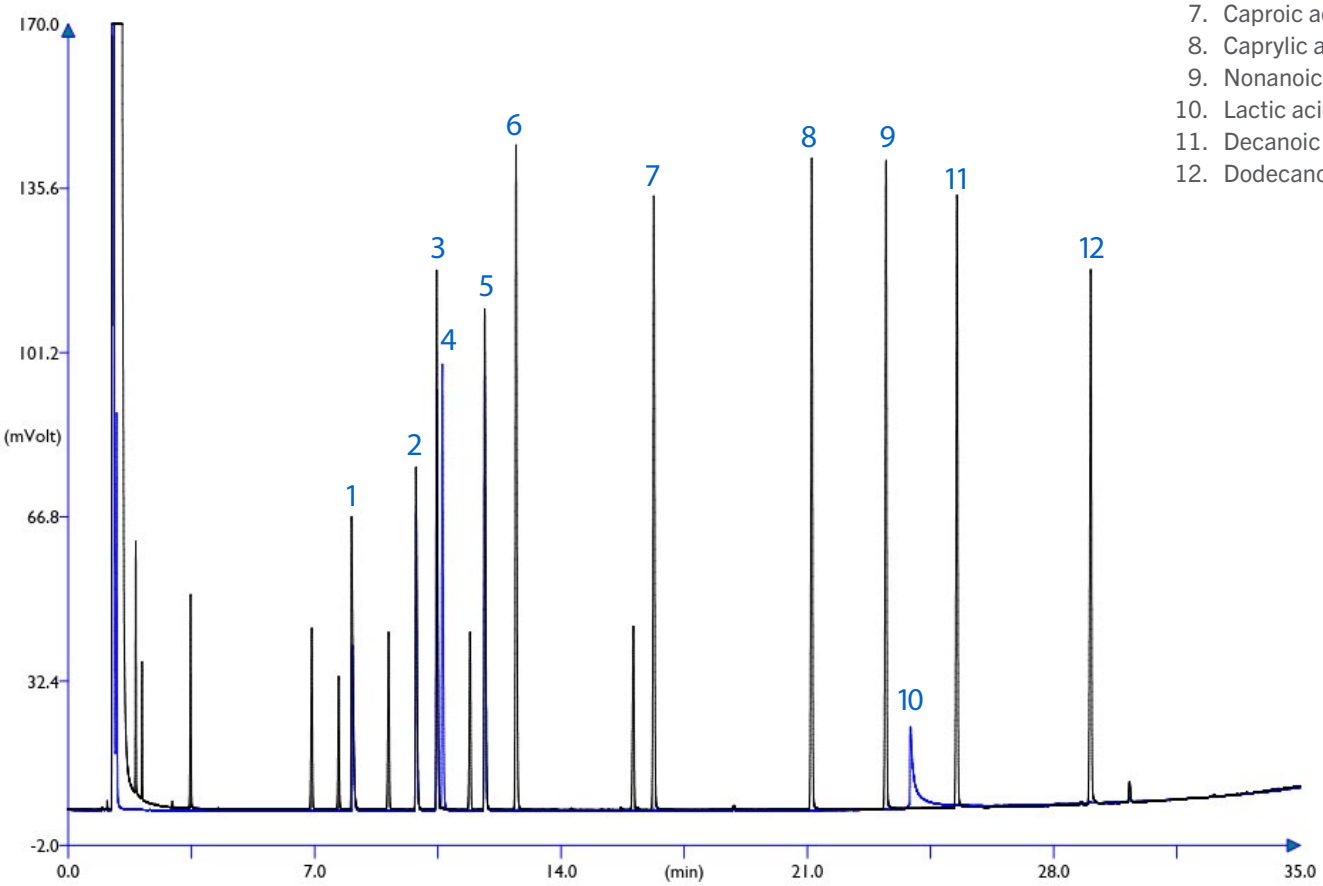
TEST CONDITIONS

Column:	Avantor® Hichrom HI-FFAP EXT, 0.5 µm, 0.32 mm x 30 m
Part Number:	HI443205030
Oven Program:	70 °C (0.5 min), 5 °C/min, 242.5 °C
Carrier Gas:	Helium, 90 kPa
Injector:	Split, 250 °C
Detector:	FID, 250 °C

The HI-FFAP EXT is a nitroterephthalic acid modified polyethylene glycol (PEG) phase of high polarity, with an extended temperature range and aqueous sample compatibility. Useful for the analysis of free acids, flavor compounds, alcohols and polar compounds.

ANALYTES

1. Acetic acid
2. Propionic acid
3. Iso-butyric acid
4. Pivalic acid
5. Butyric acid
6. Iso-valeric acid
7. Caproic acid
8. Caprylic acid
9. Nonanoic acid
10. Lactic acid
11. Decanoic acid
12. Dodecanoic acid



Hop Acids — HPLC

Hops are primarily made up of essential oils and alpha and beta acids. They have many benefits in the beer brewing process, including their antiseptic nature and bitterness flavor they give to the beer. Alpha and beta acids from the International Calibration Standard Extract (ICE-3) are separated on a HALO® Biphenyl column.

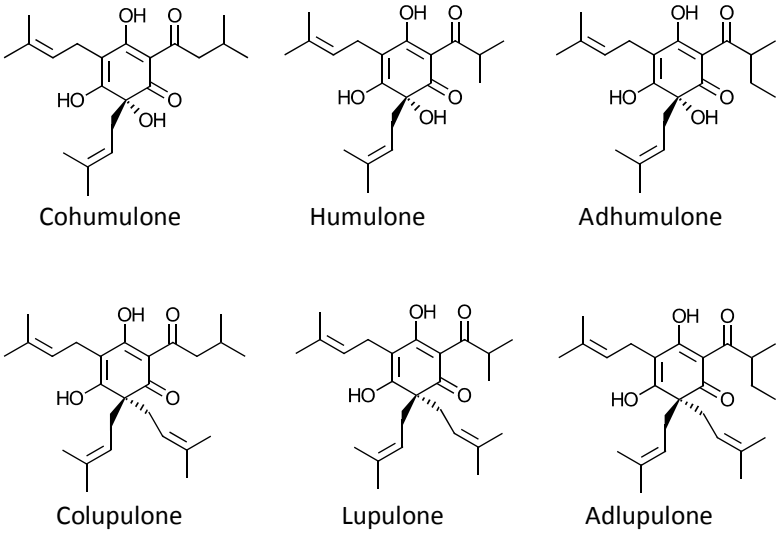
TEST CONDITIONS

Columns:	HALO® Biphenyl, 90 Å, 5 µm, 4.6 x 150 mm
Part Number:	95814-711
Mobile Phase	A: Water, 0.1% Formic acid
Mobile Phase	B: Acetonitrile, 0.1% Formic acid
Gradient:	

Time (min)	%B
0.0	60
3.0	60
6.0	80

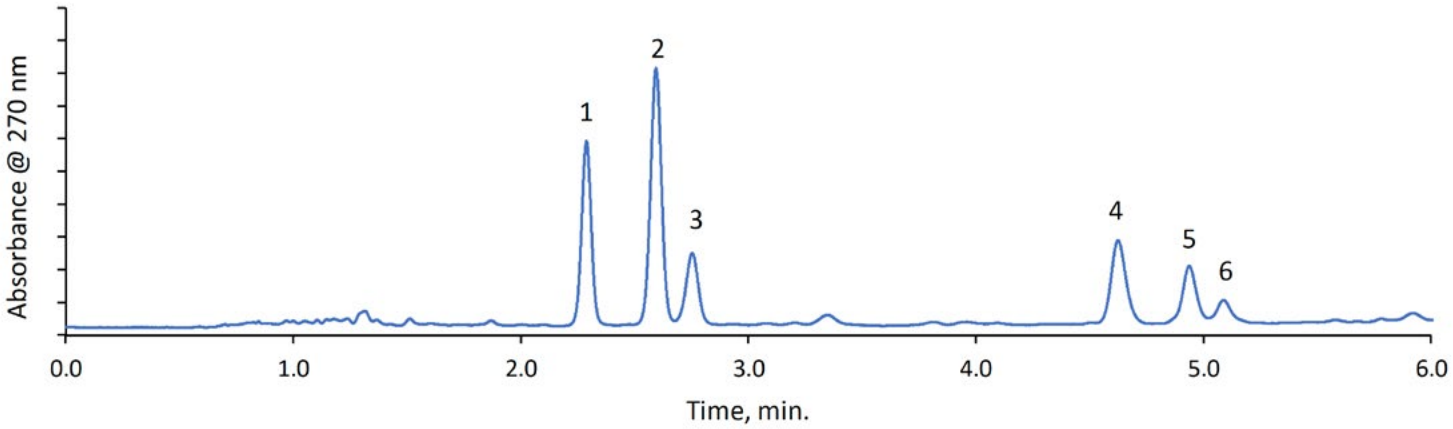
Flow Rate:	2.0 mL/min
Initial Pressure:	236 bar
Temperature:	30 °C
Detection:	270 nm, PDA
Injection Volume:	5 µL
Sample Solvent:	Acetonitrile
Data Rate:	100 Hz
Response Time:	0.025 sec
Flow Cell:	1 µL
LC System:	Shimadzu Nexera X2

STRUCTURES



ANALYTES

Alpha acids	Beta acids
1. Cohumulone	4. Colupulone
2. Humulone	5. Lupulone
3. Adhumulone	6. Adlupulone



LC-MS Separation of Corn Oil — LC-MS

LC TEST CONDITIONS

Columns: HALO® C18, 90 Å, 2.7 µm, 2.1 x 150 mm
Part Number: 92812-702
Columns: HALO® C30, 160 Å, 2.7 µm, 2.1 x 150 mm
Part Number: 92112-730
Mobile Phase A: Methanol
Mobile Phase B: IPA/0.1% Formic acid

Gradient:

Time (min)	%B
0.00	10
10.00	10
14.00	40
22.00	40
22.01	10
24.00	END

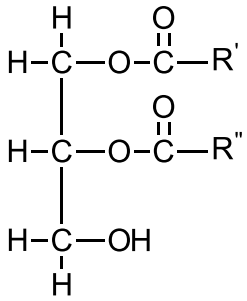
Flow Rate: 0.3 mL/min
Initial Pressure: 325 bar
Temperature: Ambient
Injection Volume: 2 µL
Sample Solvent: MeOH
LC System: Shimadzu Nexera X2

Corn oil, composed mainly of long chain fatty acids and esters, is an edible oil which comprises approximately 5-10% of edible oil consumption. In recent years, corn oil has been used in biodiesel, pharmaceutical, and cosmetic applications as well. The use of a C18 column for the analysis of edible oils is difficult due to the high concentration of hydrophobic triglycerides (TAGs); therefore, the C30 phase has seen increased application in this area. Here we show a comparison between the C18 and C30 phase, and demonstrate that the 2.7 µm HALO® C30 is an ideal choice for the separation and resolution of high mass triglycerides found in edible oils such as corn oil. C30 offers superior specificity compared to C18 columns by exhibiting higher shape selectivity, enabling better separation of hydrophobic, long-chain, structures.

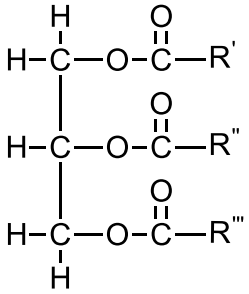
MS TEST CONDITIONS

MS system: Shimadzu LCMS-2020
Ionization: +ESI
Spray voltage: 4.50 kV
Drying line temp: 300 °C
Heat Block: 450 °C

STRUCTURES



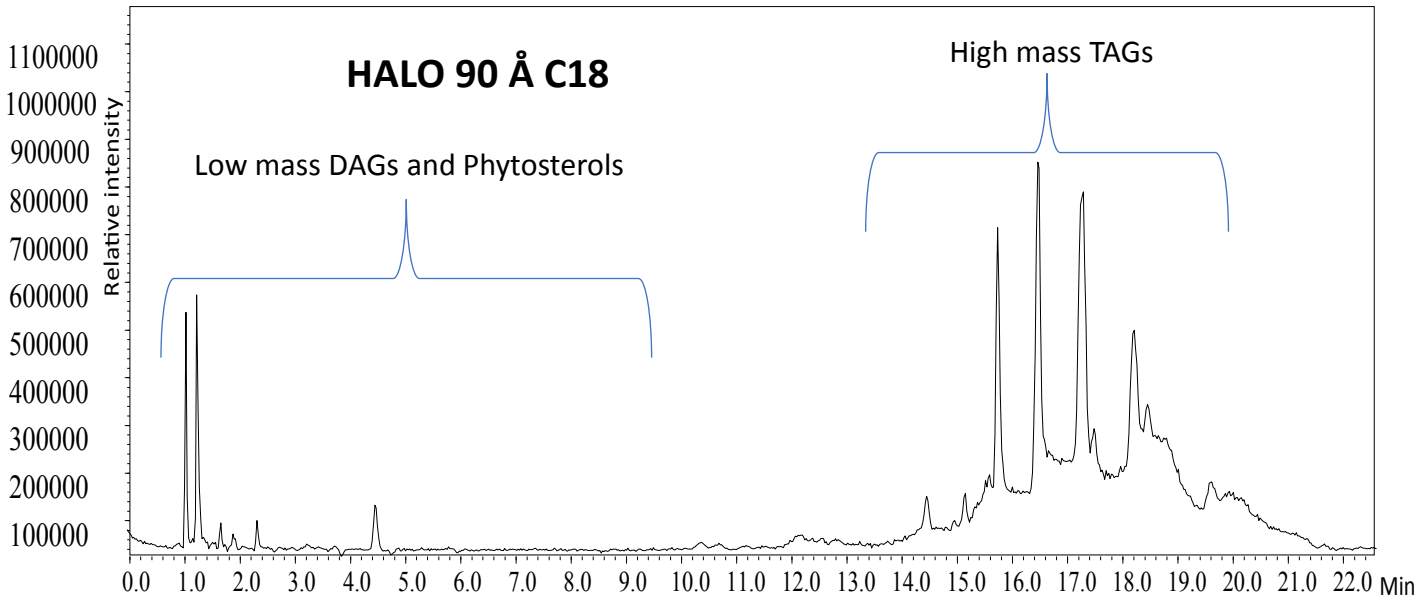
DAGs

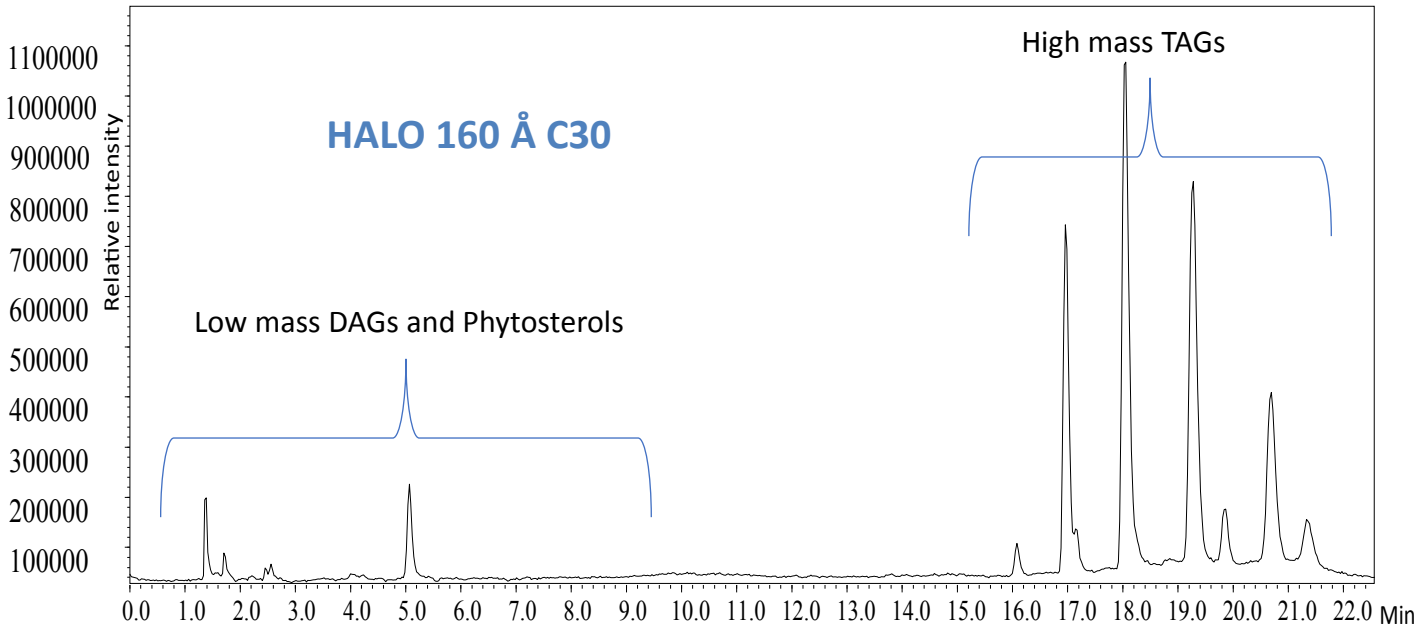


TAGs

LC-MS Separation of Corn Oil

(continued)





DAGs = diacylglycerols or diglycerides
TAGs = triacylglycerols or triglycerides

Organic Acids (I) — HPLC

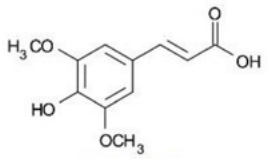
TEST CONDITIONS

Column:	Avantor® ACE® Excel C18-Amide, 100 Å, 3 µm, 2.1 x 250 mm
Part Number:	EXL11122502U
Mobile Phase:	20 mM H_3PO_4 in MeOH/H_2O (40:60 v/v)
Flow Rate:	0.21 mL/min
Temperature:	20 °C
Injection Volume:	5 µL
Detection:	UV, 210 nm

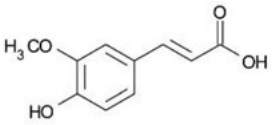
ANALYTES

1. Sinapic acid
2. Ferulic acid
3. 3,4-Dimethoxycinnamic acid
4. Cinnamic acid
5. 4-Methoxycinnamic acid

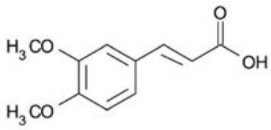
STRUCTURES



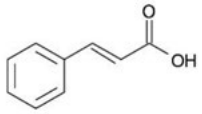
Sinapic acid



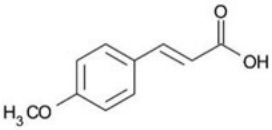
Ferulic acid



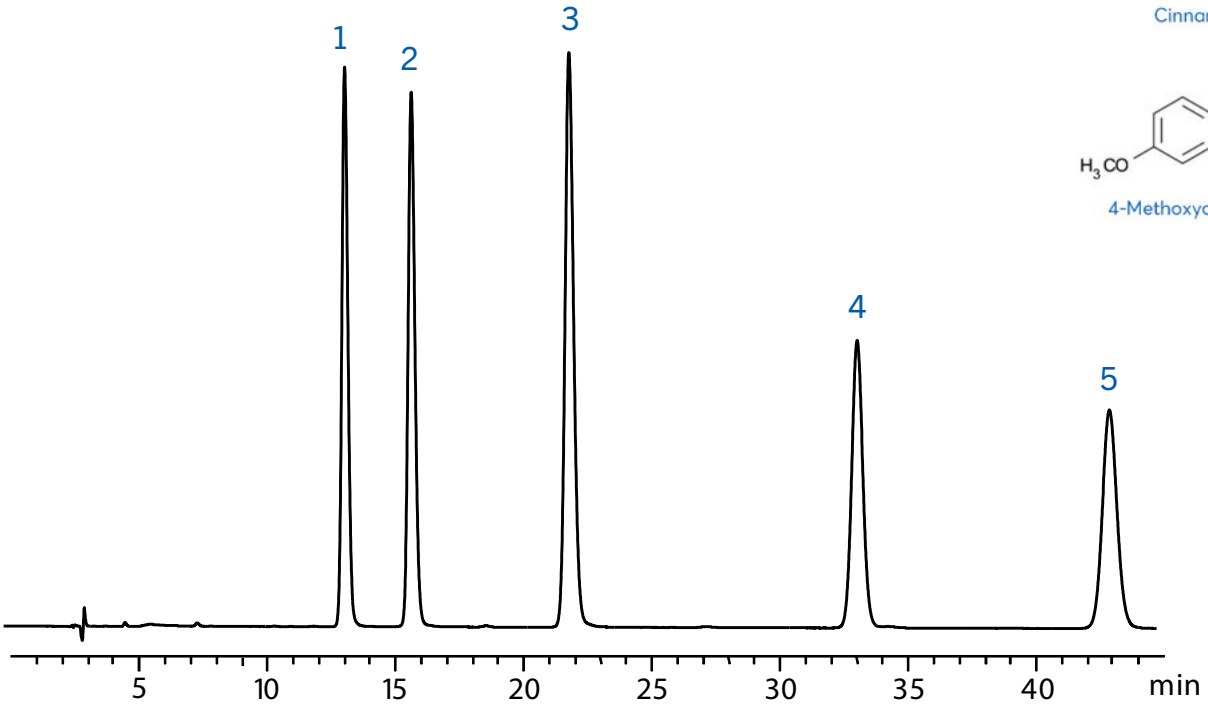
3,4-Dimethoxycinnamic acid



Cinnamic acid



4-Methoxycinnamic acid



Phenolic Acids on a 2 µm RP-Amide Column — UHPLC

TEST CONDITIONS

Column: HALO® RP-Amide, 90 Å, 2 µm, 2.1 x 100 mm
Part Number: 91812-607
Mobile Phase A: 20mM Phosphoric acid
Mobile Phase B: Methanol
Gradient:

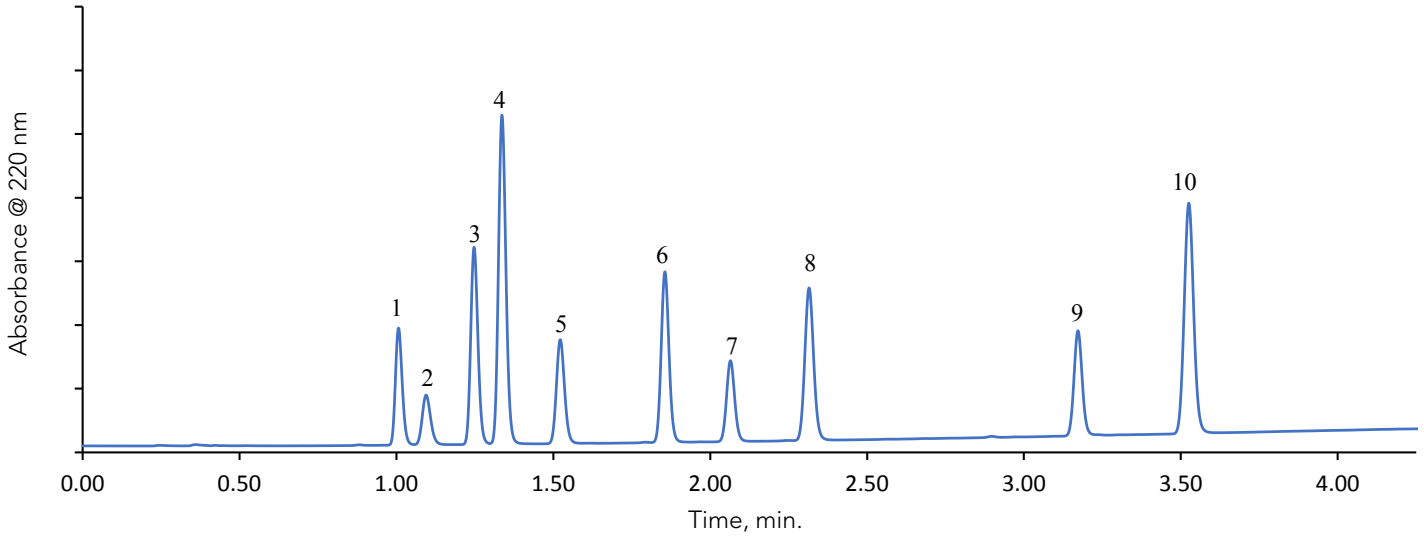
Time (min)	%B
0.00	30
3.75	60
4.25	60

Flow Rate: 0.5 mL/min
Initial Pressure: 716 bar
Temperature: 35 °C
Detection: UV 220 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: Methanol
Data Rate: 40 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

Phenolic acids can be found in many plant-based foods and beverages. Fruits, vegetables, and even olive oils all contain different varieties of these acids. For example, sinapic acid can be found in wine and caffeic acid can be found in coffee. These compounds act as antioxidants and can also help anti-inflammatory conditions in the body. They also affect the flavors of the food or oil.

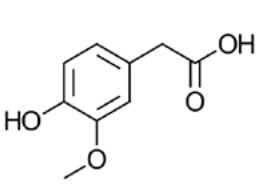
ANALYTES

1. Homovanillic acid
2. Caffeic acid
3. Syringic acid
4. Vanillic acid
5. Chlorogenic acid
6. Sinapic acid
7. Ferulic acid
8. *p*- Coumaric acid
9. *trans*- Cinnamic acid

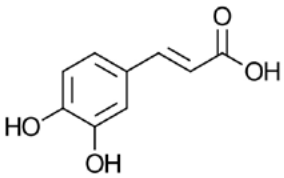


Phenolic Acids on a 2 µm RP-Amide Column *(continued)*

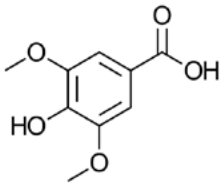
STRUCTURES OF PHENOLIC ACIDS



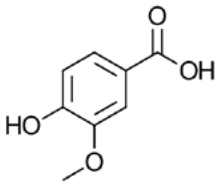
Homovanillic acid



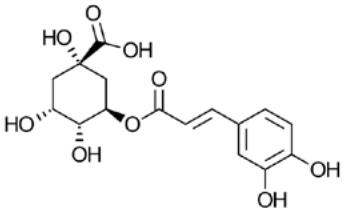
Caffeic acid



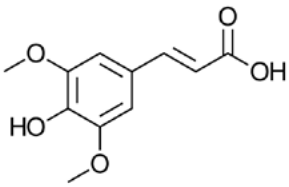
Syringic acid



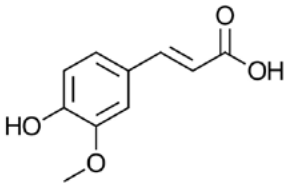
Vanillic acid



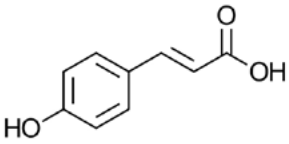
Chlorogenic acid



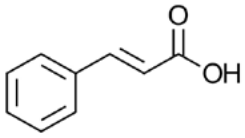
Sinapic acid



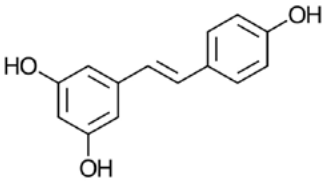
Ferulic acid



p- Coumaric acid



trans- Cinnamic acid



Resveratrol

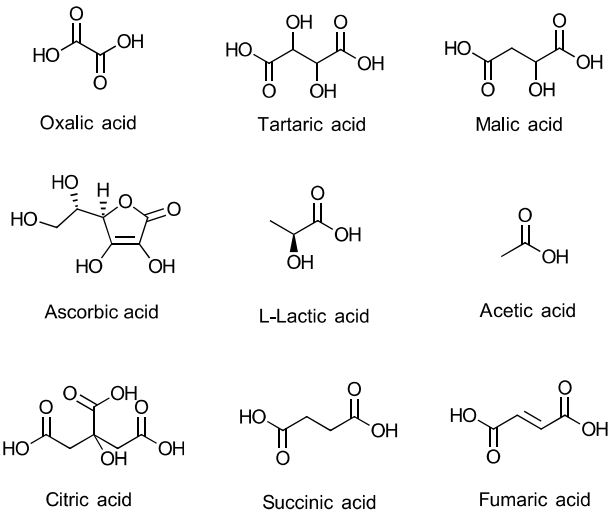
Polar Organic Acids on an AQ-C18 Column — HPLC/UHPLC

TEST CONDITIONS

Column:	HALO® AQ-C18, 90 Å, 2.7 µm, 4.6 x 250 mm
Part Number:	92814-922
Isocratic:	20 mM Potassium phosphate buffer pH: 2.7
Flow Rate:	1.0 mL/min
Pressure:	307 bar
Temperature:	40 °C
Detection:	UV 214 nm, PDA
Injection Volume:	20 µL
Sample Solvent:	Mobile phase
Data Rate:	100 Hz
Response Time:	0.025 sec
Flow Cell:	1 µL
LC System:	Shimadzu Nexera X2

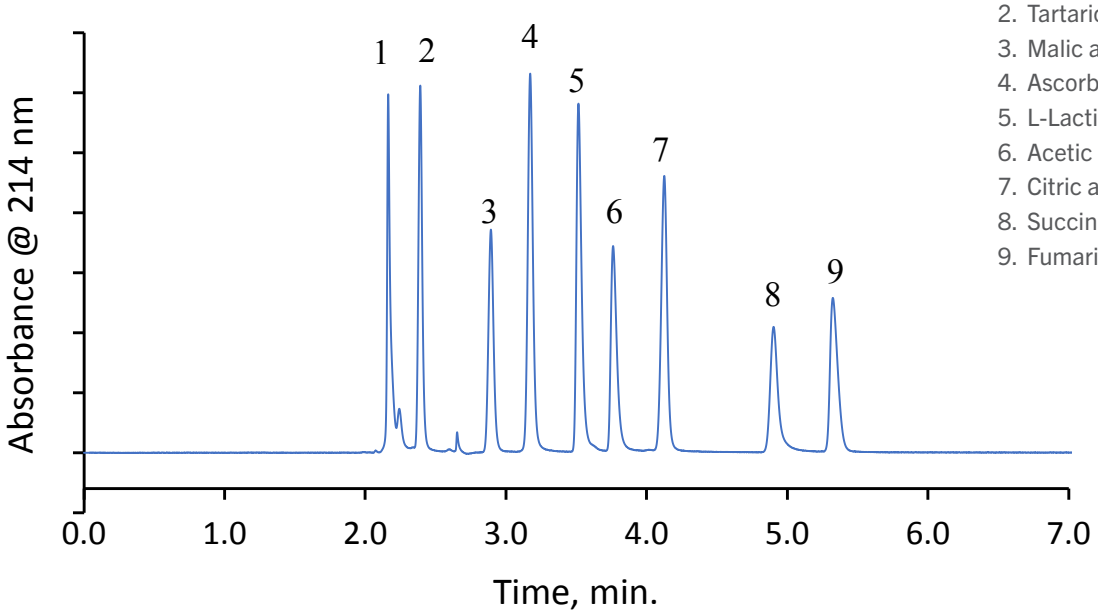
Organic acids are common in the food and beverage industry and can be found in many sample types such as fruits, vegetables, and wines. This separation of nine polar organic acids is performed on a HALO® AQ-C18 column using 100% aqueous mobile phase at low pH. The 250 mm column length was chosen to provide excellent resolution with reasonable run time for this polar mixture.

STRUCTURES



ANALYTES

1. Oxalic acid
2. Tartaric acid
3. Malic acid
4. Ascorbic acid
5. L-Lactic acid
6. Acetic acid
7. Citric acid
8. Succinic acid
9. Fumaric acid

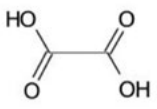


Wine Acid Analysis — HPLC

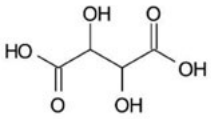
TEST CONDITIONS

Column:	Avantor® ACE® Excel C18-Amide, 100 Å, 3 µm, 2.1 x 250 mm
Part Number:	EXL11122502U
Mobile Phase:	40 mM Ammonium phosphate pH 2.5 in H_2O
Flow Rate:	0.21 mL/min
Temperature:	25 °C
Injection:	5 µL
Detection:	UV, 214 nm

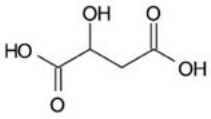
STRUCTURES



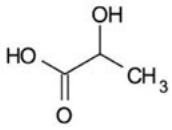
Oxalic acid



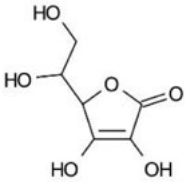
Tartaric acid



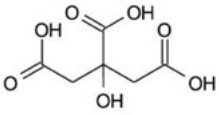
Malic acid



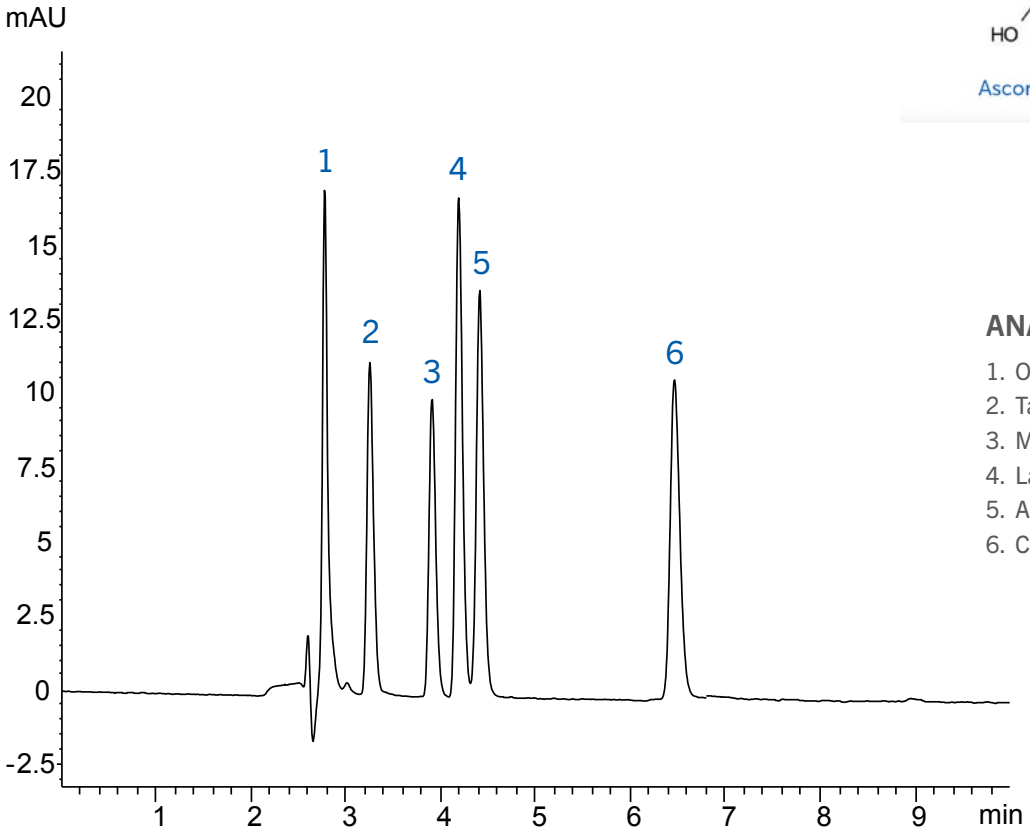
Lactic acid



Ascorbic acid



Citric acid



ANALYTES

1. Oxalic acid
2. Tartaric acid
3. Malic acid
4. Lactic acid
5. Ascorbic acid
6. Citric acid

Anthocyanins in Blueberries — HPLC/UHPLC

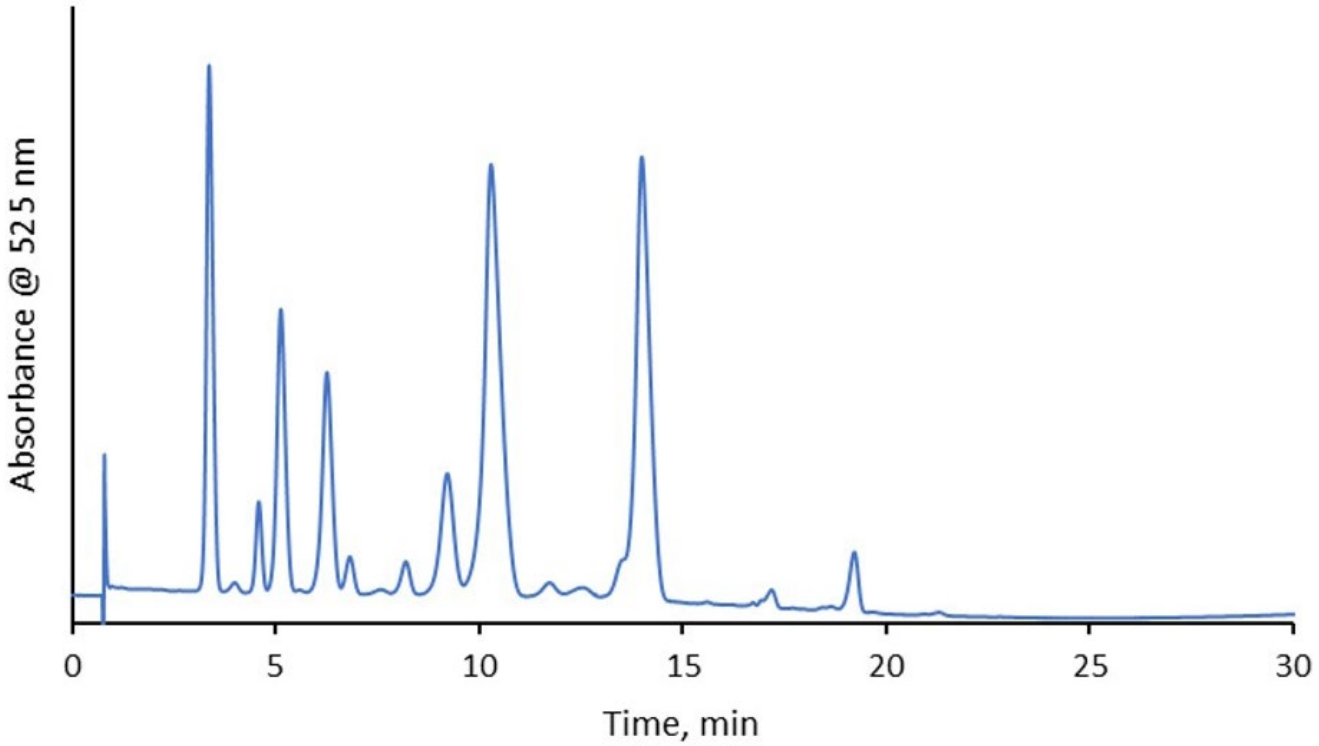
TEST CONDITIONS

Column: HALO® LPH-C18, 90 Å, 2.7 µm, 2.1 x 100 mm
Part Number: 92822-616
Mobile Phase A: Water/ 3% Phosphoric acid (pH: 1.4)
Mobile Phase B: Methanol
Gradient:

Time (min)	%B
0.0	23
10.8	26
29.8	60

Flow Rate: 0.27 mL/min
Pressure: 144 bar
Temperature: 30 °C
Detection: UV 525 nm, PDA
Injection Volume: 4.5 µL
Sample Solvent: Water
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µl
LC System: Shimadzu Nexera X2

Anthocyanins, a category of polyphenols, are a type of pigment found in plants that offer several health benefits. These flavonoids have antioxidant effects that can be found in a variety of different fruits and vegetables, including blueberries. A separation of anthocyanins is performed on a HALO® 90 Å LPH-C18 column, which is ideal for the low pH conditions of this method. Blueberries were mixed with methanol, water, and formic acid followed by being spun down and filtered. Due to the sterically protected ligand, the LPH-C18 column can withstand these low pH conditions and maintain stable retention times while standard C18 columns will show retention loss over time.



Aminoglycosides in Eggs — UHPLC

TEST CONDITIONS

Columns: Avantor® ACE® Excel C18-PFP, 100 Å, 2 µm, 2.1 x 100 mm
Part Number: EXL10101002U
Mobile Phase: A: 20 mM HFBA in H_2O/MeCN (98:2 v/v)
B: 20 mM HFBA in MeCN/H_2O (98:2 v/v)

Gradient:

Time (min)	%B
0.0	5.0
2.0	15.0
4.5	19.0
5.5	19.5
6.0	22.0
7.0	35.0
9.0	48.0
9.5	5.0
12.0	5.0

Flow Rate: 0.4 mL/min
Temperature: 40 °C
Detection: Positive ESI MRM (transitions as shown)
Sample: Extraction at low pH, clean up with WCX SPE cartridge

Egg sample spiked at 100 ug/kg (CCa)

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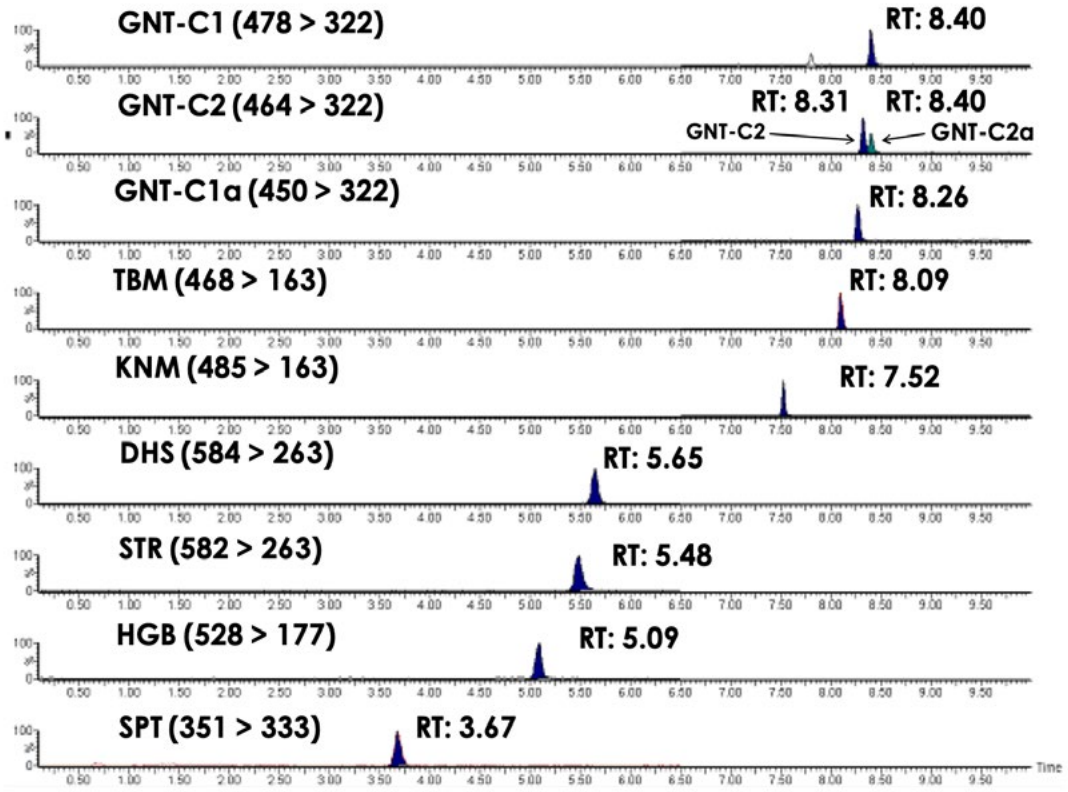


GNT	Gentamicin
TBM	Tobramycin
KNM	Kanamycin
DHS	Dihydrostreptomycin
STR	Streptomycin
HGB	Hygromycin-B
SPT	Spectinomycin

Green Tea Extract — UHPLC

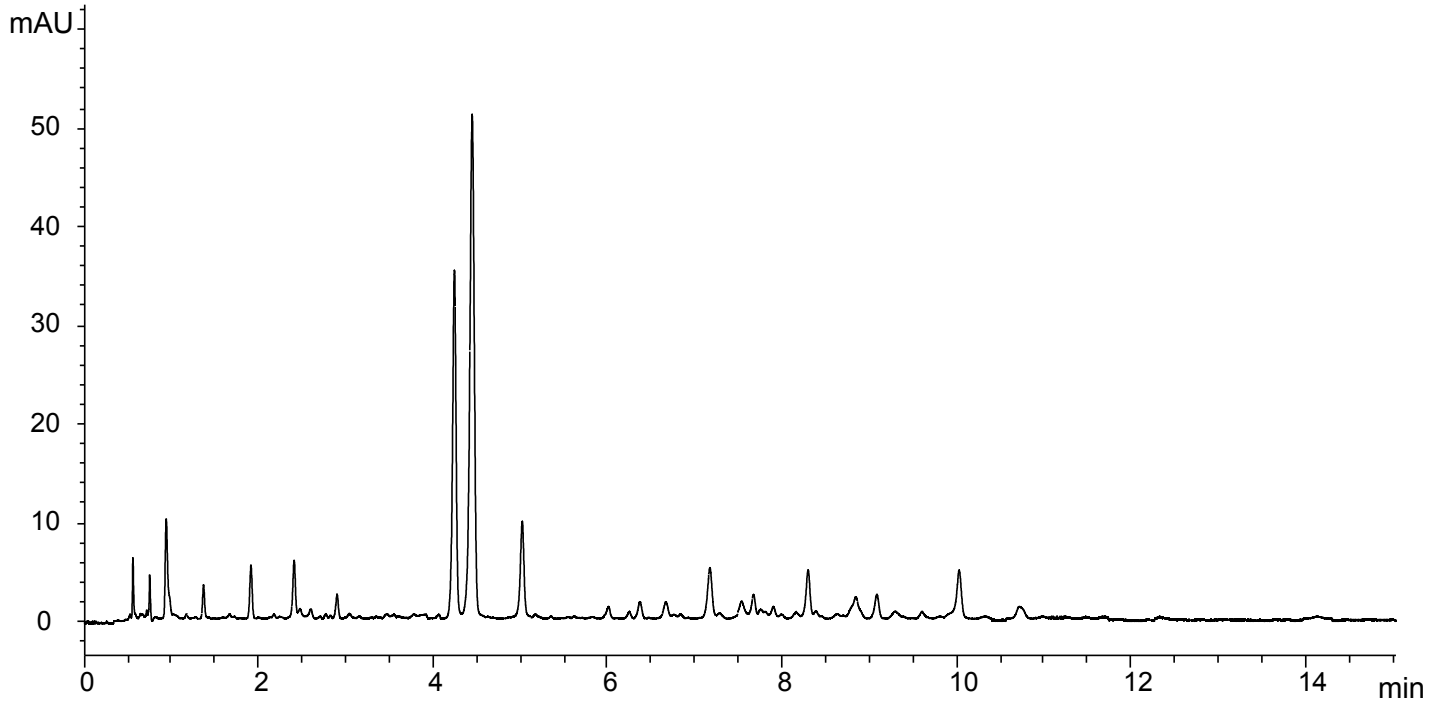
TEST CONDITIONS

Column: Avantor® ACE® Excel SuperC18, 100 Å, 1.7 µm, 3.0 x 100 mm
Part Number: EXL17111003U
Mobile Phase: A: 0.1% Formic acid in H_2O
B: 0.1% Formic acid in MeCN

Gradient:

Time (min)	%B
0	5
15	20
17	95
18	95
20	5
27	5

Flow Rate: 0.8 mL/min
Temperature: 80 °C
Injection: 2 µL
Detection: UV, 260 nm
Sample: Tablet ground to fine powder and extracted with MeCN/H_2O (1:1 v/v) with ultrasonication. Supernatant diluted with H_2O and filtered using Whatman Mini-Uniprep syringless filter.
System: Chromaster Ultra Rs



HPLC Analysis of Chlorogenic Acid in Green Coffee Extract — HPLC/UHPLC

TEST CONDITIONS

Column: HALO® C18, 90 Å, 2.7 µm, 3.0 x 100 mm
Part Number: 92813-602
Mobile Phase: A/B
A= Water + 0.1% Formic acid
B= Acetonitrile + 0.1% Formic acid

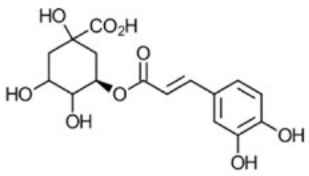
Gradient:

Time (min)	%B
0.0	10
4.0	10
9.0	50
11.0	100
13.0	100

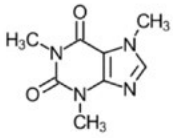
Flow Rate: 0.75 mL/min
Pressure: 250 Bar (initial pressure)
Temperature: 30 °C
Detection: UV 254, 325 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 water/acetonitrile
Response Time: 0.02 sec
Data rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
ECV: ~14 µL

Green coffee extract is sold as a dietary supplement to aid in weight loss. Chlorogenic acid is the active ingredient. The chlorogenic acid and caffeine can be easily analyzed using HPLC. A bottle of green coffee extract capsules was purchased at a local pharmacy. The powder from one capsule was placed into a vial containing 20 mL of 50/50: acetonitrile/methanol and vortexed to mix. The vial was sonicated for 10 minutes and then allowed to settle. Five mL of the supernate was filtered through a 13 mm, 0.45 µm porosity Nylon syringe filter. A portion of this solution was diluted 1:10 using a 50/50 mixture of water and acetonitrile. The diluted sample was injected into the HPLC. Caffeine was only detectable using the 254 nm wavelength, while chlorogenic acid was detected with increased sensitivity at the 325 nm wavelength. The solvent gradient extends past 10 minutes to remove some minor late eluting peaks.

STRUCTURES



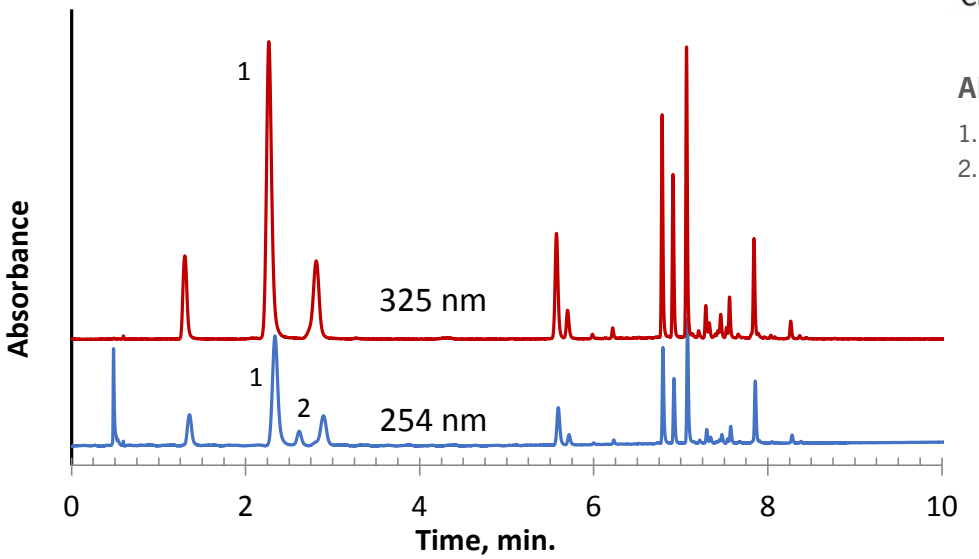
Chlorogenic Acid



Caffeine

ANALYTES

1. Chlorogenic acid
2. Caffeine

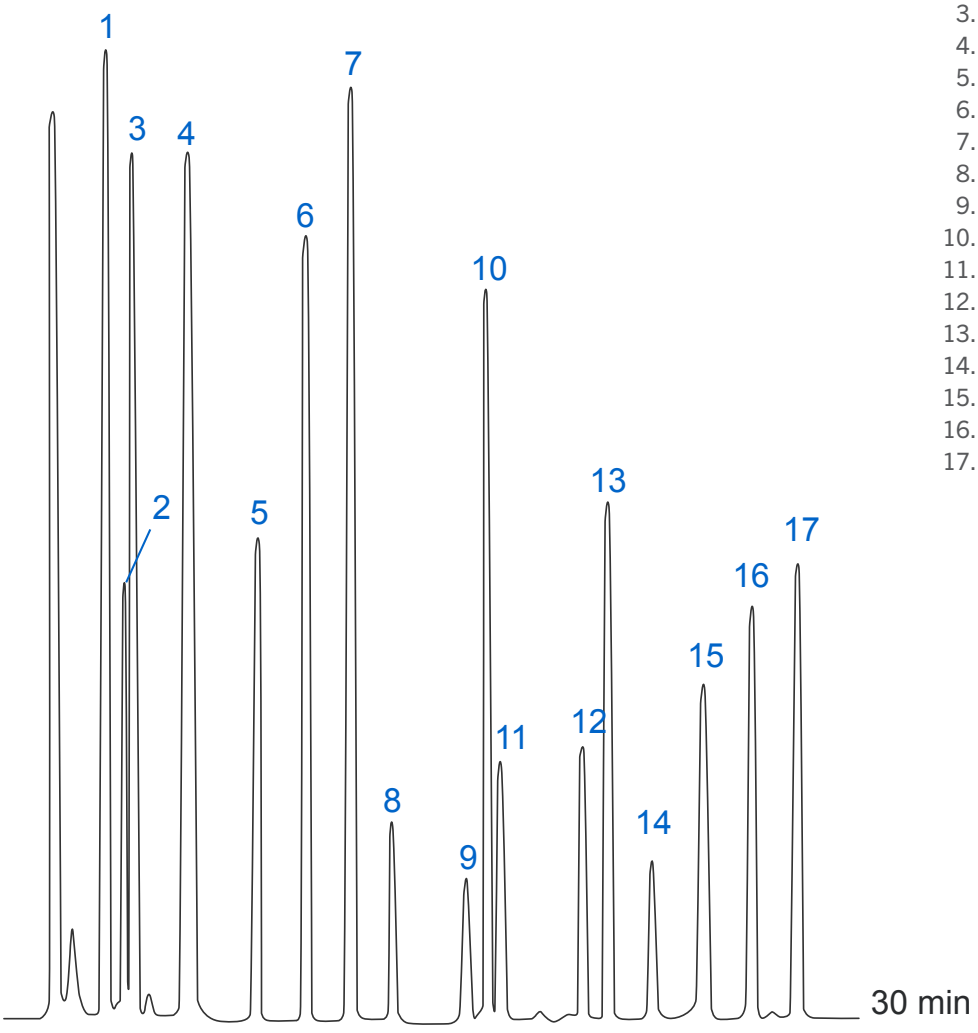


Flavor and Fragrance Compounds — GC

The HI-ACID (FFAP) column is a nitroterephthalic acid modified polyethylene glycol (PEG) phase of high polarity. Useful for the analysis of free fatty acids, flavors and fragrances, amides, alcohols and glycols.

TEST CONDITIONS

Column:	Avantor® Hichrom HI-ACID (FFAP), 0.25 µm, 0.25 mm x 30 m
Part Number:	HI102502530
Oven Program:	75 °C, 2 °C/min, 140 °C
Carrier Gas:	Helium, 1.5 mL/min
Injector:	Split, 250 °C
Detector:	FID, 250 °C



ANALYTES

1. Isoamyl Acetate
2. alpha-Phellandrene
3. Cumene
4. 1-Pentanol
5. 6-Methyl-5-hepten-2-one
6. 2-Nonanone
7. Ethyl Octanoate
8. Furfural
9. Benzaldehyde
10. Linalool
11. Isobutyric acid
12. n-Butyric acid
13. Ethyl Decanoate
14. Furfuryl alcohol
15. alpha and beta-Terpineol
16. Carvone
17. Methyl Salicylate

LC-MS of PAHs in Grilled Meat — HPLC/UHPLC

TEST CONDITIONS

Column:	HALO® EnviroClass PAH, 90 Å, 2.7 µm, 2.1 x 100 mm
Part Number:	92842-612
Flow Rate:	0.4 mL/min
Pressure:	289 bar
Column Temperature:	30 °C
Injection Volume:	1 µL
Sample Solvent:	Methanol
LC System:	Shimadzu Nexera
Mobile Phase	A: Water/0.1% Formic acid
	B: Acetonitrile/0.1% Formic acid

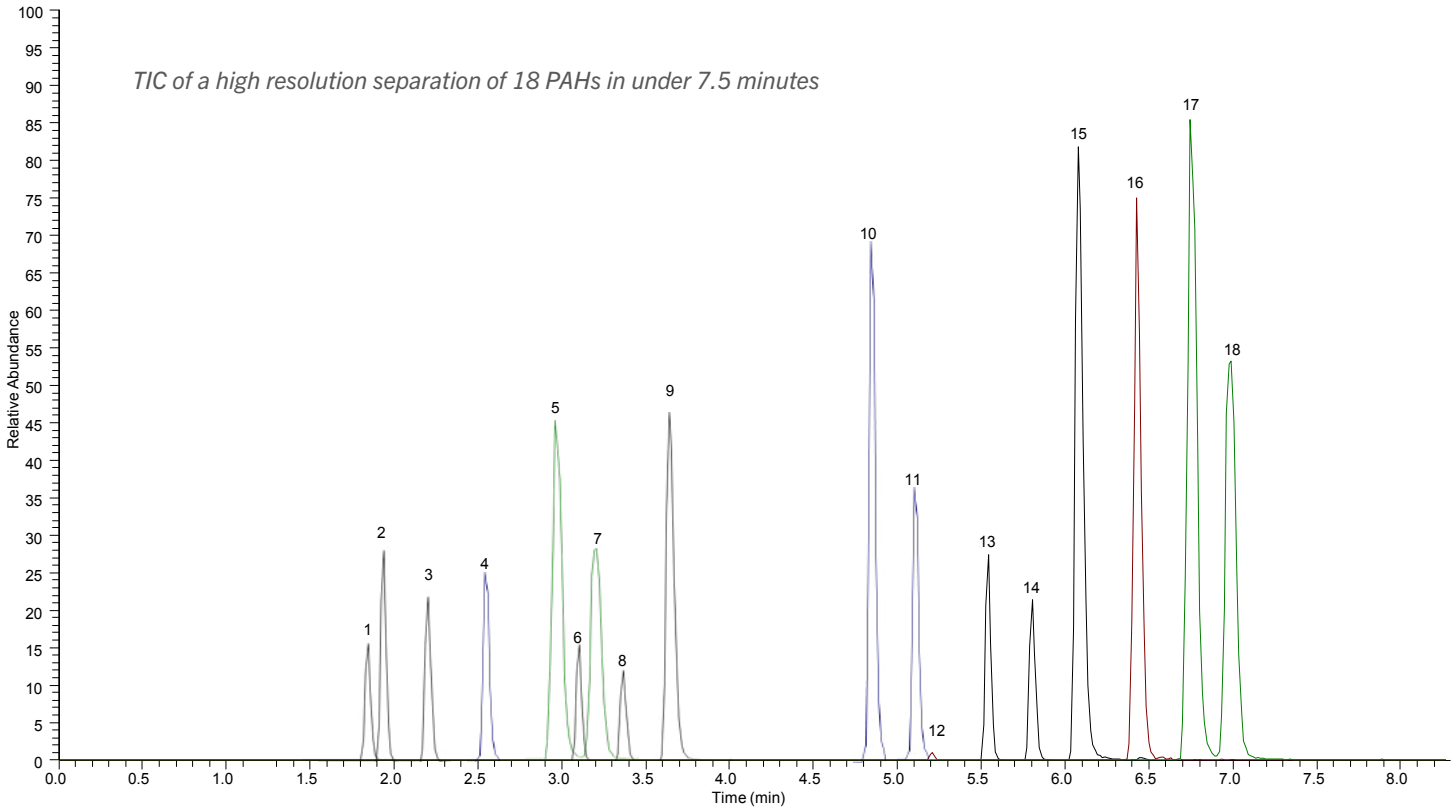
Gradient:

Time (min)	%B
0.0	40
5.0	100
8.0	100
8.01	40

MASS SPECTROMETRY CONDITIONS

MS System:	Thermo Scientific™ Q Exactive™ HF
ESI voltage:	5.5 kV
Heater Temp:	400 °C
Sheath gas:	35 (arbitrary units)
Aux gas:	8 (arbitrary units)
Tube lens voltage:	40 Vt

TIC of a high resolution separation of 18 PAHs in under 7.5 minutes



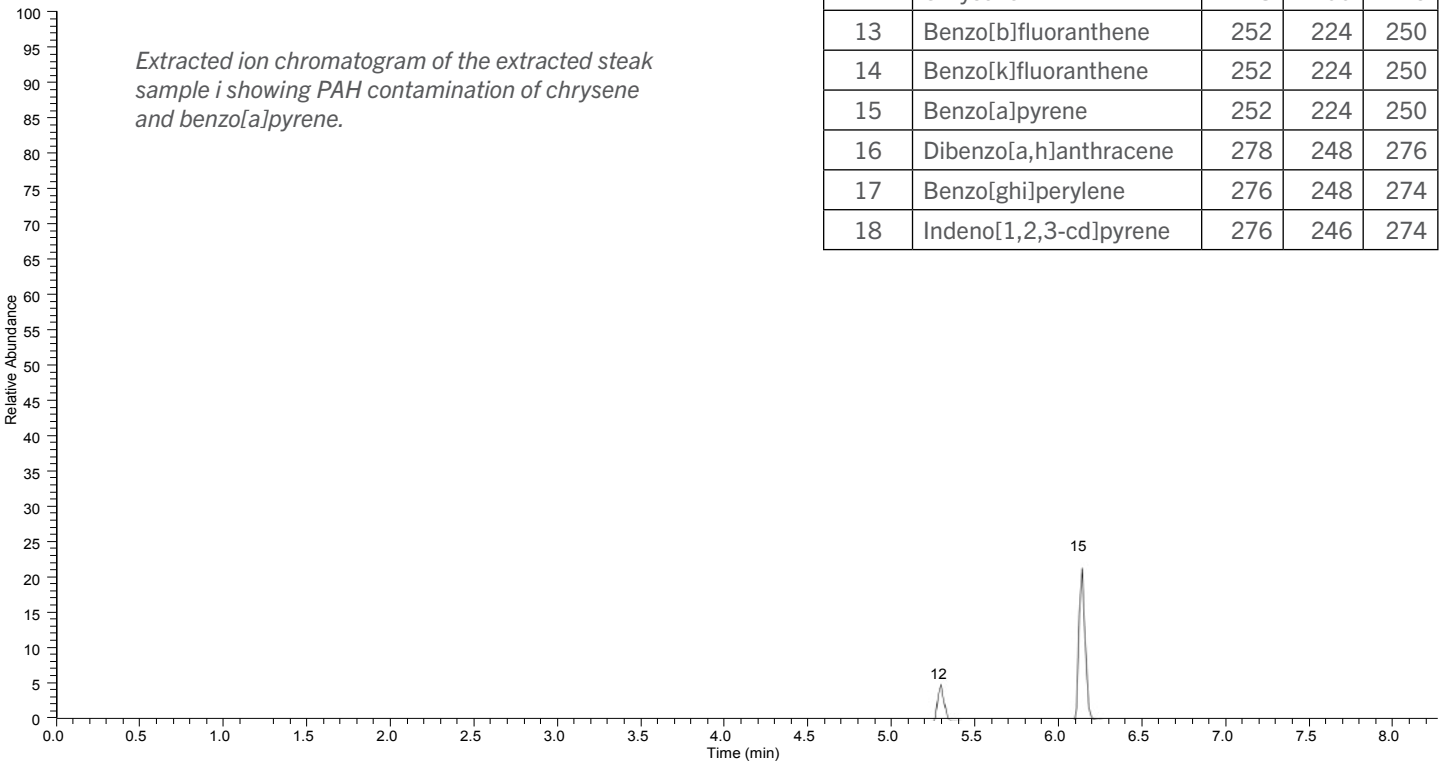
LC-MS of PAHs in Grilled Meat *(continued)*

The HALO® PAH column continues in the tradition of HALO® products by offering high resolution separations, in high throughput time frames. 18 PAH compounds with 6 sets of isomeric compounds were able to be quickly and efficiently resolved in under 8 minutes. In addition, the high resolution separation of the HALO® PAH column, enabled chrysene and benzo[a]pyrene to be resolved from a complex meat matrix, enabling quantitation of PAH contamination present in barbequed steak. The concentration of PAHs in the sample, were below those established by the EU, and demonstrates that not only can the HALO® PAH column be used in the stringent regulatory testing of current established methods, but also be relied upon as future regulations dictate the establishment of new methods, requiring lower limits of detection. The HALO® PAH column offers a rugged and reproducible particle design meeting the needs of complex matrix testing. Fused-Core® technology is ideal for PAH analysis in particular, enabling customers to achieve analytical goals of speed, accuracy, and precision LC separations.

ANALYTES AND ELUTION ORDER

Peak #	Compound	Precursor Ion	Fragment 1	Fragment 2
1	Naphthalene	128	78	102
2	Acenaphthylene	152	126	151
3	1-Methylnaphthalene	142	89	115
4	2-Methylnaphthalene	142	115	141
5	Acenaphthene	154	126	153
6	Fluorene	166	115	165
7	Phenanthrene	178	151	176
8	Anthracene	178	152	176
9	Fluoranthene	202	150	200
10	Pyrene	202	150	200
11	Benzo[a]anthracene	228	150	226
12	Chrysene	228	200	226
13	Benzo[b]fluoranthene	252	224	250
14	Benzo[k]fluoranthene	252	224	250
15	Benzo[a]pyrene	252	224	250
16	Dibenzo[a,h]anthracene	278	248	276
17	Benzo[ghi]perylene	276	248	274
18	Indeno[1,2,3-cd]pyrene	276	246	274

Extracted ion chromatogram of the extracted steak sample i showing PAH contamination of chrysene and benzo[a]pyrene.



Artificial Food Colors — HPLC

TEST CONDITIONS

Column: Avantor® ACE® C18, 100 Å, 3 µm, 4.6 x 100 mm
Part Number: ACE1111046
Mobile Phase: A: 1.0120 g Tetrabutylammonium bromide and .6804 g KH_2PO_4 in 1 L H_2O
B: 1.0120 g Tetrabutylammonium bromide in 1 L MeOH

Gradient:

Time (min)	%B
0	45
12	60
25	45

Flow Rate: 0.8 mL/min
Temperature: Ambient
Injection: 10 µL
Detection: UV-Vis, 480 nm

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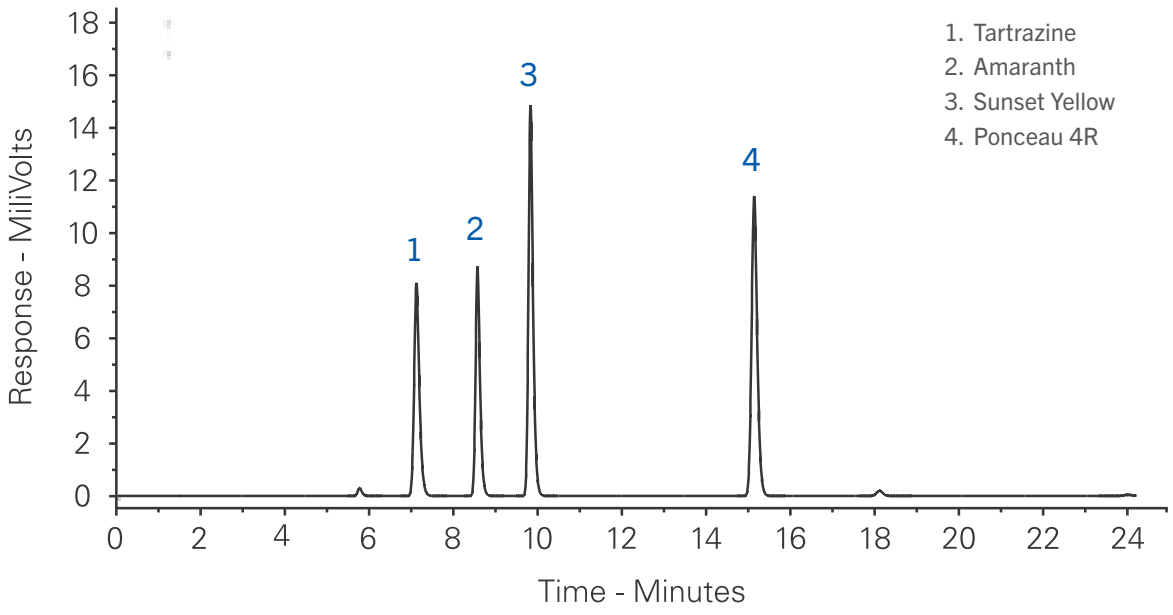

ANALYTES

1. Tartrazine
2. Amaranth
3. Sunset Yellow
4. Ponceau 4R

Illegal Dyes in Spices — HPLC

TEST CONDITIONS

Column: Avantor® ACE® C18, 100 Å, 3 µm, 4.6 x 100 mm
Part Number: ACE1111046
Mobile Phase: A: H_2O
B: MeOH
C: 0.06 M Tetrabutylammonium bromide and 0.5 M KH_2PO_4 in H_2O pH 2.55

Gradient:

Time (min)	%A	%B	%C
0	45	50	5
45	3	92	5
65	3	92	5
66	45	50	5
75	45	50	5

Flow Rate: 1 mL/min
Temperature: Ambient
Injection: 10 µL
Detection: UV-Vis, 500 nm

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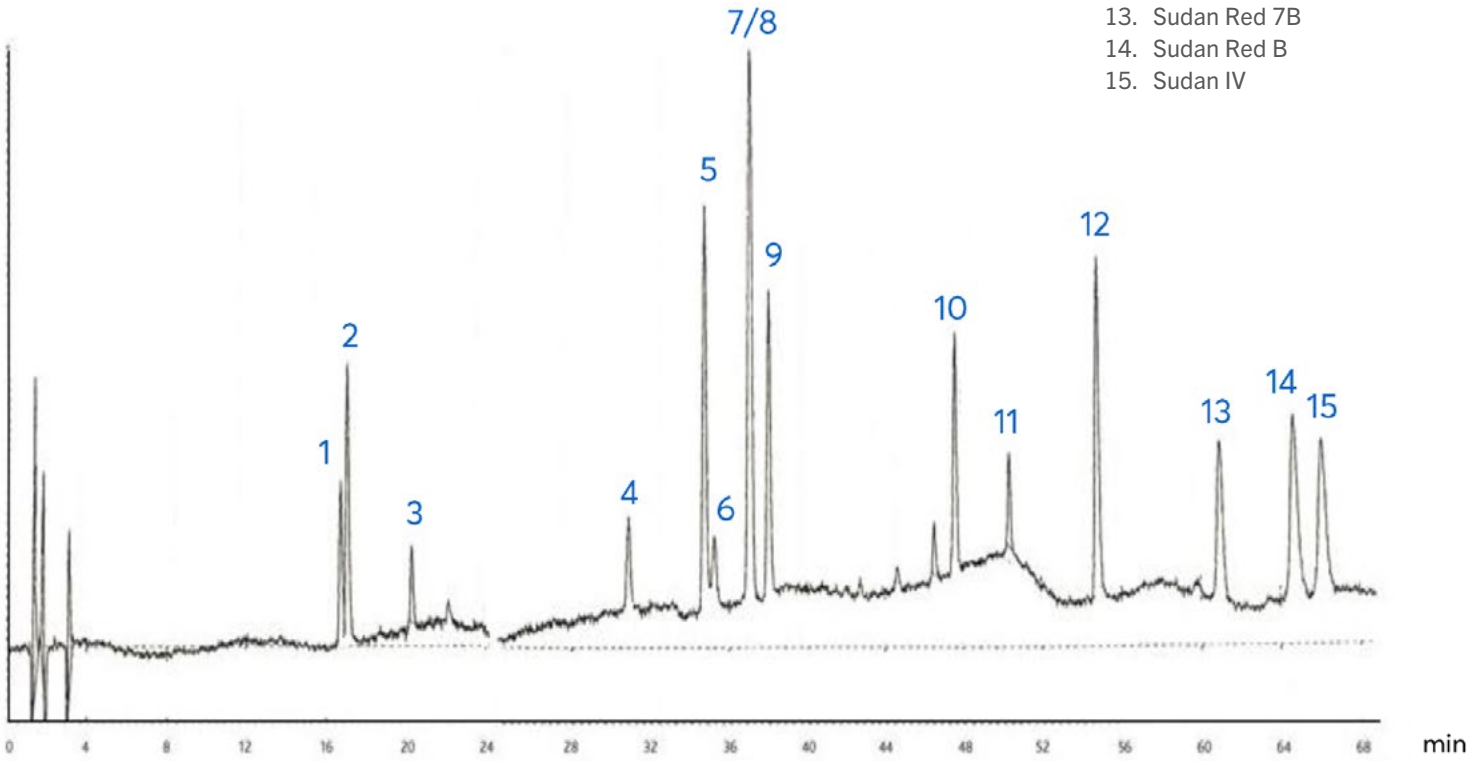
ANALYTES

1. Rhodamine B
2. Orange II
3. Metanil Yellow
4. Butter Yellow
5. Para Red
6. Sudan Orange G
7. Toluidine Red
8. Sudan I
9. Sudan Red G
10. Sudan II
11. Sudan Black B
12. Sudan III
13. Sudan Red 7B
14. Sudan Red B
15. Sudan IV

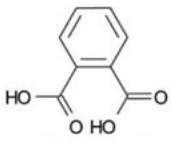


Preservatives (I) — UHPLC

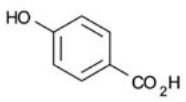
TEST CONDITIONS

Column:	Avantor® ACE® Excel C18, 100 Å, 1.7 µm, 3.0 x 50 mm
Part Number:	EXL1710503U
Mobile Phase:	20 mM Potassium phosphate pH 2.5 in MeCN/H_2O (30:70 v/v)
Flow Rate:	0.43 mL/min
Temperature:	20 °C
Injection Volume:	0.7 µL
Detection:	UV, 230 nm

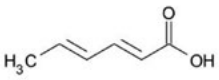
STRUCTURES



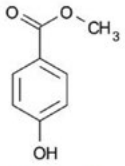
Phthalic acid



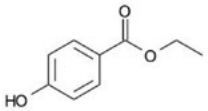
4-Hydroxybenzoic acid



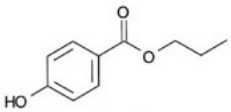
Sorbic acid



Methyl paraben



Ethyl paraben



Propyl paraben

ANALYTES

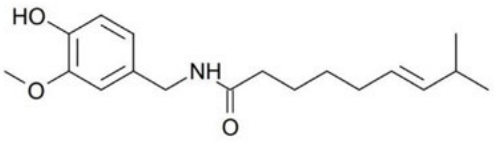
1. Phthalic acid
2. 4-Hydroxybenzoic acid
3. Sorbic acid
4. Methyl acid
5. Ethyl acid
6. Propyl paraben

Capsaicins — UHPLC

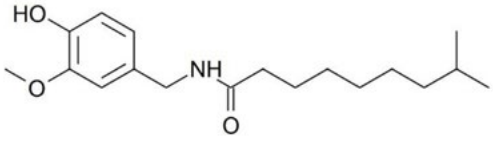
TEST CONDITIONS

Column:	Avantor® ACE® Excel C18, 100 Å, 2 µm, 3.0 x 100 mm
Part Number:	EXL1011003U
Mobile Phase:	0.1% H_3PO_4 in MeCN/H_2O (60:40 v/v)
Flow Rate:	0.6 mL/min
Temperature:	40 °C
Injection Volume:	1 µL
Detection:	UV, 220 nm
Sample Preparation:	Extraction with 10 mL ethanol
	Sonication for 20 minutes
	Centrifugation for 10 minutes at 6,000 RPM
	Filtration with 0.45 µm Whatman Mini-UniPrep syringeless filter vial

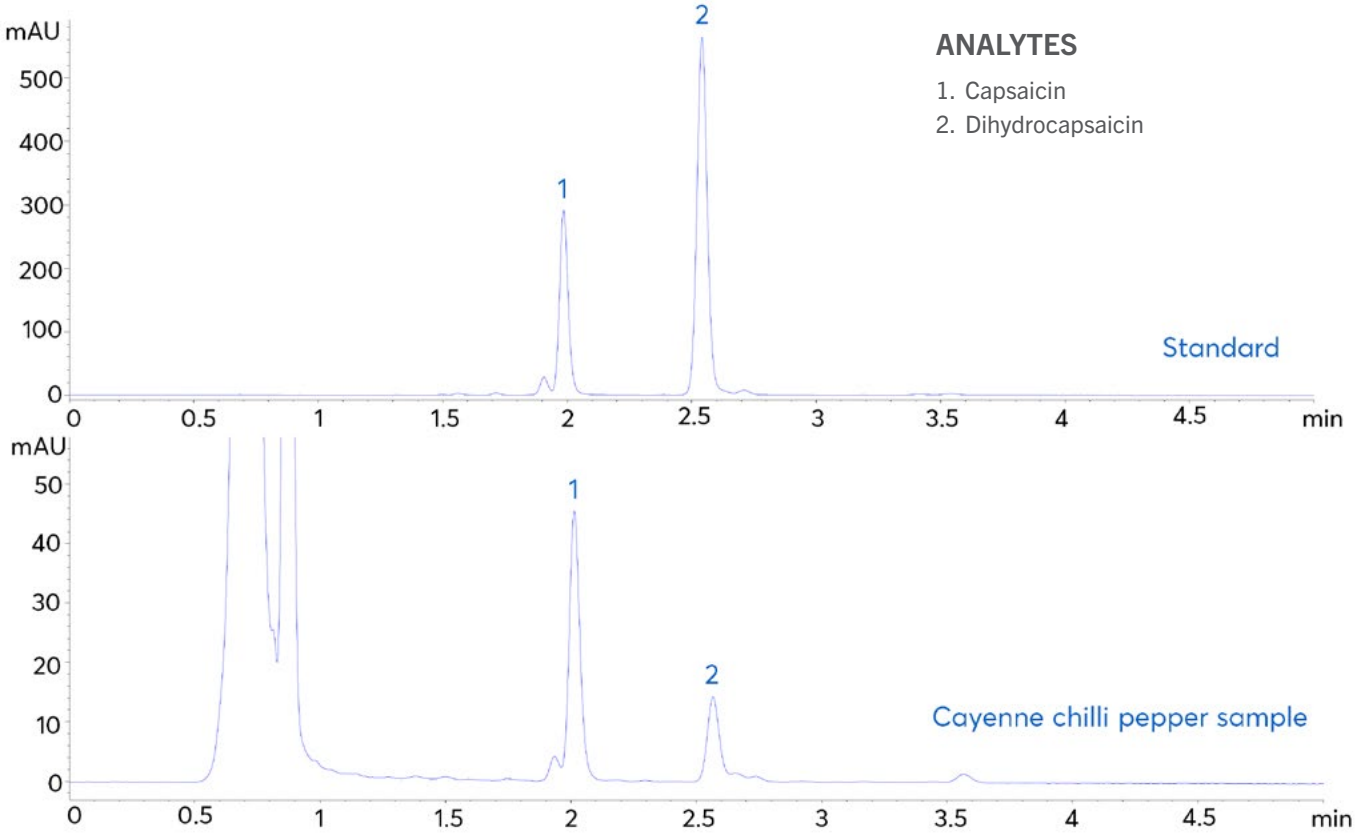
STRUCTURES



Capsaicin



Dihydrocapsaicin



ANALYTES

1. Capsaicin
2. Dihydrocapsaicin

Capsaicins in Chili Powder — HPLC/UHPLC

TEST CONDITIONS

Column:	HALO® C18, 90 Å, 2.7 µm, 3.0 x 100 mm
Part Number:	92813-602
Mobile Phase:	A/B
	A= water
	B= acetonitrile

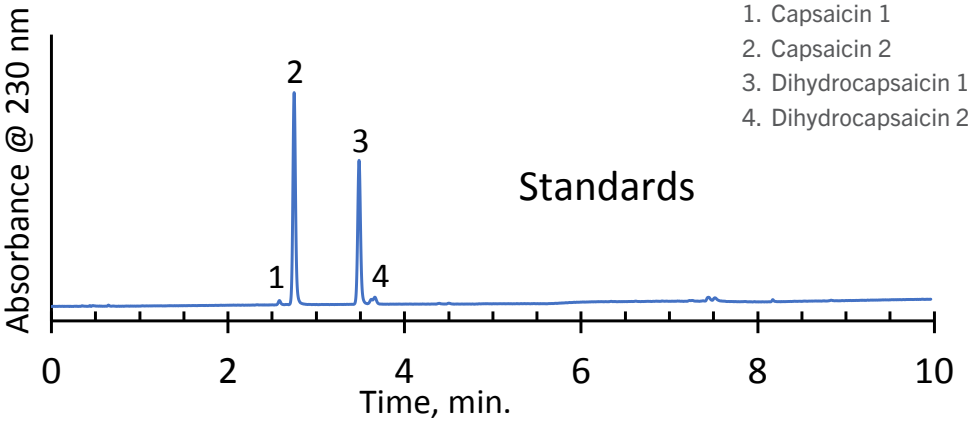
Gradient:

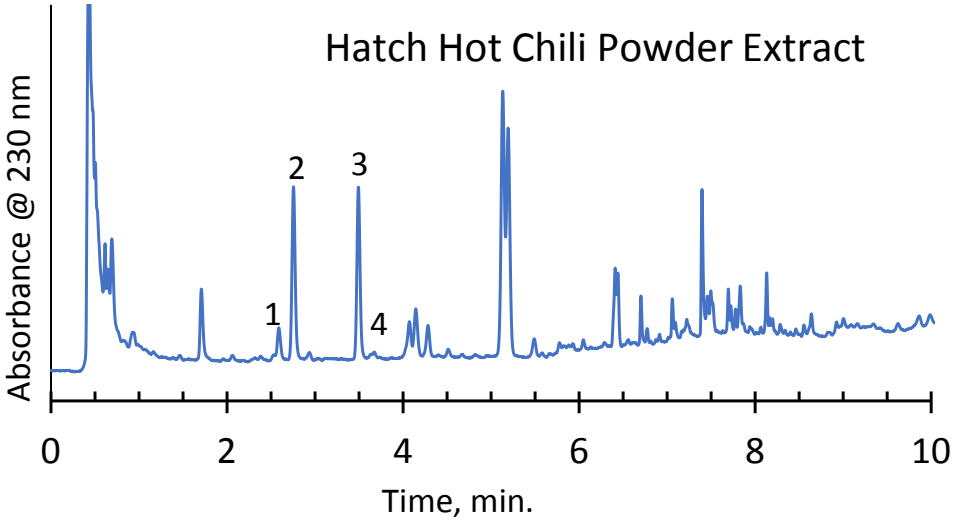
Time (min)	%B
0.0	40
5.0	60
7.0	100
20.0	100

Flow Rate:	0.8 mL/min
Pressure:	223 bar starting pressure
Temperature:	40 °C
Injection Volume:	1.0 µL
Sample Solvent:	Acetonitrile
Detection:	UV 230 nm, VWD
Response Time:	0.02 sec
Data rate:	25 Hz
Flow Cell:	2.5 µL semi-micro
LC System:	Shimadzu Prominence UFLC XR
ECV:	~14 µL

ANALYTES

1. Capsaicin 1
2. Capsaicin 2
3. Dihydrocapsaicin 1
4. Dihydrocapsaicin 2





Capsaicin and dihydrocapsaicin are two of the main components of chili powder that give it the "heat" when making a batch of chili. The amount of heat is often measured by a subjective test and then rated in terms of Scoville units that are a dilution factor beyond which the capsaicins and other hot compounds cannot be detected. One can also use HPLC to measure these compounds more objectively. Here these two ingredients are separated from an acetonitrile extract using a HALO® C18 column.

Curcumin Analysis in Turmeric on a 1.5 mm Stainless Steel Column — HPLC/UHPLC

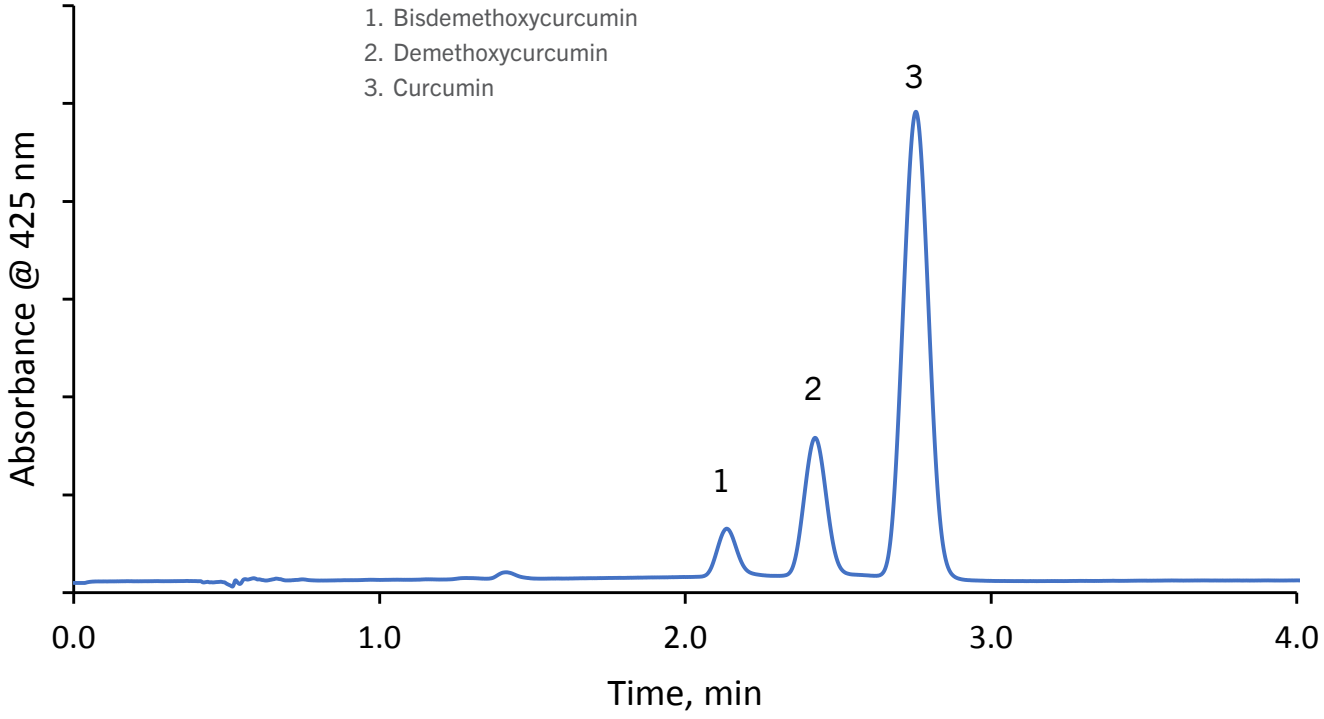
TEST CONDITIONS

Column:	HALO® C18, 90 Å, 2.7 µm, 1.5 x 100 mm
Part Number:	9281X-602
Mobile Phase A:	Water/ 0.1% Formic acid
Mobile Phase B:	ACN/ 0.1% Formic acid
Isocratic:	50 %B
Flow Rate:	0.2 mL/min
Back Pressure:	337 bar
Temperature:	30 °C
Detection:	UV 425 nm, PDA
Injection Volume:	1 µL
Sample Solvent:	Ethanol
Data Rate:	100Hz
Response Time:	0.025 sec
Flow Cell:	1 µL
Instrument:	Shimadzu Nexera X2

Turmeric - along with its most active com-pound, curcumin have many proven health benefits such as improved heart health, antioxidant properties, and a potent anti-in-flammatory. A separation of curcumins is performed on a HALO® 1.5 mm C18 column showing excellent resolution and sensitivity. Turmeric capsules were dissolved in ethanol at 0.5 mg/ml and filtered through a 0.45 um syringe filter before injection.

ANALYTES

1. Bisdemethoxycurcumin
2. Demethoxycurcumin
3. Curcumin



Common Catechins Found in Tea via LC-MS — LC-MS

TEST CONDITIONS

Column: HALO® LPH-C18, 90 Å, 2 µm, 2.1 x 100 mm
Part Number: 91822-616
Mobile Phase A: Water, 0.2% Formic acid (pH 2.45)
Mobile Phase B: Acetonitrile, 0.2% Formic acid
Gradient:

Time (min)	%B
0.0	10
1.0	10
6.0	21
7.0	21

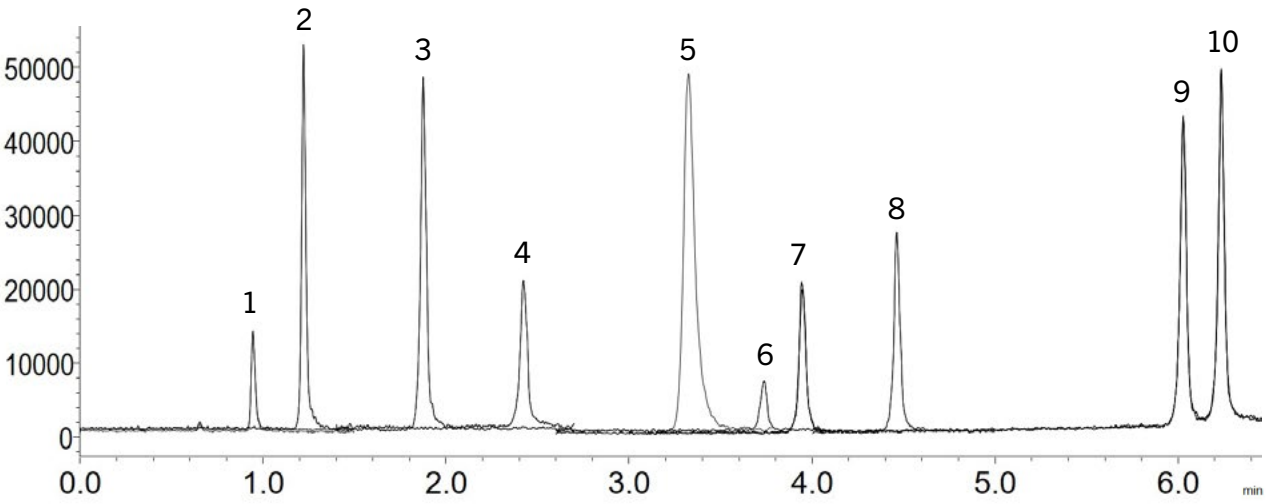
Flow Rate: 0.3 mL/min
Pressure: 438 bar
Temperature: 40 °C
Detection: +/- ESI MS/MS
Injection Volume: 2 µL
Sample Solvent: Water
MS System: Shimadzu 8040
LC System: Shimadzu Nexera X2

MS CONDITIONS:

Nebulizer Gas Flow: 2 L/min DL
Temperature: 250 °C
Heat Block Temperature: 400 °C
Drying Gas Flow: 10 L/min

Catechins belong to the subgroup of polyphenols called flavonoids. These compounds contain antioxidant properties and exist in food and medicinal plants, including tea. An LC-MS separation of catechins and caffeine is demonstrated on a HALO® LPH-C18 column showing excellent resolution using purified standards. This column is ideal for low pH separations due to its sterically protected ligand, preventing acid hydrolysis and reducing retention drift over time.

Peak #	Compound	M/Z
1	Gallic acid	169
2	Gallocatechin	305
3	Epigallocatechin	305
4	Catechin	289
5	Caffeine	195
6	Epicatechin	289
7	Epigallocatechin gallate	457
8	Gallocatechin gallate	457
9	Epicatechin gallate	441
10	Catechin gallate	441



Cyclodextrin-Encapsulated Flavor Compounds in Beer — HPLC

TEST CONDITIONS

Column:	Avantor® ACE® C18, 100 Å, 3 µm, 4.0 x 150 mm
Part Number:	ACE1111504
Mobile Phase:	0.1% Phosphoric acid in MeOH/H_2O (53:47 v/v)
Flow Rate:	0.5 mL/min
Temperature:	35 °C
Injection Volume:	20 µL
Detection:	UV, 225 nm (261 nm for 2-Methoxy-4-vinylphenol)

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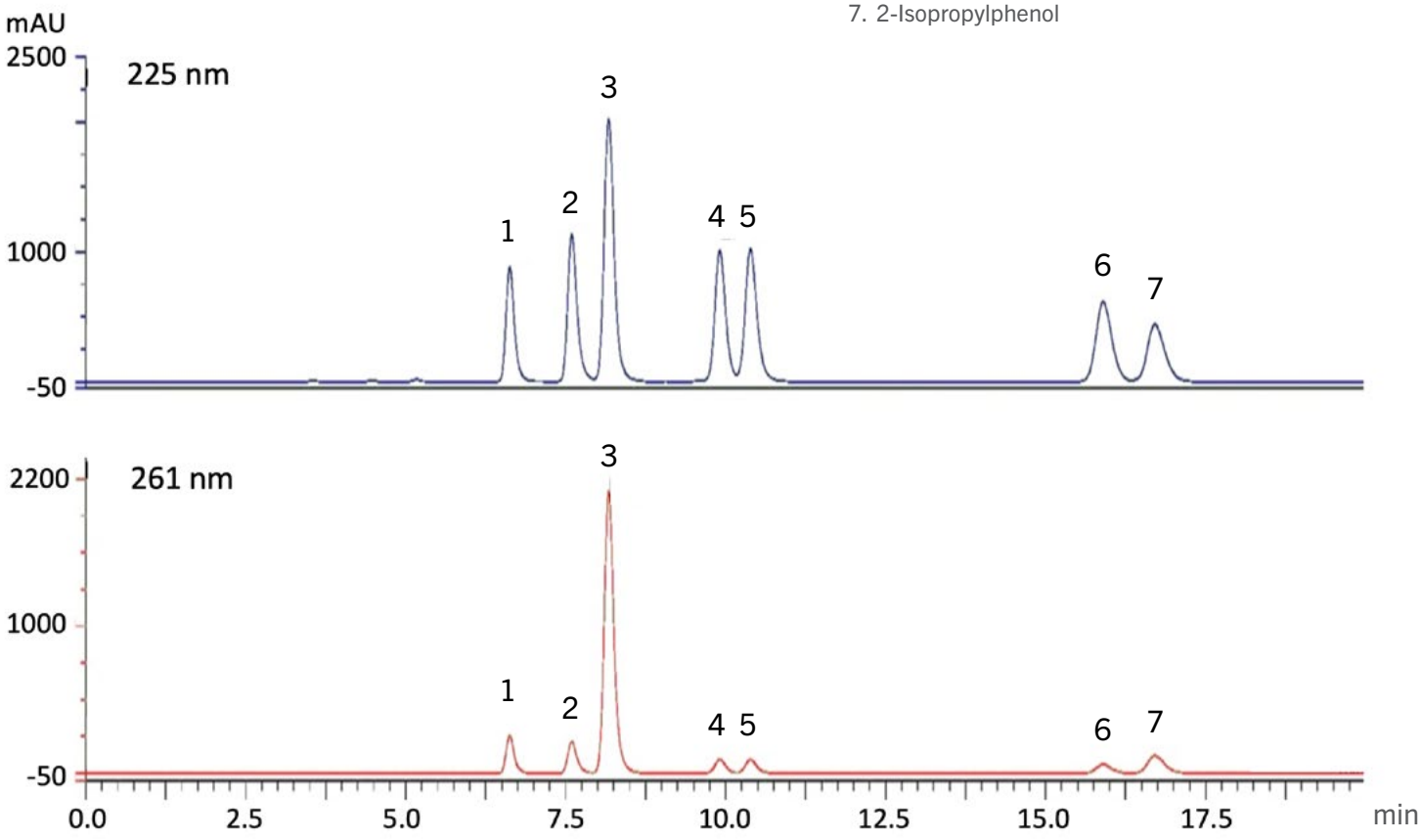
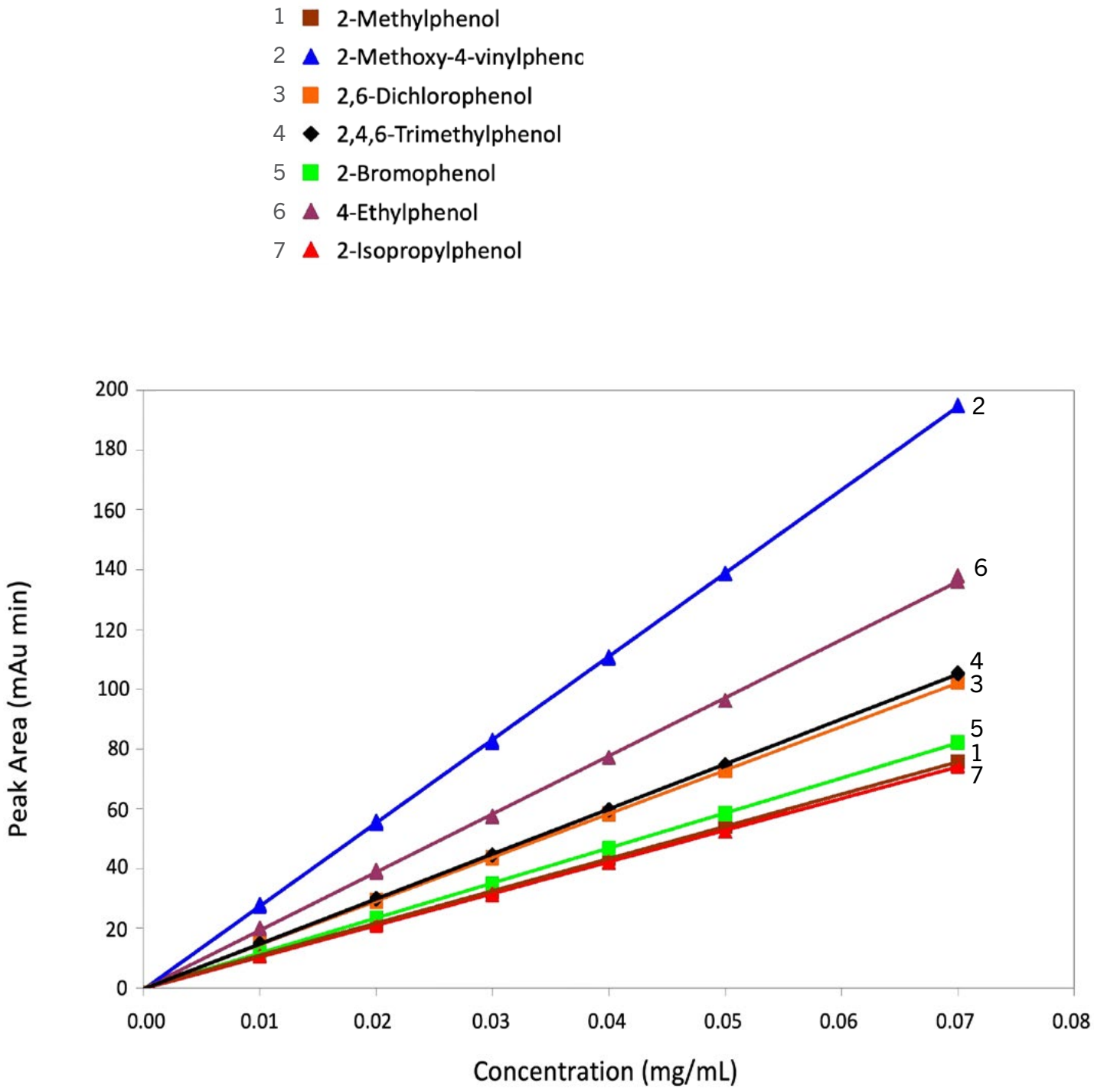
ANALYTES

1. 2-Methylphenol
2. 2-Bromophenol
3. 2-Methoxy-4-vinylphenol
4. 4-Ethylphenol
5. 2,4-Dichlorophenol
6. 2,4,6-Trimethylphenol
7. 2-Isopropylphenol



Cyclodextrin-Encapsulated Flavor Compounds in Beer *(continued)*

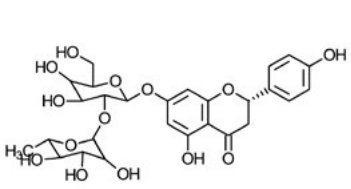


Five Flavonoids — UHPLC

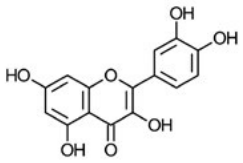
TEST CONDITIONS

Column:	HALO® C8, 90 Å, 2 µm, 2.1 x 100 mm
Part Number:	91812-608
Mobile Phase:	75/25: A/B A= 0.025 M Ammonium formate, pH=3 B= Acetonitrile
Flow Rate:	0.5 mL/min
Pressure:	473 bar
Temperature:	40 °C
Detection:	UV 276 nm, PDA
Injection Volume:	0.1 µL
Sample Solvent:	Methanol
Response Time:	0.025 sec
Flow Cell:	1 µL
LC System:	Shimadzu Nexera
Data rate:	100 Hz
ECV:	~ 7 µL

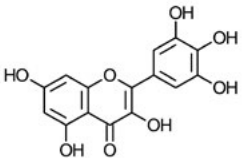
STRUCTURES



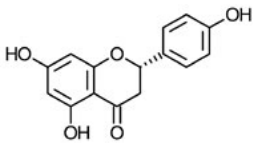
Naringin



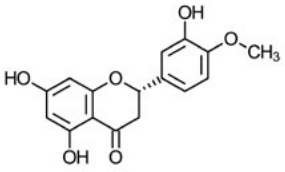
Quercetin



Myricetin



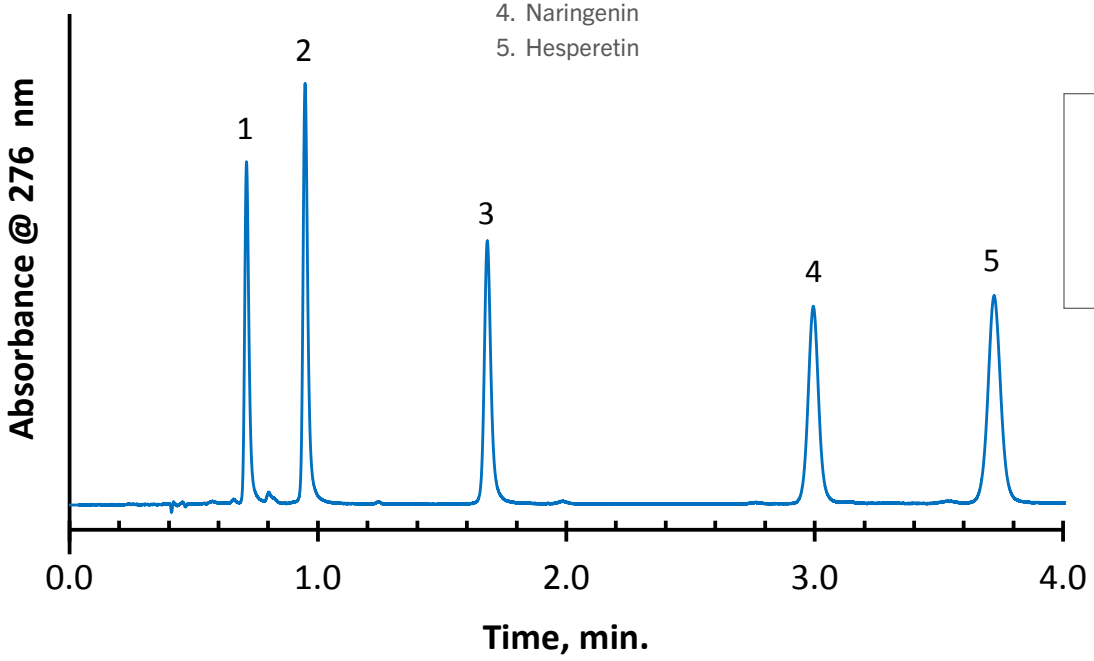
Naringenin



Hesperetin

ANALYTES

1. Naringin
2. Myricetin
3. Quercetin
4. Naringenin
5. Hesperetin



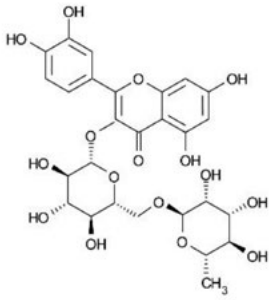
This separation of five flavonoids shows symmetrical peak shape on a HALO® C8 column using a mass-spec compatible mobile phase.

Four Flavonoids — HPLC

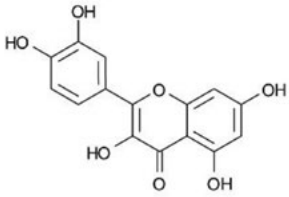
TEST CONDITIONS

Column:	Avantor® ACE® C18, 100 Å, 5 µm, 4.6 x 150 mm
Part Number:	ACE1211546
Mobile Phase:	MeCN/0.1% Formic acid in H_2O (40:60 v/v)
Flow Rate:	1 mL/min
Temperature:	Ambient
Injection Volume:	1 µL
Detection:	UV, 254 nm

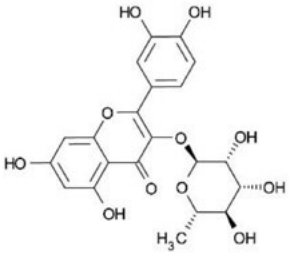
STRUCTURES



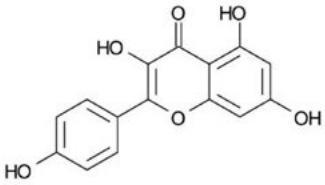
Rutin



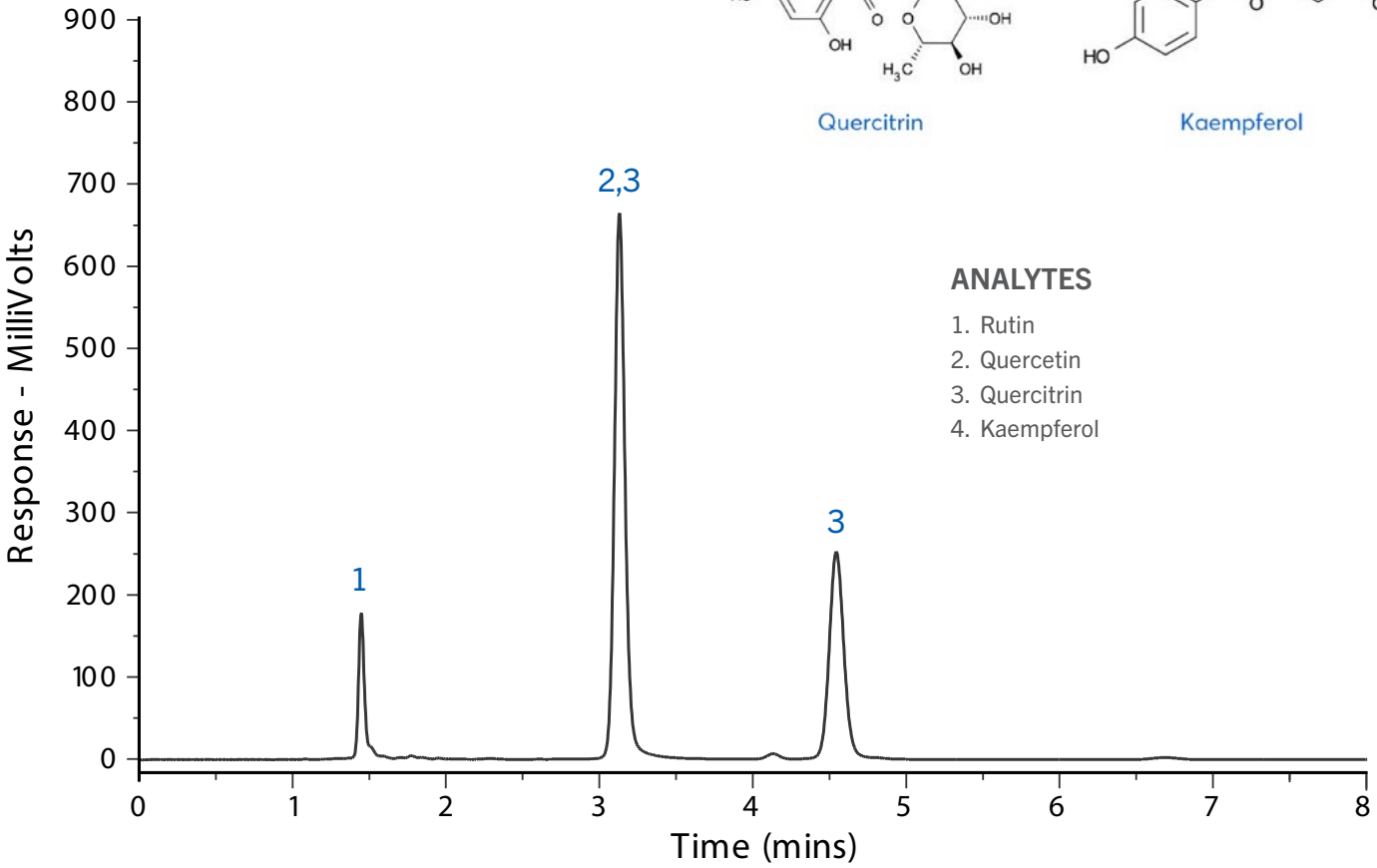
Quercetin



Quercitrin



Kaempferol



ANALYTES

1. Rutin
2. Quercetin
3. Quercitrin
4. Kaempferol

Vanillins — HPLC

TEST CONDITIONS

Column: Avantor® ACE® Excel C18-Amide, 100 Å, 3 µm, 4.6 x 150 mm

Part Number: EXL11121546U

Mobile Phase:
A: 0.1% Formic acid in H_2O
B: 0.1% Formic acid in MeCN

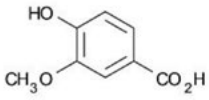
Gradient:

Time (min)	%B
0	30
10	55
10	55
15	30

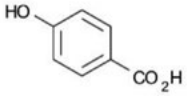
Post time 5 minutes

Flow Rate: 1 mL/min
Temperature: 40 °C
Injection: 5 µL
Detection: UV, 260 nm

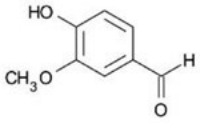
STRUCTURES



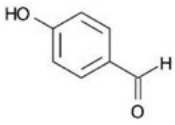
Vanillic acid



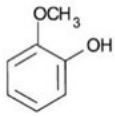
4-Hydroxybenzoic acid



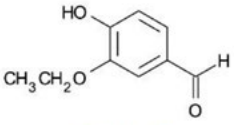
Vanillin



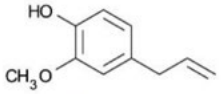
4-Hydroxybenzaldehyde



Guaiacol



Ethyl Vanillin



Eugenol

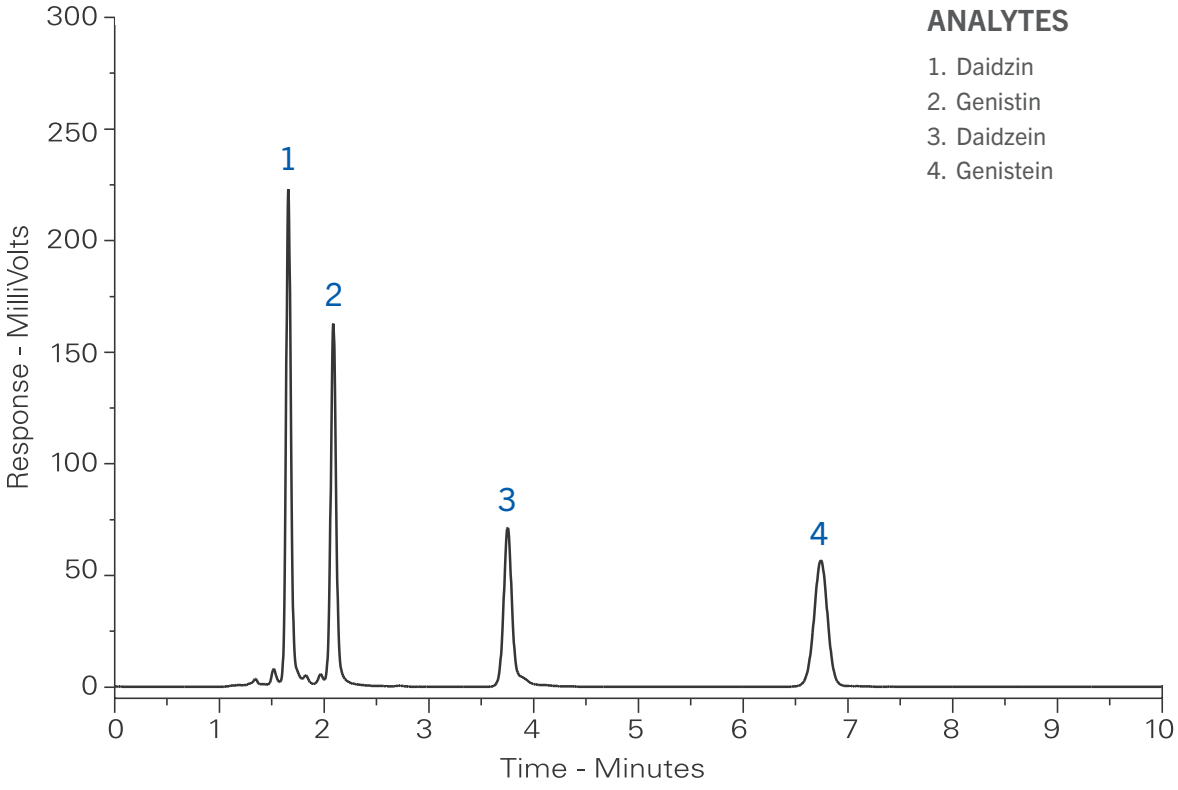
ANALYTES

1. Vanillic acid
2. 4-Hydroxybenzoic acid
3. Vanillin
4. 4-Hydroxybenzaldehyde
5. Guaiacol
6. Ethyl Vanillin
7. Eugenol

Isoflavones — HPLC

TEST CONDITIONS

Column:	Avantor® ACE® C18, 100 Å, 5 µm, 4.6 x 150 mm
Part Number:	ACE1211546
Mobile Phase:	MeCN/0.1% Formic acid in H_2O (35:65 v/v)
Flow Rate:	1 mL/min
Temperature:	Ambient
Injection Volume:	1 µL
Detection:	UV, 254 nm



ANALYTES

1. Daidzin
2. Genistin
3. Daidzein
4. Genistein

Polyphenols in Wine — HPLC/UHPLC

TEST CONDITIONS

Column:	HALO® LPH-C18, 90 Å, 2.7 µm, 2.1 x 100 mm
Part Number:	92822-616
Mobile Phase A:	Water/ 0.1% Formic acid
Mobile Phase B:	Acetonitrile/ 0.1% Formic acid

Gradient:

Time (min)	%B
0.0	0
3.5	8
7.1	10
25.0	30
26.0	40
27.0	100
29.0	100
30.0	0
35.0	0

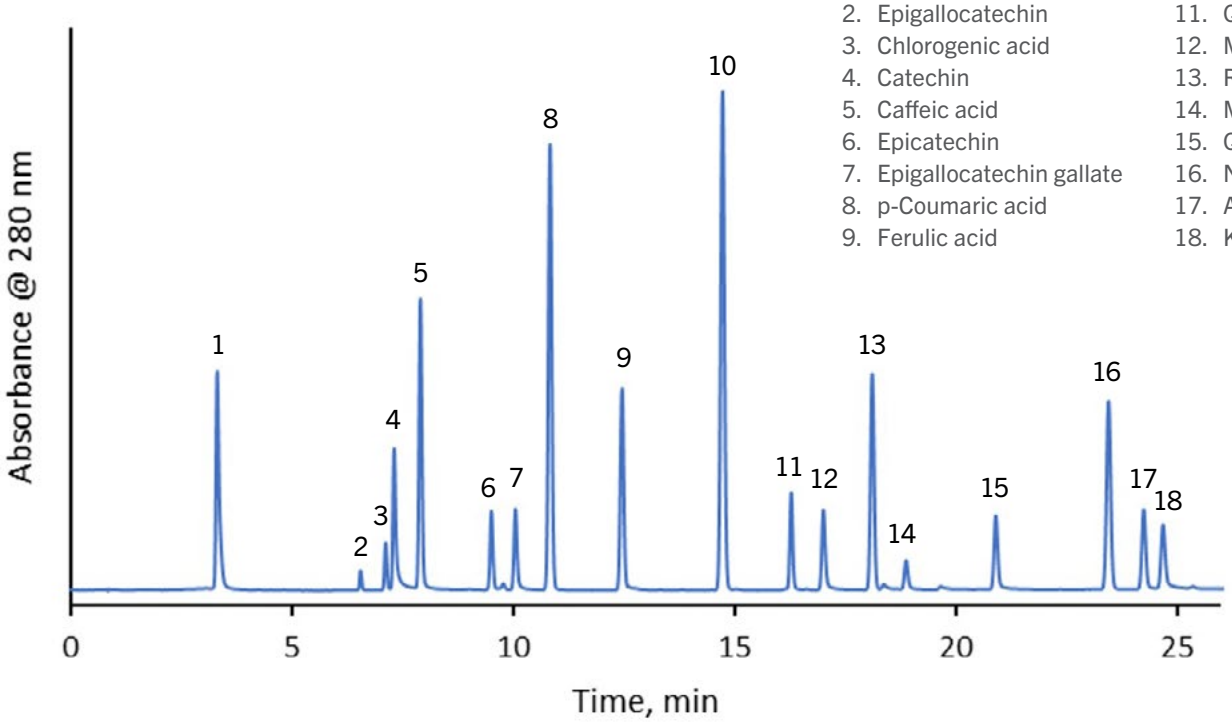
Flow Rate:	0.3 mL/min
Pressure:	159 bar
Temperature:	30 °C
Detection:	UV 280 nm, PDA

Injection Volume:	0.7 µL
Sample Solvent:	Water
Data Rate:	100 Hz
Response Time:	0.025 sec
Flow Cell:	1 µL
LC System:	Shimadzu Nexera X2

Polyphenols can be found in a wide variety of plant-based foods and are packed with antioxidants and potential health benefits. There are more than 8,000 of these types of compounds which contain multiples of phenol units. Common polyphenols found in wine are separated using a HALO® 90 Å LPH-C18 column using analytical standards. This stationary phase contains a sterically protected ligand which is ideal for high stability under low pH conditions.

ANALYTES

1. Gallic acid
2. Epigallocatechin
3. Chlorogenic acid
4. Catechin
5. Caffeic acid
6. Epicatechin
7. Epigallocatechin gallate
8. p-Coumaric acid
9. Ferulic acid
10. o-Coumaric acid
11. Quercitrin
12. Myricetin
13. Resveratrol
14. Morin
15. Quercetin
16. Naringenin
17. Apigenin
18. Kaempferol

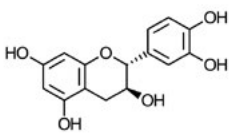


Six Flavonoids — HPLC/UHPLC

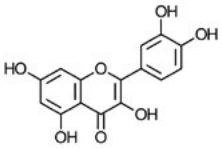
TEST CONDITIONS

Column:	HALO® C18, 90 Å, 2.7 µm, 4.6 x 50 mm
Part Number:	92814-402
Mobile Phase:	70/30: A/B
	A= 0.02 M Phosphate buffer, pH=2.9, (adj.)
	B= Acetonitrile
Flow Rate:	2.0 mL/min
Pressure:	224 bar
Temperature:	30 °C
Detection:	UV 240 nm, VWD
Injection Volume:	1.0 µL
Sample Solvent:	Methanol
Response Time:	0.02 sec
Flow Cell:	2.5 µL semi-micro
LC System:	Shimadzu Prominence UFLC XR
ECV:	~14µL
Data rate:	25 Hz

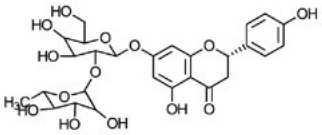
STRUCTURES



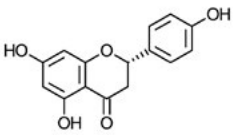
Catechin



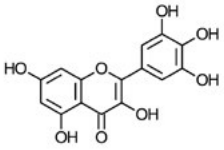
Quercetin



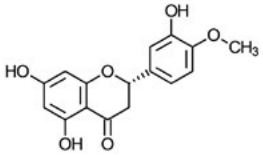
Naringin



Naringenin



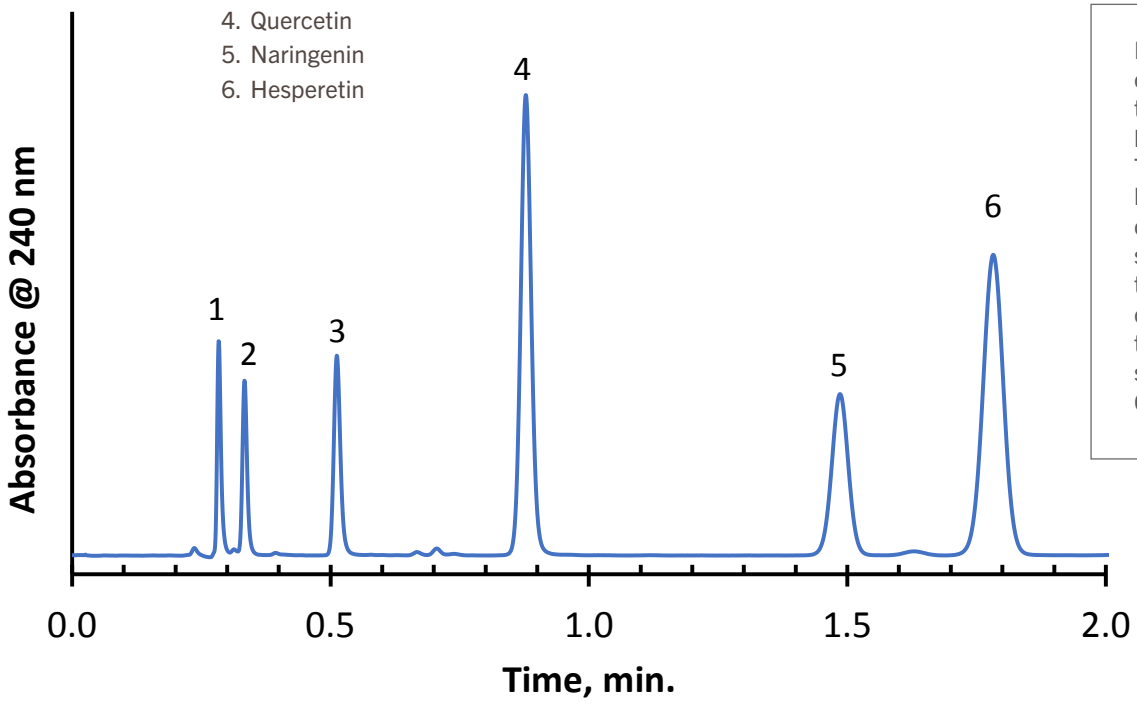
Myricetin



Hesperetin

ANALYTES

1. Catechin
2. Naringin
3. Myricetin
4. Quercetin
5. Naringenin
6. Hesperetin



Flavonoids are naturally occurring polyphenols that are found in plant leaves, flowers and seeds. They have beneficial health effects and are often taken as dietary supplements. Analysis of this flavonoids mixture can be carried out in less than 2 minutes using a short HALO® Fused Core C18 column.

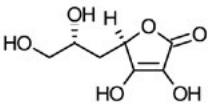
Food Additives — HPLC/UHPLC

TEST CONDITIONS

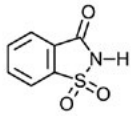
Column:	HALO® Phenyl-Hexyl, 90 Å, 2.7 µm, 4.6 x 50 mm HALO® RP-Amide, 90 Å, 2.7 µm, 4.6 x 50 mm
Part Numbers:	92814-406, 92814-407, respectively
Mobile Phase:	70/30—A/B A= 0.025 M phosphate buffer, pH=2.5 B= Methanol
Flow Rate:	1.5 mL/min
Pressure:	approximately 220 Bar
Temperature:	40 °C
Detection:	UV 220 nm, VWD
Injection Volume:	2.0 µL
Sample Solvent:	50/50-Water/methanol
Response Time:	0.02 sec
Flow Cell:	2.5 µL semi-micro
LC System:	Shimadzu Prominence UFLC XR
Extra column volume:	~14µL

These compounds are often added to foods to sweeten or preserve them. They can be rapidly analyzed using HALO® Phenyl-Hexyl or RP-Amide phases. Note the difference in retention and selectivity of the two phases when run under the same conditions. This allows for flexibility in method development and optimization of the separation.

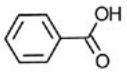
STRUCTURES



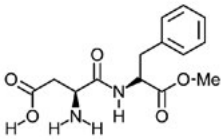
Ascorbic acid



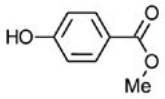
Saccharin



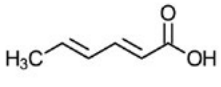
Benzoic acid



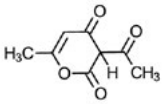
Aspartame



Methyl paraben



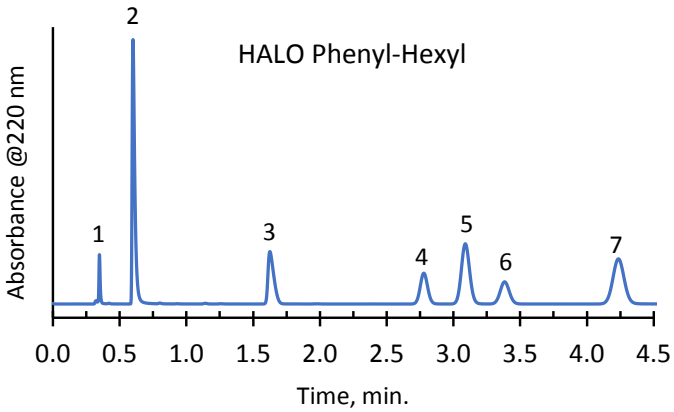
Sorbic acid

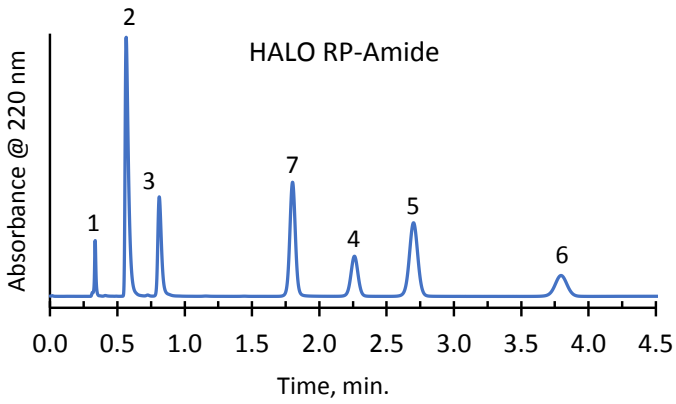


Dehydroacetic acid

ANALYTES

1. Ascorbic acid
2. Saccharin
3. Aspartame
4. Sorbic acid
5. Benzoic acid
6. Methyl paraben
7. Dehydroacetic acid





Food Additives Assay — HPLC/UHPLC

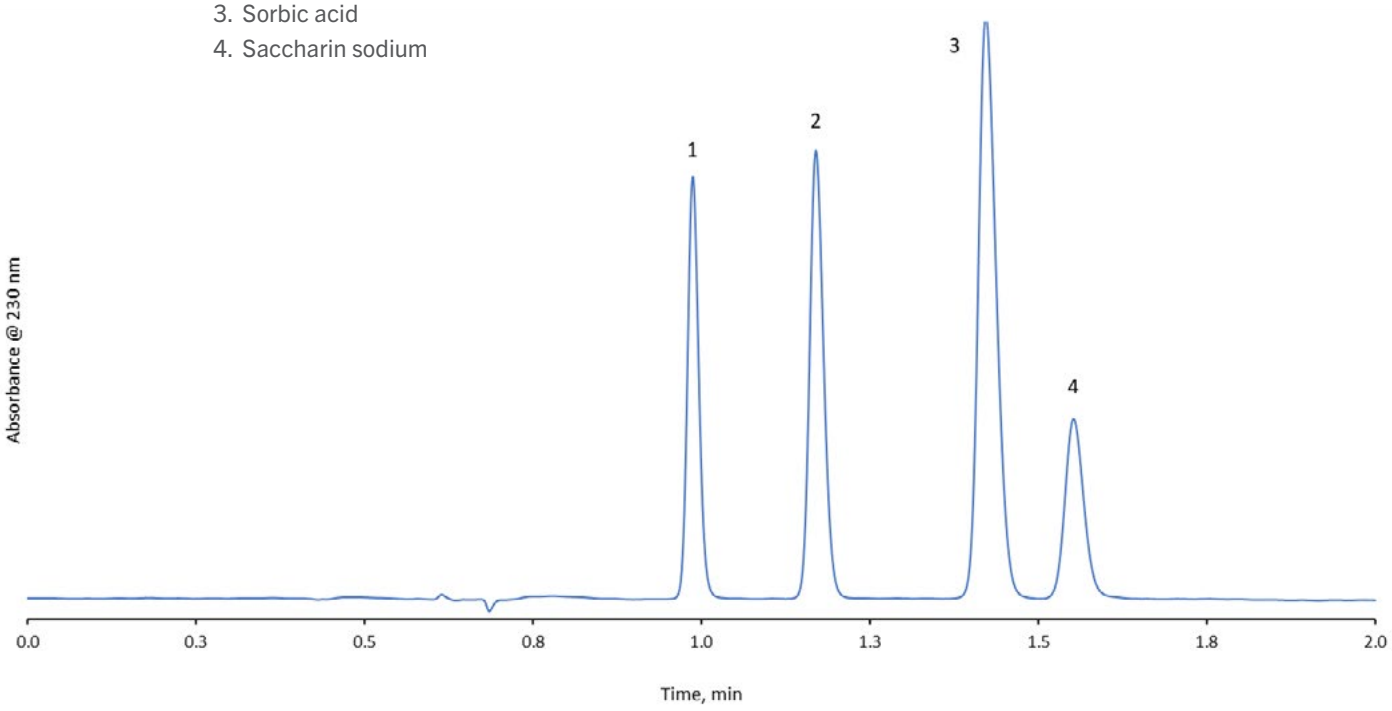
TEST CONDITIONS

Column:	HALO® AQ-C18, 90 Å, 5 µm, 4.6 x 150 mm
Part Number:	95814-722
Mobile Phase A:	20 mM Ammonium acetate
Mobile Phase B:	Methanol
Isocractic:	90/10 A/B
Flow Rate:	2 mL/min
Pressure:	336 bar
Temperature:	30 °C
Detection wavelength:	230 nm
Injection Volume:	10 µL
Sample Solvent:	Mobile phase
Data Rate:	100 hz
Response Time:	0.025 sec
Flow Cell:	1 µL
LC System:	Shimadzu Nexera X2

A rapid and highly efficient assay <400 bar for food security and safety measurements is demonstrated with a HALO® 90 À AQ-C18 5 um, 4.6 x 150 mm column. Determination of acesulfame, benzoic acid, sorbic acid and saccharin sodium food additives are specified in China's national standard regulation methods GB 5009.28-2016 and GB 5009.140-2016. These compounds are used as anti-septic/anti-microbial agents to prevent spoilage of food products by microorganisms. A baseline resolution separation is completed <1.7 min; modernization of this method is as easy as exploiting the 5 micron HALO® column - compatible with HPLC and UHPLC instruments.

ANALYTES

1. Acesulfame
2. Benzoic acid
3. Sorbic acid
4. Saccharin sodium



Determination of Sterols (TMS Derivatives) in Sunflower Oil — GC

TEST CONDITIONS

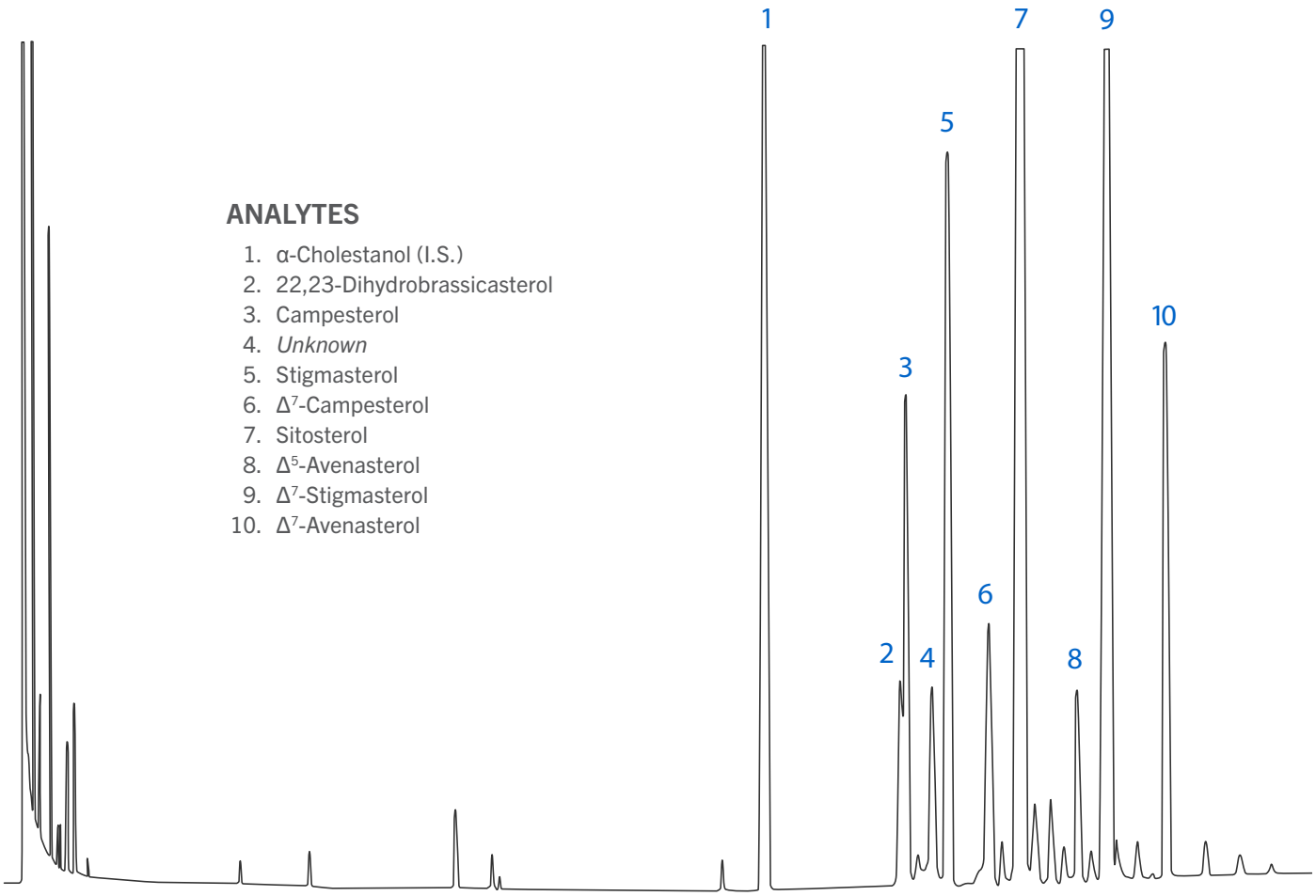
Column:	Avantor® Hichrom HI-LAP, 0.1 µm, 0.32 mm x 25 m
Part Number:	HI463201025
Oven Program:	260 °C Isothermal
Carrier Gas:	Hydrogen, 2 mL/min
Injector:	Split, 300 °C, 1 µL, 1:80 Split Ratio
Detector:	FID, 300 °C

Acknowledgement: Dr C. Mariani, Stazione Sperimentale Oli e Grassi, Via Guiseppe Colombo 79, Milan, Italy

The HI-LAP (Lipid Analysis Phase) is a dedicated and unique stationary phase especially developed for the analysis of lipids, sterols and saturated and unsaturated triglyceride separations.

ANALYTES

1. α-Cholestanol (I.S.)
2. 22,23-Dihydrobrassicasterol
3. Campesterol
4. *Unknown*
5. Stigmasterol
6. Δ^7-Campesterol
7. Sitosterol
8. Δ^5-Avenasterol
9. Δ^7-Stigmasterol
10. Δ^7-Avenasterol

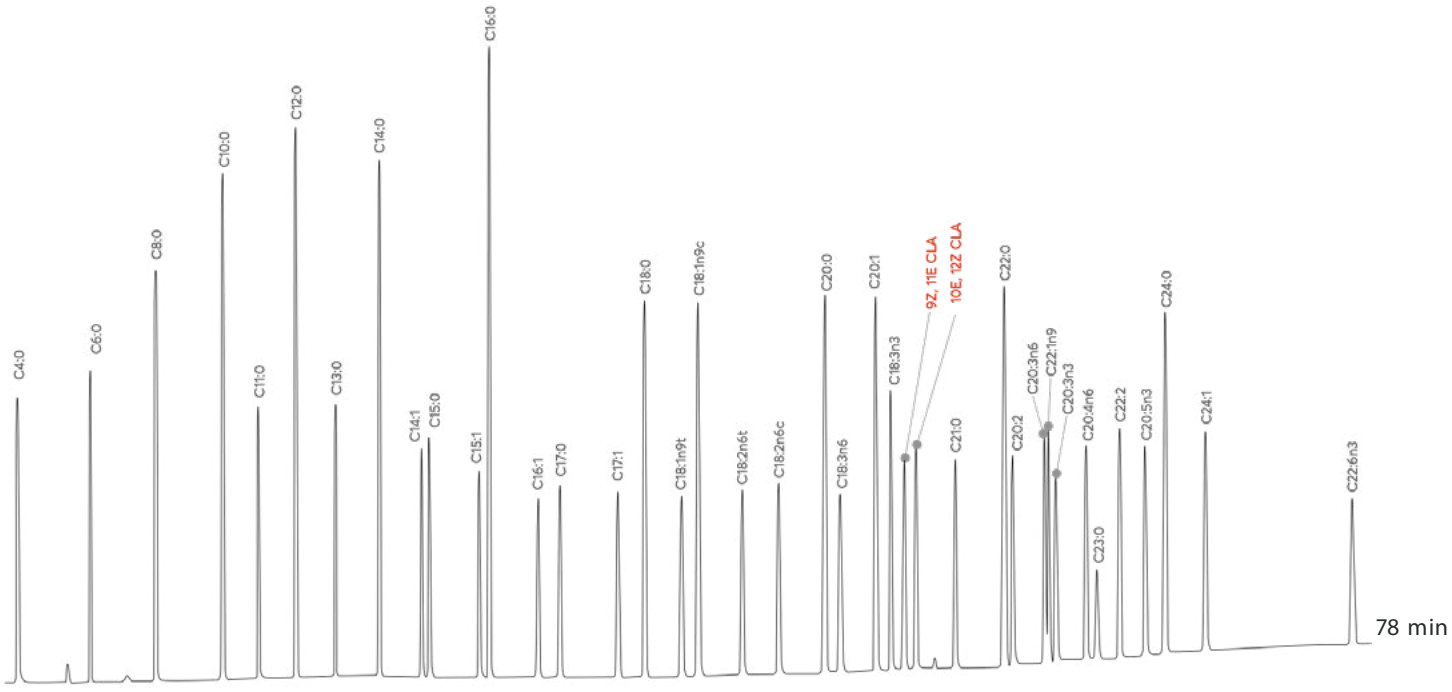


FAMEs and Conjugated Linoleic Acid (OLA) Isomers — GC

TEST CONDITIONS

Column:	Avantor® Hichrom HI-10, 0.2 µm, 0.25 mm x 100 m
Part Number:	HI2625020100
Oven Program:	40 °C (4 min), 10 °C/min, 120 °C (1min), 5 °C/min, 180 °C (18 min), 2 °C /min, 200 °C (1 min), 2 °C/min, 240 °C
Carrier Gas:	Hydrogen, 1.2 mL/min, constant flow
Injector:	Split, 250 °C, 1:30 split ratio
Detector:	FID, 250 °C

The HI-10 is a high polarity phase with a 100% cyanopropyl polysiloxane composition. It is well suited for the analysis of FAMEs, especially for the separation of cis/trans isomers of FAMEs.

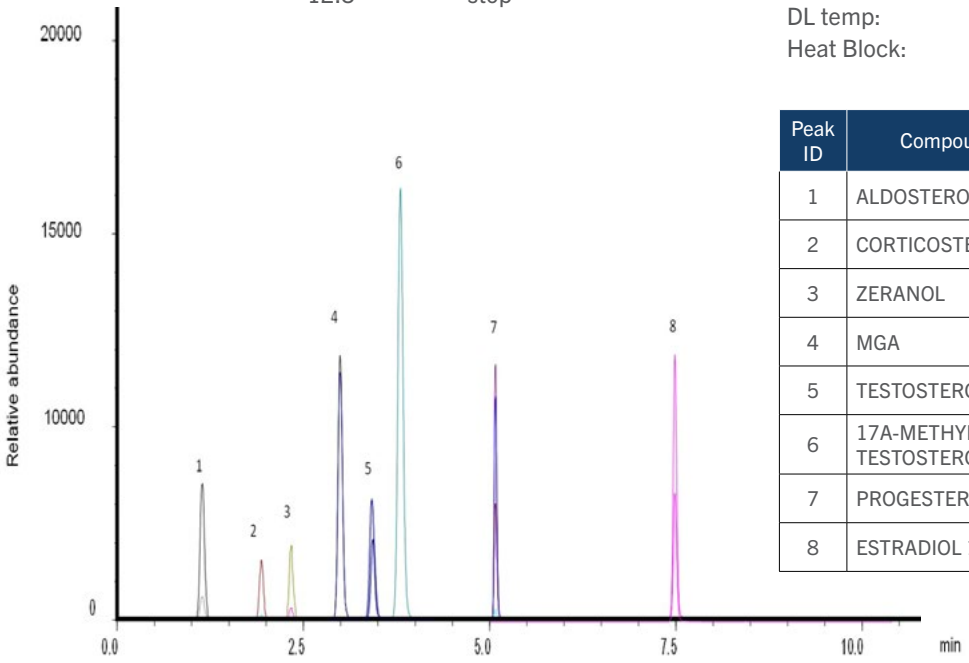


Steroids Spiked in Ground Beef — HPLC/UHPLC

TEST CONDITIONS

Column:	HALO® C18, 90 Å, 2.7 µm, 2.1 x 100 mm
Part Number:	92812-602
Mobile Phase A:	Water, 5 mM Ammonium formate, 0.1 % Formic acid pH 4.0
Mobile Phase B:	Methanol
Flow Rate:	0.3 mL/min
Pressure:	190 bar
Temperature:	50 °C
Injection Volume:	2.0 µL
Sample Solvent:	45/55/ MEOH/H_2O
Detection:	+ESI/ -ESI MS/MS
LC System:	Shimadzu Nexera X2
ESI LCMS system:	Shimadzu LCMS-8040

Time (min)	%B
2.0	14
3.0	60
3.5	60
8.0	100
10.0	100
10.5	0
12.5	stop



For over fifty years, the Food and Drug Administration (FDA) has approved the use of a number of steroids in beef cattle, including natural estrogen, progesterone, testoster-one, and their synthetic versions such as trenbolone acetate (TBA). The function of these drugs is to increase growth rate and the efficiency by which the animals convert the feed they eat into muscle/meat. The drugs are usually administered as implants (dosing of 100-200 days), which are placed under the skin on the back side of the animal's ear. The implants dissolve slowly under the skin and are not re-moved. Although cooking the meat does have some effect on the stability of the steroids in beef, it does not eliminate the exposure, as many steroids are stable at elevated tem-peratures. A standard pahel of steroids spiked into ground beef, and then run on the HALO® 90 Å C18, shows a highly resolved separation of all compounds. The panel consisted of common growth promotors and those used for therapeutic purposes, and was chosen to represent the most common steroids that can be expected to be found in beef, through therapeutic or growth promotion utilization.

MS SOURCE CONDITIONS:

Spray Voltage:	3.0 kV
Nebulizing gas:	2 L/min
Drying gas:	15 L/min
DL temp:	250 °C
Heat Block:	400 °C

Peak ID	Compound	Transition	RT (Min)
1	ALDOSTERONE	361.0000>343.1000	1.154
2	CORTICOSTERONE	347.6000>109.0000	1.965
3	ZERANOL	321.0000>277.0000	2.355
4	MGA	395.0000> 325.1000	3.100
5	TESTOSTERONE	289.0000>109.0000	3.366
6	17A-METHYL-TESTOSTERONE	303.1000>97.0000	3.839
7	PROGESTERONE	315.0000>109.1000	5.085
8	ESTRADIOL 17B	272.4000>159.1000	7.501

250 Pesticide Screen using LC-MS/MS — LC-MS

TEST CONDITIONS

Column: Avantor® ACE® Excel C18, 100 Å, 2 µm, 2.1 x 100 mm
Part Number: EXL1011002U
Mobile Phase: A: 10 mM Ammonium formate + 0.05% Formic acid in H_2O
B: 10 mM Ammonium formate + 0.05% Formic acid in MeOH

Gradient:

Time (min)	%B
0.00	2
0.25	30
10.00	100
12.00	100
12.50	2
14.50	2

Flow Rate: 0.5 mL/min
Temperature: 50 °C
Detection: TSQ Quantiva triple quad MS
Positive mode HESI
Spray voltage: 3500 V
Ion transfer tube temperature: 350 °C
Vaporizer temperature: 300 °C

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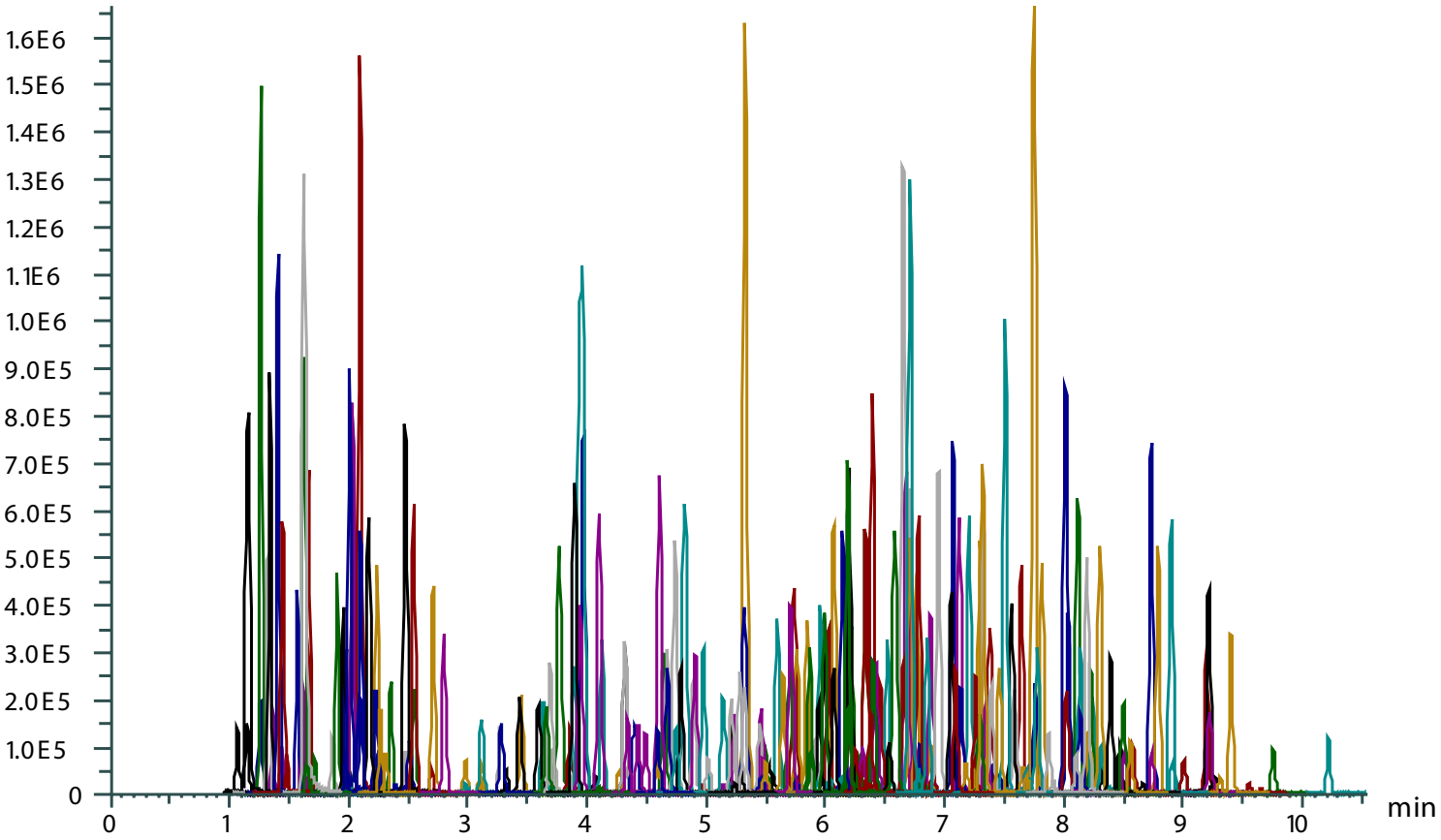


250 Pesticide Screen using LC-MS/MS *(continued)*

Analyte	t_R mins	Adduct	Precursor Ion m/z	Quant Ion m/z	Conf Ion m/z
3-OH Carbofuran	2.25	$[M+H]^+$	238.1	181.2	163.1
5-OH Thiabendazole	1.66	$[M+H]^+$	218.0	147.2	191.1
Abamectin	9.45	$[M+NH_4]^+$	890.5	305.3	567.5
Acephate	1.26	$[M+H]^+$	184.0	143.1	125.1
Acetamiprid	2.24	$[M+H]^+$	223.1	126.1	90.1
Aldicarb	2.95	$[M+NH_4]^+$	208.1	116.1	89.0
Aldicarb sulfone	1.44	$[M+NH_4]^+$	240.1	148.0	86.0
Aldicarb sulfoxide	1.37	$[M+NH_4]^+$	224.1	132.0	89.1
Allethrin	8.33	$[M+H]^+$	303.2	135.1	123.1
Ametoctradin	7.64	$[M+H]^+$	276.2	149.1	176.2
Atrazine	4.64	$[M+H]^+$	216.1	174.0	104.0
Azinphos ethyl	6.30	$[M+H]^+$	346.0	132.1	233.0
Azinphos methyl	5.14	$[M+H]^+$	318.0	132.0	124.9
Azinphos methyl OA	2.98	$[M+H]^+$	302.0	132.2	160.0
Azoxystrobin	5.59	$[M+H]^+$	404.1	372.1	344.1
Bendiocarb	3.72	$[M+H]^+$	224.1	167.1	109.1
Benoxacor	5.23	$[M+H]^+$	260.1	134.1	120.1
Bifenazate	6.27	$[M+H]^+$	301.1	198.0	170.1
Bitertanol	7.41	$[M+H]^+$	338.2	269.3	99.1
Boscalid	5.85	$[M+H]^+$	343.0	307.0	140.0
Bupirimate	6.68	$[M+H]^+$	317.2	210.2	237.3
Buprofezin	8.24	$[M+H]^+$	306.1	201.1	106.1
Cadusafos	7.58	$[M+H]^+$	271.1	159.0	131.0
Carbaryl	4.07	$[M+NH_4]^+$	219.1	145.1	127.0
Carbendazim	2.10	$[M+H]^+$	192.1	160.1	132.1
Carbofuran	3.77	$[M+H]^+$	222.1	165.2	123.2
Carboxin	3.97	$[M+H]^+$	236.1	143.0	93.0
Carfentrazone ethyl	6.88	$[M+H]^+$	412.0	346.1	366.0
Chlorantraniliprole	5.24	$[M+H]^+$	484.0	286.0	194.0
Chlorfenvinphos	7.21	$[M+H]^+$	359.0	170.0	99.1
Chlorimuron ethyl	5.73	$[M+H]^+$	415.1	186.0	83.0
Chlorpyrifos	8.47	$[M+H]^+$	349.9	198.0	97.0

Analyte	t_R mins	Adduct	Precursor Ion m/z	Quant Ion m/z	Conf Ion m/z
Chlorpyrifos OA	6.65	$[M+H]^+$	334.0	278.0	197.9
Clethodim	7.71	$[M+H]^+$	360.3	164.1	136.1
Clofentezine	7.38	$[M+H]^+$	303.0	138.1	102.0
Cloransulam methyl	4.13	$[M+H]^+$	430.0	398.1	370.0
Clothianidin	1.99	$[M+H]^+$	250.0	169.1	132.0
Coumaphos	7.07	$[M+H]^+$	363.0	227.1	307.1
Crotoxyphos	5.86	$[M+NH_4]^+$	332.1	127.1	193.1
Crufomate	6.77	$[M+H]^+$	292.1	236.1	108.1
Cyantraniliprole	4.33	$[M+2+H]^+$	475.0	286.0	444.1
Cyazofamid	6.52	$[M+H]^+$	325.1	108.1	261.2
Cyflufenamid	7.42	$[M+H]^+$	413.1	295.1	203.0
Cymoxanil	2.48	$[M+H]^+$	199.1	128.1	111.1
Cyphenothrin	9.27	$[M+NH_4]^+$	393.2	151.2	123.2
Cyprosulfamide	3.30	$[M+H]^+$	375.1	135.1	254.1
Cyromazine	1.15	$[M+H]^+$	167.1	125.2	68.2
DEF	9.20	$[M+H]^+$	315.1	169.0	113.0
Demeton-S sulfone	2.55	$[M+H]^+$	291.1	235.1	263.1
Dialifos	7.46	$[M+H]^+$	394.0	208.1	181.0
Diazinon	7.12	$[M+H]^+$	305.1	169.1	153.2
Diazinon OA	5.32	$[M+H]^+$	289.1	153.2	233.1
Dichlormid	3.85	$[M+H]^+$	208.0	140.0	81.2
Dichlorvos	3.63	$[M+H]^+$	221.0	109.1	127.0
Dicrotophos	1.87	$[M+H]^+$	238.1	112.2	193.1
Diethofencarb	5.53	$[M+H]^+$	268.2	124.1	180.2
Diflubenzuron	6.66	$[M+H]^+$	311.0	158.0	141.0
Dimethenamid	5.70	$[M+H]^+$	276.1	244.1	168.2
Dimethoate	2.23	$[M+H]^+$	230.1	199.0	125.0
Dimethomorph	5.76, 6.07	$[M+H]^+$	388.1	301.0	165.1
Dinotefuran	1.36	$[M+H]^+$	203.1	129.1	114.2
Dioxacarb	2.26	$[M+H]^+$	224.1	123.1	167.1
Dioxathion	8.10	$[M-C_4H_{10}O_2PS_2]^+$	271.1	97.0	125.0
Disulfoton sulfone	4.59	$[M+H]^+$	307.0	261.1	125.0

250 Pesticide Screen using LC-MS/MS *(continued)*

Analyte	t_R mins	Adduct	Precursor Ion m/z	Quant Ion m/z	Conf Ion m/z
Disulfoton sulfoxide	4.49	$[M+H]^+$	291.0	185.1	213.1
Diuron	4.82	$[M+H]^+$	233.0	72.1	160.0
DMST	3.90	$[M+H]^+$	215.1	106.1	151.0
Dodine	7.56	$[M+H]^+$	228.3	186.3	60.1
Emamectin	8.57	$[M+H]^+$	886.5	158.1	126.1
Ethiofencarb	4.27	$[M+H]^+$	226.1	107.1	169.1
Ethiofencarb sulfone	1.90	$[M+NH_4]^+$	275.1	107.1	201.1
Ethiofencarb sulfoxide	1.98	$[M+H]^+$	242.1	107.1	185.0
Ethion	8.31	$[M+H]^+$	385.0	199.1	143.0
Ethion monoxon	6.73	$[M+H]^+$	369.0	199.0	143.0
Ethiprole	5.77	$[M+NH_4]^+$	413.9	351.0	255.0
Ethofumesate	5.55	$[M+H]^+$	287.1	121.1	241.1
Ethoprop	6.46	$[M+H]^+$	243.1	173.0	131.0
Etofenprox	9.75	$[M+NH_4]^+$	394.2	177.2	107.1
Etoxazole	8.73	$[M+H]^+$	360.2	141.0	304.2
Famoxadone	7.24	$[M+NH_4]^+$	392.2	331.1	238.0
Fenamidone	5.76	$[M+H]^+$	312.1	236.1	92.2
Fenamiphos	6.71	$[M+H]^+$	304.1	217.1	202.0
Fenamiphos sulfone	4.10	$[M+H]^+$	336.1	266.1	188.1
Fenamiphos sulfoxide	3.96	$[M+H]^+$	320.1	233.1	171.1
Fenazaquin	9.21	$[M+H]^+$	307.2	161.2	57.2
Fenhexamid	6.39	$[M+H]^+$	302.1	178.0	97.2
Fenobucarb	5.49	$[M+H]^+$	208.1	95.0	152.0
Fenoxaprop ethyl	8.04	$[M+H]^+$	362.1	288.1	91.1
Fenoxycarb	6.80	$[M+H]^+$	302.1	88.1	116.1
Fenpropimorph	6.42	$[M+H]^+$	304.3	147.2	119.1
Fenpyroximate	8.90	$[M+H]^+$	422.2	366.1	214.2
Fensulfothion	4.89	$[M+H]^+$	309.0	235.0	281.1
Fenuron	2.17	$[M+H]^+$	165.1	72.1	77.1
Flonicamid	1.66	$[M+H]^+$	230.1	203.0	98.0
Fluazifop P butyl	8.12	$[M+H]^+$	384.1	282.2	328.2
Fludioxonil	5.76	$[M+NH_4]^+$	266.1	158.1	131.0

Analyte	t_R mins	Adduct	Precursor Ion m/z	Quant Ion m/z	Conf Ion m/z
Flufenoxuron	8.79	$[M+H]^+$	489.0	158.1	141.1
Flufenpyr ethyl	6.72	$[M+H]^+$	409.1	335.0	307.0
Flumetsulam	2.03	$[M+H]^+$	326.1	129.1	109.0
Flumiclorac pentyl	8.13	$[M+NH_4]^+$	441.1	308.1	354.1
Fluometuron	4.31	$[M+H]^+$	233.1	72.2	46.3
Fluopicolide	6.00	$[M+H]^+$	383.0	173.0	145.0
Fluopyram	6.33	$[M+H]^+$	397.1	173.0	208.0
Fluoxastrobin	6.40	$[M+H]^+$	459.1	427.2	188.1
Fluridone	5.32	$[M+H]^+$	330.1	309.1	290.0
Flusilazole	6.77	$[M+H]^+$	316.1	247.2	165.1
Fluthiacet methyl	6.88	$[M+H]^+$	404.0	344.0	273.9
Flutolanil	5.95	$[M+H]^+$	324.1	262.0	282.0
Flutriafol	4.74	$[M+H]^+$	302.1	70.1	123.1
Fluxapyroxad	6.02	$[M+H]^+$	382.1	342.1	314.1
Forchlorfenuron	4.78	$[M+H]^+$	248.1	129.1	93.1
Formetanate HCl	1.26	$[M+H]^+$	222.0	165.1	120.0
Fosthiazate	4.40	$[M+H]^+$	284.1	104.1	228.1
Hexaconazole	7.29	$[M+H]^+$	314.1	158.9	70.0
Hexythiazox	8.51	$[M+H]^+$	353.1	228.0	168.0
Imazalil	5.14	$[M+H]^+$	297.1	159.1	255.1
Imazosulfuron	5.28	$[M+H]^+$	413.0	153.0	156.1
Imidacloprid	1.96	$[M+H]^+$	256.1	209.1	175.0
Imiprothrin	6.34	$[M+H]^+$	319.2	151.1	123.1
Indaziflam	6.58	$[M+H]^+$	302.2	158.1	145.1
Indoxacarb	7.75	$[M+H]^+$	528.1	249.0	150.1
Ipconazole	7.81	$[M+H]^+$	334.2	70.1	125.0
Iprovalicarb	6.31	$[M+H]^+$	321.2	119.1	186.2
Isofenphos	7.39	$[M+H]^+$	346.1	217.0	245.1
Isoprocarb	4.67	$[M+H]^+$	194.1	95.1	152.2
Isoproturon	4.79	$[M+H]^+$	207.2	72.2	165.2
Kresoxim methyl	6.90	$[M+H]^+$	314.1	267.2	222.1
Lactofen	8.22	$[M+NH_4]^+$	479.1	344.1	223.0

250 Pesticide Screen using LC-MS/MS *(continued)*

Analyte	t_R mins	Adduct	Precursor Ion m/z	Quant Ion m/z	Conf Ion m/z
Lenacil	4.67	$[M+H]^+$	235.1	153.1	136.1
Leptophos OA	7.75	$[M+2+H]^+$	396.9	155.1	364.9
Linuron	5.46	$[M+H]^+$	249.0	182.1	160.1
Malathion	5.92	$[M+H]^+$	331.0	127.1	285.1
Malathion OA	3.89	$[M+H]^+$	315.1	127.1	99.0
Mandipropamid	5.94	$[M+H]^+$	412.1	328.2	356.2
Mefenpyr diethyl	7.26	$[M+H]^+$	373.1	327.1	160.0
Mepanipyrim	6.21	$[M+H]^+$	224.1	106.2	77.1
Mesotrione	2.01	$[M+H]^+$	340.1	228.1	104.1
Metaflumizone	8.30	$[M+H]^+$	507.1	178.0	287.1
Metalaxyl	4.91	$[M+H]^+$	280.1	220.1	192.1
Metaldehyde	2.02	$[M+NH_4]^+$	194.1	62.2	45.3
Metconazole	7.32	$[M+H]^+$	320.2	70.1	125.0
Methamidophos	1.16	$[M+H]^+$	142.0	94.2	125.1
Methidathion	4.97	$[M+NH_4]^+$	320.0	145.1	85.1
Methiocarb	5.64	$[M+H]^+$	226.1	169.2	121.1
Methiocarb sulfone	2.35	$[M+NH_4]^+$	275.0	122.1	201.1
Methiocarb sulfoxide	2.10	$[M+H]^+$	242.1	185.1	122.1
Methomyl	1.61	$[M+H]^+$	163.1	106.1	88.1
Methoxyfenozide	6.04	$[M+H]^+$	369.2	149.1	313.1
Metolcarb	3.28	$[M+H]^+$	166.1	109.1	94.1
Metribuzin	3.59	$[M+H]^+$	215.1	187.1	131.1
Mevinphos	2.70	$[M+NH_4]^+$	242.1	193.1	127.1
Monocrotophos	1.71	$[M+H]^+$	224.1	193.0	127.0
Monolinuron	4.16	$[M+H]^+$	215.1	126.1	148.1
Myclobutanil	6.15	$[M+H]^+$	289.1	125.0	70.1
Nicosulfuron	3.45	$[M+H]^+$	411.1	182.0	213.0
Norflurazon	4.98	$[M+H]^+$	304.0	160.0	140.0
Norflurazon desmethyl	4.43	$[M+H]^+$	290.0	179.0	140.0
Omethoate	1.33	$[M+H]^+$	214.0	183.0	125.0
Oxamyl	1.48	$[M+NH_4]^+$	237.1	72.0	90.0
Oxamyl oxime	1.34	$[M+H]^+$	163.1	72.1	90.1

Analyte	t_R mins	Adduct	Precursor Ion m/z	Quant Ion m/z	Conf Ion m/z
Oxydemeton methyl	1.57	$[M+H]^+$	247.0	169.1	109.1
Oxydemeton methyl sulfone	1.62	$[M+H]^+$	263.0	169.0	109.0
Parathion methyl OA	3.10	$[M+H]^+$	248.0	202.0	109.1
Parathion OA	4.61	$[M+H]^+$	276.1	220.1	248.1
Pencycuron	7.50	$[M+H]^+$	329.1	125.1	89.1
Penflufen	6.95	$[M+H]^+$	318.2	234.1	141.0
Penthiopyrad	7.05	$[M+H]^+$	360.1	177.1	276.1
Phenothrin	9.56	$[M+H]^+$	351.2	183.1	168.0
Phenthoate	6.81	$[M+H]^+$	321.0	247.1	79.1
Phorate OA	5.10	$[M+H]^+$	245.0	75.2	47.2
Phorate OA Sulfone	2.51	$[M+H]^+$	277.0	155.0	127.0
Phorate OA Sulfoxide	2.31	$[M+H]^+$	261.0	153.0	81.0
Phorate Sulfone	4.61	$[M+H]^+$	293.0	114.9	171.0
Phorate Sulfoxide	4.49	$[M+H]^+$	277.0	170.9	199.0
Phosalone	7.35	$[M+H]^+$	368.0	182/0	111.1
Phosmet	5.21	$[M+H]^+$	318.0	160.1	133.1
Phosmet OA	3.12	$[M+H]^+$	302.0	160.0	133.0
Phosphamidon	3.43	$[M+H]^+$	300.1	127.1	174.1
Phoxim	7.25	$[M+H]^+$	299.1	77.2	129.1
Picoxystrobin	6.79	$[M+H]^+$	368.1	145.0	115.0
Pirimicarb	4.24	$[M+H]^+$	239.2	182.1	72.0
Pirimicarb Desmethyl	2.71	$[M+H]^+$	225.1	168.2	72.1
Pirimiphos Methyl	7.34	$[M+H]^+$	306.1	164.2	108.1
Prallethrin	7.69	$[M+H]^+$	301.2	133.0	151.2
Prochloraz	7.39	$[M+H]^+$	376.0	308.1	70.1
Profoxydim	7.71, 9.00	$[M+H]^+$	466.2	280.0	180.0
Promecarb	5.88	$[M+H]^+$	208.1	109.0	151.1
Propamocarb	1.41	$[M+H]^+$	189.1	102.0	144.0
Propaquizafop	8.21	$[M+H]^+$	444.1	299.2	371.2
Propargite	8.74	$[M+NH_4]^+$	368.2	231.2	175.1
Propetamphos	6.13	$[M+H]^+$	282.1	138.1	156.1
Propoxur (S)	3.69	$[M+H]^+$	210.1	168.2	111.1

250 Pesticide Screen using LC-MS/MS *(continued)*

Analyte	t_R mins	Adduct	Precursor Ion m/z	Quant Ion m/z	Conf Ion m/z
Prosulfuron	5.29	$[M+H]^+$	420.1	167.1	141.1
Pymetrozine	1.44	$[M+H]^+$	218.1	105.1	78.1
Pyraclostrobin	7.30	$[M+H]^+$	388.1	163.1	194.1
Pyraflufen Ethyl	7.13	$[M+H]^+$	413.0	339.0	253.1
Pyrazophos	7.31	$[M+H]^+$	374.1	222.2	194.1
Pyridaben	9.22	$[M+H]^+$	365.1	309.0	147.1
Pyridalyl	10.21	$[M+2+H]^+$	492.0	110.9	164.0
Pyrimethanil	5.45	$[M+H]^+$	200.1	107.1	168.1
Pyriproxyfen	8.39	$[M+H]^+$	322.1	96.0	227.1
Quinalphos	6.78	$[M+H]^+$	299.1	163.1	147.1
Quinoxyfen	8.50	$[M+H]^+$	308.0	197.1	214.1
Quizalofop Ethyl	8.01	$[M+H]^+$	373.1	299.2	255.1
Resmethrin	9.40	$[M+H]^+$	339.2	128.1	171.1
Rimsulfuron	3.94	$[M+H]^+$	432.1	182.1	139.0
Rotenone	6.71	$[M+H]^+$	395.2	213.2	192.1
Saflufenacil	5.32	$[M+H]^+$	501.1	349.1	198.0
Sedaxane	6.20, 6.54	$[M+H]^+$	332.2	159.0	139.0
Sethoxydim	8.03	$[M+H]^+$	328.2	178.0	220.1
Simazine	3.66	$[M+H]^+$	202.1	104.1	132.1
Spinetoram	8.14	$[M+H]^+$	748.5	142.1	203.1
Spinosad A	7.69	$[M+H]^+$	732.5	142.1	98.0
Spinosad D	8.10	$[M+H]^+$	746.5	142.1	98.0
Spirodiclofen	8.91	$[M+H]^+$	411.1	313.1	71.1
Spiromesifen	8.66	$[M+NH_4]^+$	388.1	273.1	187.0
Spiromesifen Alcohol	5.01	$[M+H]^+$	273.2	187.1	179.1
Spirotetramat	6.38	$[M+H]^+$	374.2	302.3	216.2
Spiroxamine	5.95	$[M+H]^+$	298.3	144.2	100.2
Sulfoxaflor	2.39	$[M+NH_4]^+$	295.2	174.1	154.1
Sulprofos	8.56	$[M+H]^+$	323.0	219.1	139.1

Analyte	t_R mins	Adduct	Precursor Ion m/z	Quant Ion m/z	Conf Ion m/z
TCMTB	5.48	$[M+H]^+$	239.0	180.0	136.0
Tebufenozide	6.78	$[M+H]^+$	353.2	133.0	104.8
Tebufenpyrad	8.19	$[M+H]^+$	334.2	117.1	145.1
Tebuthiuron	3.89	$[M+H]^+$	229.1	172.0	116.0
Tepraloxydim	4.10, 6.19	$[M+H]^+$	342.2	250.1	166.1
Terbufos Sulfone	5.46	$[M+H]^+$	321.0	115.0	143.0
Terbufos Sulfoxide	5.49	$[M+H]^+$	305.1	97.0	187.0
Terbuthylazine	5.71	$[M+H]^+$	230.1	174.1	104.1
Tetrachlorvinphos	6.86	$[M+2+H]^+$	366.9	127.1	206.0
Tetramethrin	7.91, 8.10	$[M+H]^+$	332.2	164.1	135.1
Thiabendazole	2.48	$[M+H]^+$	202.0	175.0	131.1
Thiacloprid	2.55	$[M+H]^+$	253.0	126.1	99.1
Thiamethoxam	1.65	$[M+H]^+$	292.0	211.1	181.1
Thifensulfuron Methyl	3.28	$[M+H]^+$	388.0	167.1	205.0
Thiobencarb	7.46	$[M+H]^+$	258.1	125.0	89.0
Thiodicarb	4.34	$[M+H]^+$	355.1	163.2	88.1
Thionazin	4.74	$[M+H]^+$	249.1	193.1	97.0
Topramezone	1.63	$[M+H]^+$	364.1	334.1	125.1
Triadimefon	6.07	$[M+H]^+$	294.1	197.0	225.0
Triadimenol	6.25	$[M+H]^+$	296.1	70.2	99.0
Triazophos	6.19	$[M+H]^+$	314.1	162.1	119.1
Tribenuron Methyl	4.59	$[M+H]^+$	396.1	155.1	181.1
Trichlorfon	2.26	$[M+H]^+$	256.9	109.0	221.0
Tricyclazole	2.80	$[M+H]^+$	190.0	163.1	136.1
Trifloxystrobin	7.78	$[M+H]^+$	409.1	186.2	206.2
Triflumizole	7.87	$[M+H]^+$	346.1	278.0	73.0
Triforine	5.23	$[M+2+H]^+$	434.9	213.0	98.2
Zoxamide	7.09	$[M+H]^+$	336.0	187.0	159.0

LC-MS Screening Comparison of Mycotoxins in Craft and Home Brewed Beers — HPLC/UHPLC

TEST CONDITIONS

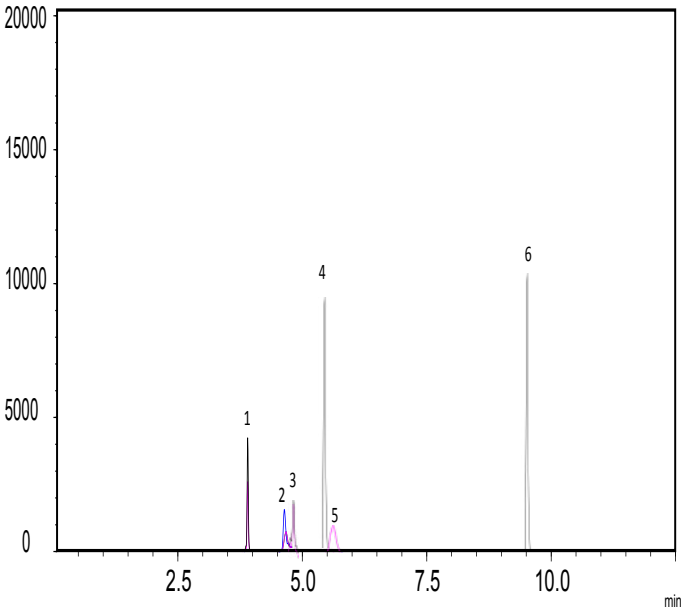
Column: HALO® PFP, 90 Å, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-609
Mobile Phase A: Water, 5 mM Ammonium formate, 0.1 % Formic acid
Mobile Phase B: Methanol, 0.1% Formic acid
Gradient:

Time (min)	%B
0.0	0
0.5	14
2.0	14
3.0	60
3.5	60
8.0	100
10.0	100
10.5	0
12.5	End

Flow Rate: 0.4 mL/min
Pressure: 290 bar
Temperature: 40 °C
Injection Volume: 7.0 µL
Sample Solvent: 49/50/1 ACN/H_2O/Acetic acid
Detection: +ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS System: Shimadzu LCMS-8040

Mycotoxin contamination can have serious health implications. Although there are no set regulatory limits for mycotoxins in beer, most governments have clear levels for mycotoxins in various types of grain and animal feed. For example, in the United States, most levels are in the mid to high ppb range. Despite relatively low levels of mycotoxin activity in the beer, given the propensity for people to indulge in excessive drinking, and the cumulative effects of the toxicity of these compounds, excessive consumption would lead to a cumulative toxic effect, which warrants further analysis and regulation. Beer analysis can be challenging due to matrix effects and interference, often resulting in low sensitivity and ambiguous results; therefore, it is critical to have a column that has superior performance. The HALO® 90 Å PFP can not only meet these challenges, but exceed them by demonstrating superior performance and sensitivity, making it an ideal column to be used in environmental, and, specifically, mycotoxin analysis.

LC-MS Screening Comparison of Mycotoxins in Craft and Home *(continued)*

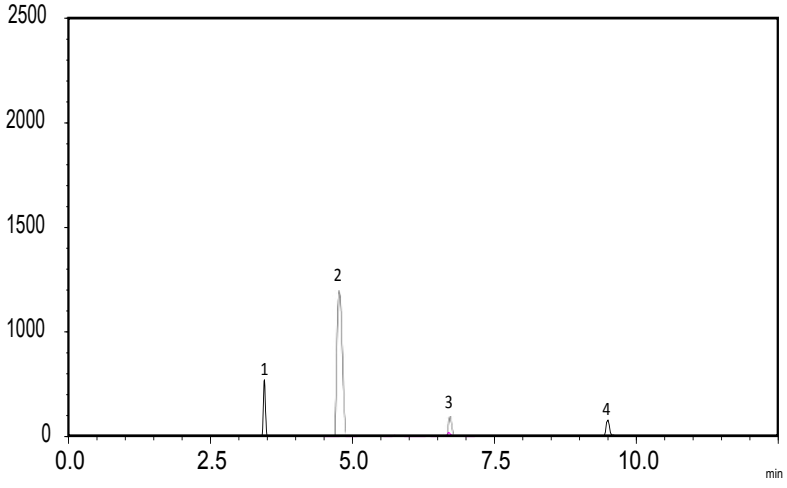


CRAFT-BREWED BEER

Peak ID	Mycotoxin	Retention Time (min)	Precursor Ion	Product Ion
1	T-2 Toxin	3.95	489.2	245.1
2	Aflatoxin G2	4.65	331.1	189.2
3	15-acetylde-oxynivalenol	4.88	339.1	321.1
4	Aflatoxin B2	5.52	315.1	287.1
5	Aflatoxin M1	5.75	329.1	273.3
6	Zearalenone	9.55	319.1	283.2

HOME BREWED BEER

Peak ID	Mycotoxin	Retention Time (min)	Precursor Ion	Product Ion
1	T-2 Toxin	3.95	489.2	245.1
2	15-acetylde-oxynivalenol	4.88	339.1	321.1
3	Aflatoxin M1	5.75	329.1	273.3
4	Zearalenone	9.55	319.1	283.2



Mycotoxins by LC-MS/MS — LC-MS

TEST CONDITIONS

Column: Avantor® ACE® Excel C18-AR, 100 Å, 2 µm, 2.1 x 50 mm
Part Number: EXL1090502U
Mobile Phase: A: 1 mM Ammonium acetate, 0.5% Acetic acid in H_2O
B: 1 mM Ammonium acetate, 0.5% Acetic acid in 95% MeOH

Gradient:

Time (min)	%B
0.0	40
1.0	40
2.4	60
6.8	87

Flow Rate: 0.6 mL/min
Temperature: 40 °C
Injection: 2 µL
Detection: AB SCIEX triple quad 5500
Positive ESI mode
Source temperature: 500 °C
IonSpray voltage: 5500 V

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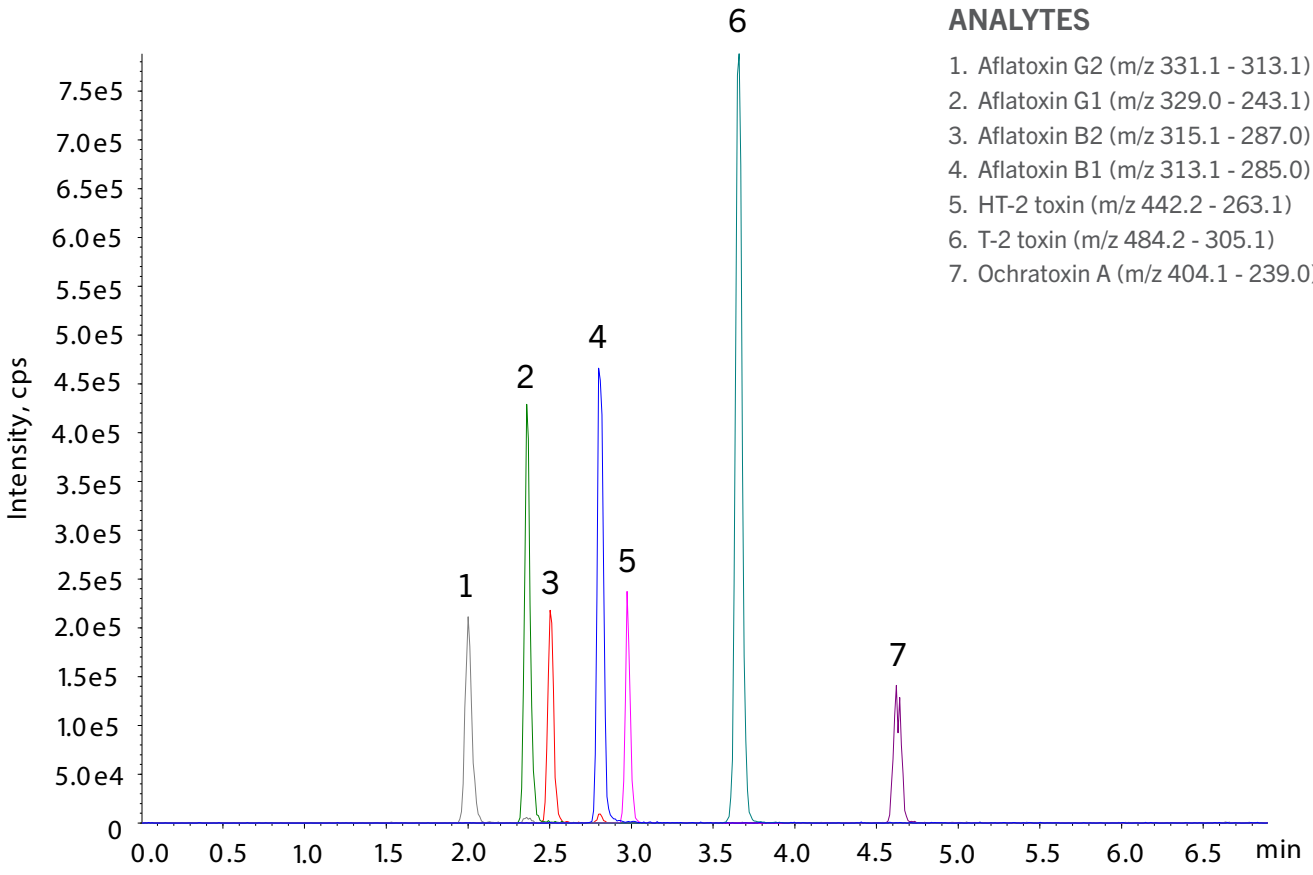

ANALYTES

1. Aflatoxin G2 (m/z 331.1 - 313.1)
2. Aflatoxin G1 (m/z 329.0 - 243.1)
3. Aflatoxin B2 (m/z 315.1 - 287.0)
4. Aflatoxin B1 (m/z 313.1 - 285.0)
5. HT-2 toxin (m/z 442.2 - 263.1)
6. T-2 toxin (m/z 484.2 - 305.1)
7. Ochratoxin A (m/z 404.1 - 239.0)

Paralytic Shellfish Poisoning (PSP) Toxins — HPLC

TEST CONDITIONS

Column: Avantor® ACE® UltraCore SuperC18, 95 Å, 5 µm, 4.6 x 150 mm
Part Number: CORE5A1546U
Mobile Phase: A: 0.1 M Ammonium formate in H_2O
B: 0.1 M Ammonium formate in H_2O/MeOH (95:5 v/v)

Gradient:

Time (min)	%B
0.00	0
2.00	0
4.00	80
5.50	80
5.51	0
7.00	0

Flow Rate: 2 mL/min
Temperature: 20 °C
Injection Volume: 30 µL
Detection: Fluorescence λ_{Ex} 340 nm, λ_{Em} 395nm
Sample: Prechromatographic oxidation with hydrogen peroxide and periodate

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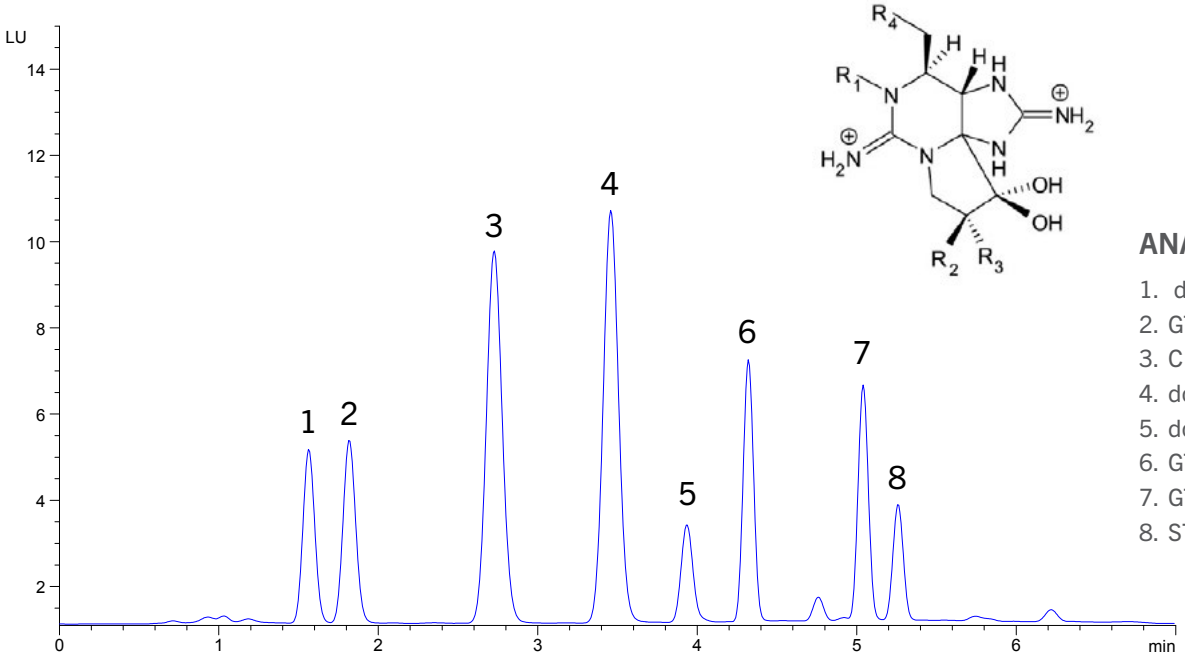
STRUCTURES

PST Variant	R1	R2	R3	R4
STX	H	H	H	H_2N-COO
NEO	OH	H	H	H_2N-COO
GTX1	OH	H	OSO_3^-	H_2N-COO
GTX2	H	H	OSO_3^-	H_2N-COO
GTX3	H	OSO_3^-	H	H_2N-COO
GTX4	OH	OSO_3^-	H	H_2N-COO
GTX5 (B1)	H	H	H	O_3S-NH-COO
C1	H	H	OSO_3^-	O_3S-NH-COO
C2	H	OSO_3^-	H	O_3S-NH-COO
dcSTX	H	H	H	OH
dcNEO	OH	H	H	OH
dcGTX1	OH	H	OSO_3^-	OH
dcGTX2	H	H	OSO_3^-	OH
dcGTX3	H	OSO_3^-	H	OH



ANALYTES

1. dcGTX2,3
2. GTX1/4 + dcGTX2,3
3. C1,2
4. dcSTX + dcNEO
5. dcSTX + NEO
6. GTX2/3 + GTX1/4
7. GTX5
8. STX + NEO

Patulin and HMF — HPLC/UHPLC

In the United States the FDA maintains different limits for mycotoxins in many foods and beverages. Patulin, a mycotoxin that is produced from mold on a variety of fruits has a limit of 50 ug/kg. For analysis, patulin was spiked into apple juice and the sample was cleaned up using solid phase extraction. Interfering analytes such as 5-(Hydroxymethyl) furfural (HMF) can make analysis more challenging. This separation shows the two compounds separated on a HALO® Biphenyl column with enough resolution to easily check for sample recovery.

TEST CONDITIONS

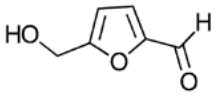
Column:	HALO® Biphenyl, 90 Å, 2.7 µm, 2.1 x 100 mm
Part Number:	92812-611
Mobile Phase A:	Water with 0.1% Acetic acid
Mobile Phase B:	Acetonitrile with 0.1% Acetic acid

Gradient:

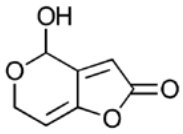
Time (min)	%B
0.0	5
2.6	90

Flow Rate:	0.6 mL/min
Initial Pressure:	285 bar
Temperature:	40 °C
Detection:	UV 276 nm, PDA
Injection Volume:	1.0 µL
Sample:	Apple Juice spiked with HMF and 50 ng/mL Patulin
Data Rate:	100 Hz
Response Time:	0.025 sec
Flow Cell:	1 µL
LC System:	Shimadzu Nexera X2

STRUCTURES



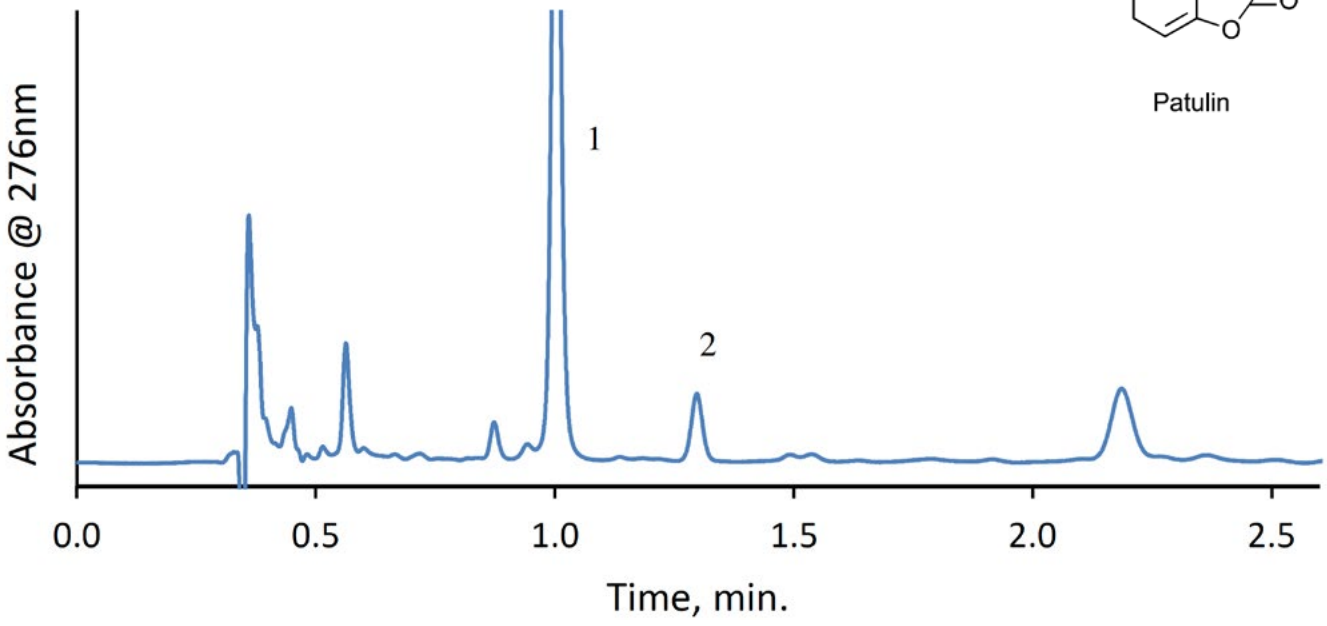
5-(Hydroxymethyl) furfural



Patulin

ANALYTES

1. 5-(Hydroxymethyl) furfural
2. Patulin



Pesticide Screening of Barley — UHPLC

Pesticide screening methods can help show whether there is a concern with your soil, crops, and even water supply. A pesticide screening is performed on a sample of barley using a HALO® 90 Å LPH-C18 column. This column is ideal for low pH testing conditions based on its sterically protected ligand which helps reduce acid hydrolysis of the stationary phase leading to an increase in column lifetime.

TEST CONDITIONS

Column: HALO® LPH-C18, 90 Å, 2 µm, 2.1 x 100 mm
Part Number: 91822-616
Mobile Phase A: Water, 0.1% Formic acid
Mobile Phase B: Acetonitrile, 0.1% Formic acid
Gradient:

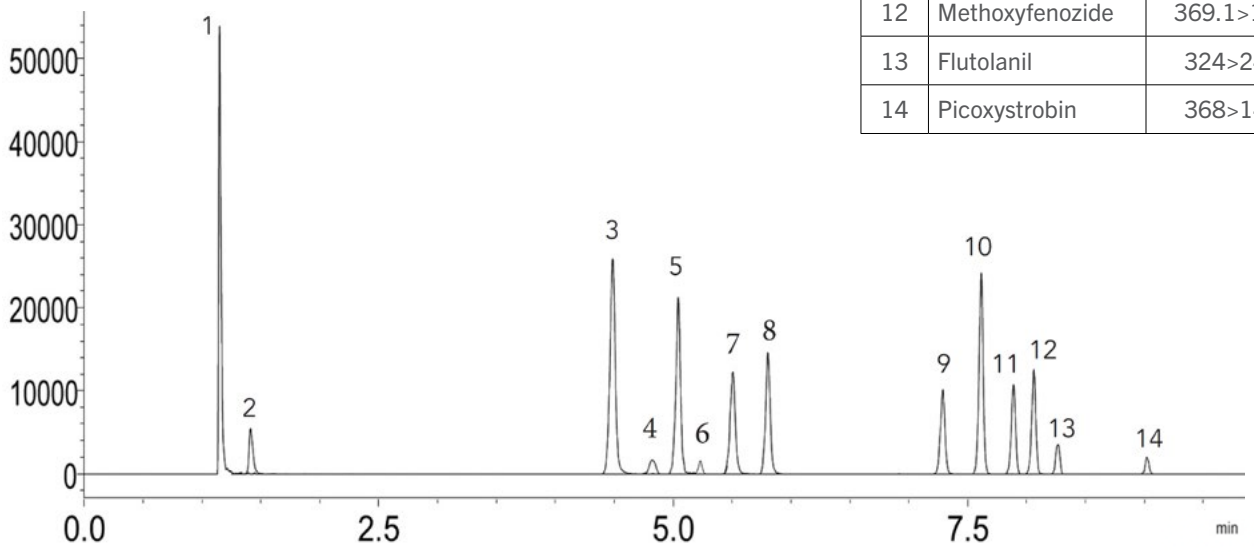
Time (min)	%B
0.0	30
1.0	30
12.0	100
16.0	100

Flow Rate: 0.2 mL/min
Pressure: 235 bar
Temperature: 30 °C
Detection: +ESI MS/MS
Injection Volume: 2 µL
Sample Solvent: Methanol
MS System: Shimadzu 8040
LC System: Shimadzu Nexera X2

MS CONDITIONS

Nebulizer Gas Flow: 3 L/min
DL Temperature: 250 °C
Heat Block Temperature: 400 °C
Drying Gas Flow: 18 L/min

Peak #	Compound	Transition	CE
1	Carbendazim	192>160.1	-21
2	Dicrotophos	238>112	-22
3	Azamethiphos	324.9>183	-17
4	Pyrimethanil	200.10>107.2	-25
5	Carbofuran	222>123	-22
6	Dodemorph	282.2>116.1	-25
7	Atrazine	216.03>174.1	-17
8	Diuron	232.94>72	-17
9	Iprovalicarb	321.1>119	-30
10	Azoxystrobin	404.04>372.1	-14
11	Fluopram	396.98>208	-25
12	Methoxyfenozide	369.1>149.1	-25
13	Flutolanil	324>242.1	-28
14	Picoxystrobin	368>145.1	-25



Whey Proteins from Whole Milk — HPLC

TEST CONDITIONS

Column: Avantor® ACE® C4-300, 300 Å, 3 µm, 2.1 x 150 mm
Part Number: ACE2131502
Mobile Phase: A: 0.5% Formic acid in H_2O
B: 0.5% Formic acid in MeCN

Gradient:

Time (min)	%B
0	35
16	43
17	80
20	80
21	35
31	35

Flow Rate: 0.4 mL/min
Temperature: 40 °C
Injection: 10 µL
Detection: ESI-MS (+ve)

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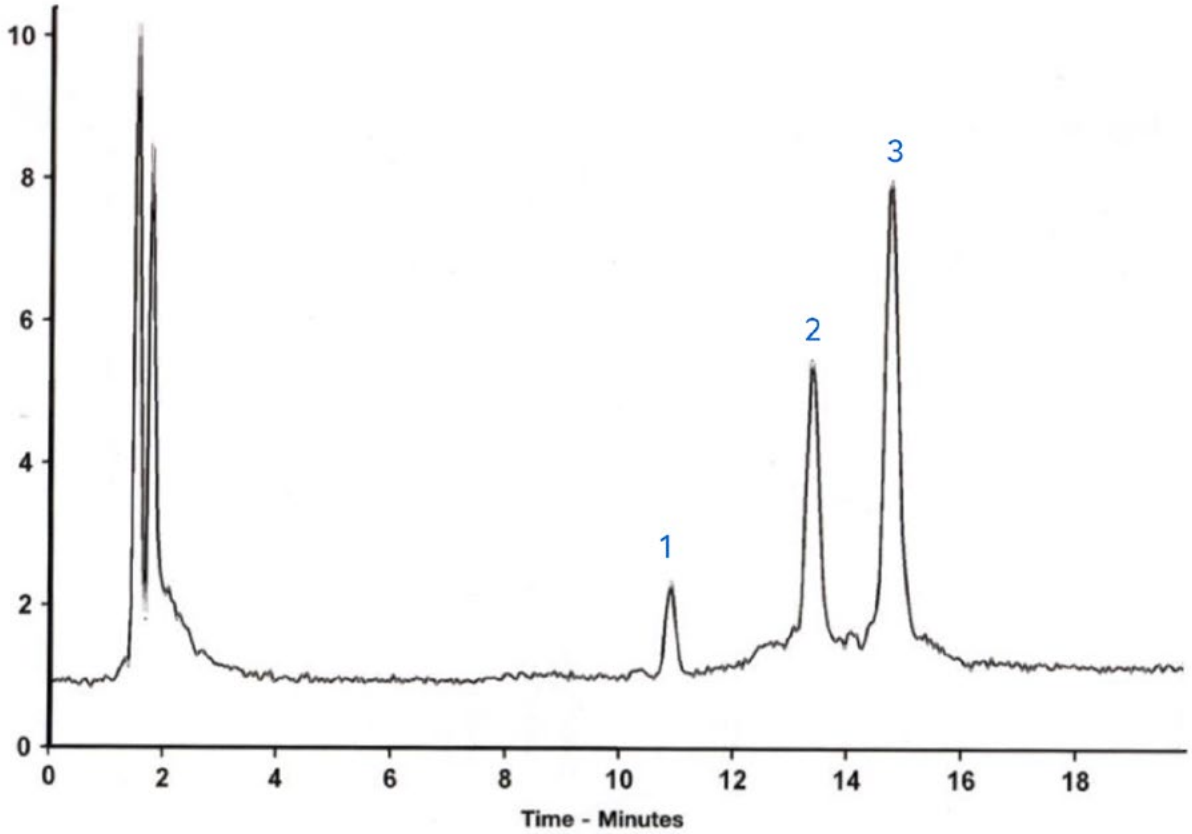
ANALYTES

1. α-Lactalbumin
2. β-Lactoglobulin B
3. β-Lactoglobulin A



Garlic Analysis (I) — HPLC

TEST CONDITIONS

Column:	Avantor® ACE® C18, 100 Å, 5 µm, 4.6 x 250 mm
Part Number:	ACE1212546
Mobile Phase:	H_2O/MeOH (50:50 v/v)
Flow Rate:	1 mL/min
Temperature:	30 °C
Injection Volume:	20 µL
Detection:	UV, 210 nm

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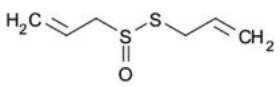
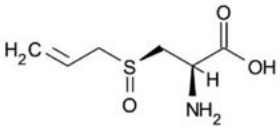
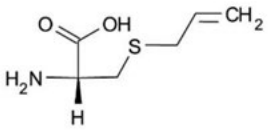
STRUCTURES



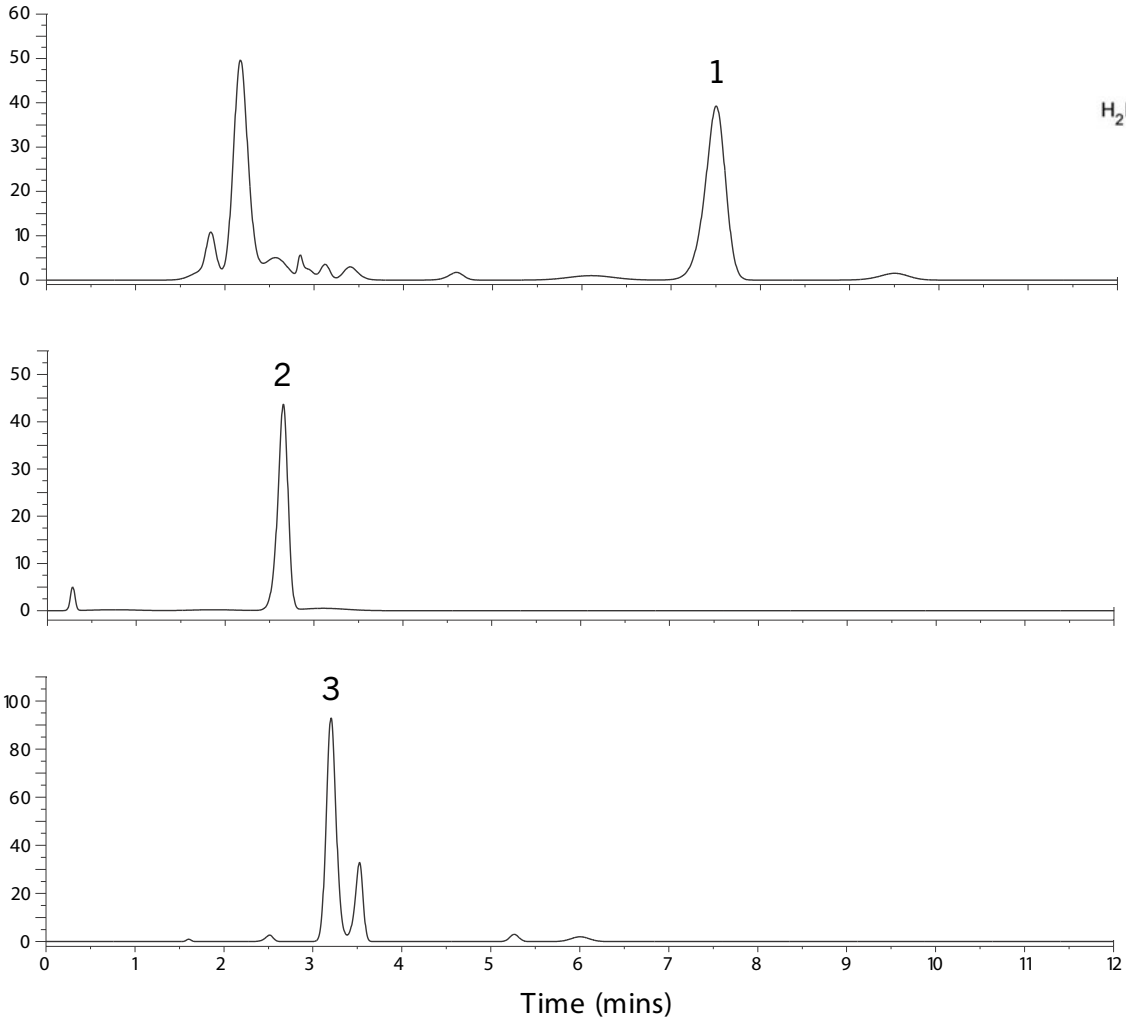
Allicin



Alliin



Deoxyalliin



ANALYTES

1. Allicin
2. Alliin
3. Deoxyalliin

Garlic Analysis (II) — HPLC

TEST CONDITIONS

Column: Avantor® ACE® C18, 100 Å, 5 µm, 4.6 x 250 mm
Part Number: ACE1212546
Mobile Phase: A: H_2O
B: MeCN

Gradient:

Time (min)	%B
0	40
20	100
25	100

Flow Rate: 1 mL/min
Temperature: 30 °C
Injection Volume: 20 µL
Detection: UV, 254 nm

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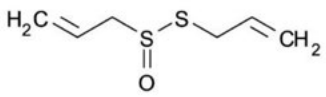
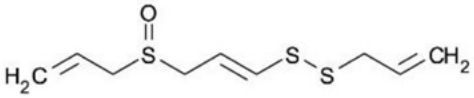
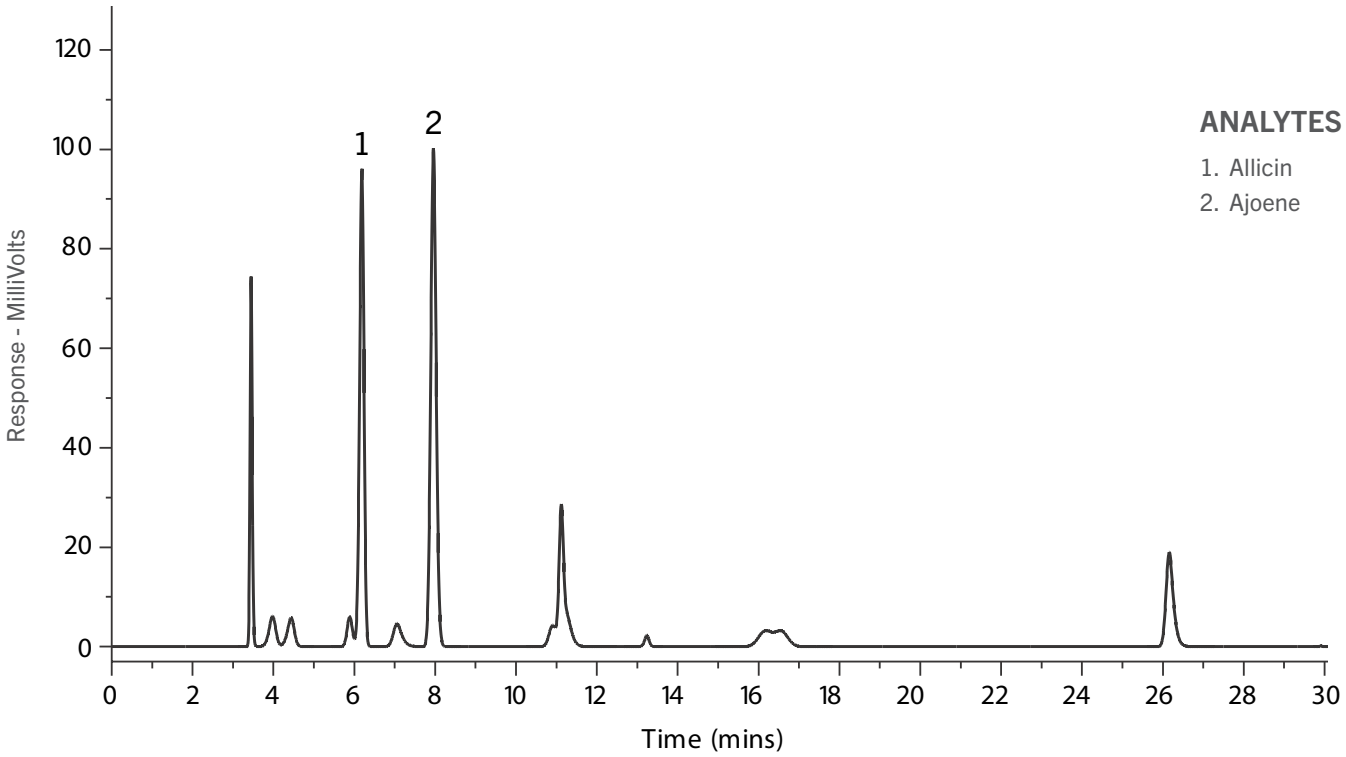
STRUCTURES



Allicin



Ajoene



ANALYTES

1. Allicin
2. Ajoene

Additives and Intense Sweeteners — HPLC

TEST CONDITIONS

Column: Avantor® ACE® C18, 100 Å, 5 µm, 4.0 x 250 mm
Part Number: ACE1212504
Mobile Phase:
A: H_2O
B: MeCN
C: 1% TFA in H_2O

Gradient:

Time (min)	%A	%B	%C
0	88	2	10
25	50	40	10
30	50	60	10
35	88	2	10

Flow Rate: 1 mL/min
Temperature: 30 °C
Detection: ELSD

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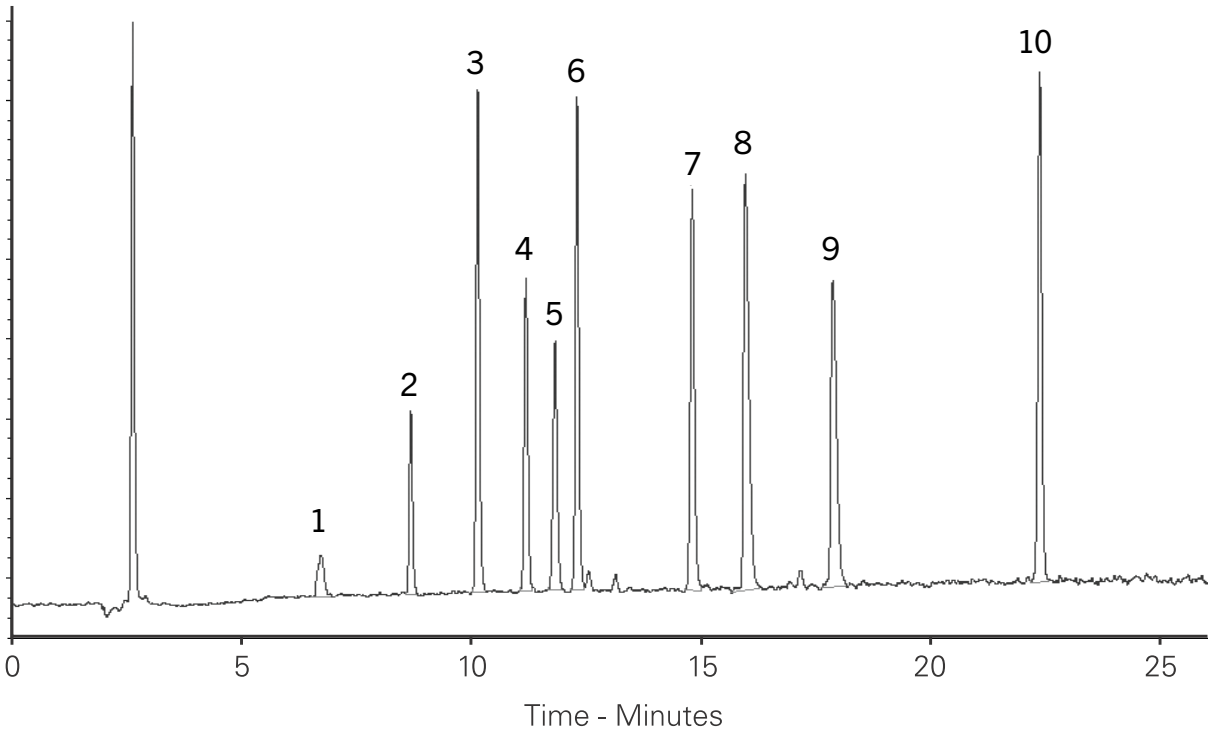
ANALYTES

1. Acesulfame K
2. Theobromine
3. Theophylline
4. Cyclamate
5. Saccharin
6. Caffeine
7. Sucralose
8. Quinine sulphate
9. Aspartame
10. Neohesperidin dihydrochalcone



Analysis of Sucrose and Lactose in Chocolate Using a Penta-HILIC Column — HPLC/UHPLC

TEST CONDITIONS

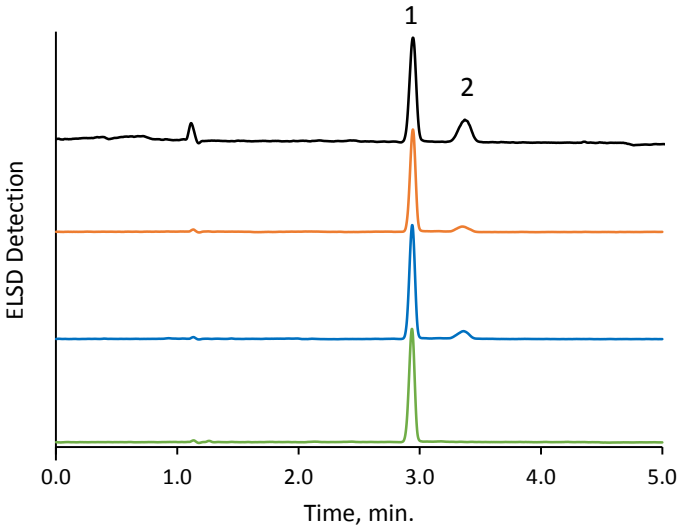
Column:	HALO® Penta-HILIC 90 Å, 2.7 µm, 4.6 x 150 mm
Part Number:	92814-705
Mobile Phase A:	Water
Mobile Phase A:	Acetonitrile
Flow Rate:	1.4 mL/min
Pressure:	213 bar
Temperature:	65 °C
Detection:	ELSD, 40 °C, 3.3 bar
Injection Volume:	15 µL
Sample Solvent:	80/20 ACN/ Water
Response Time:	0.10 sec
DataRate:	10 Hz
LC System:	Shimadzu NexeraX2

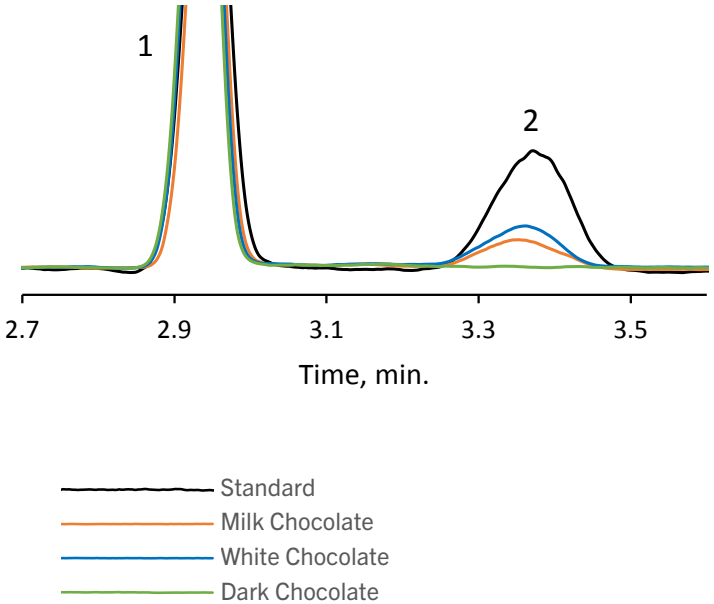
Chocolate is a very well-known, popular, food type worldwide. It is used for all occasions and can even have some health benefits as well, which include improved blood flow and brain function. There are four main types of chocolate to choose from- milk, white, dark, and raw.

Analysis of three different types of chocolate (milk, white, and dark) was carried out (or performed) in HILIC mode using an ELSD detector. The compounds of interest were sucrose and lactose. The HALO® Penta-HILIC column was used, which has a polar ligand with 5 hydroxyl groups tethered via novel proprietary linkage chemistry to Fused-Core® silica particles.

ANALYTES

1. Sucrose
2. D-(+) Lactose monohydrate





Analysis of Sugars in Juice — HPLC/UHPLC

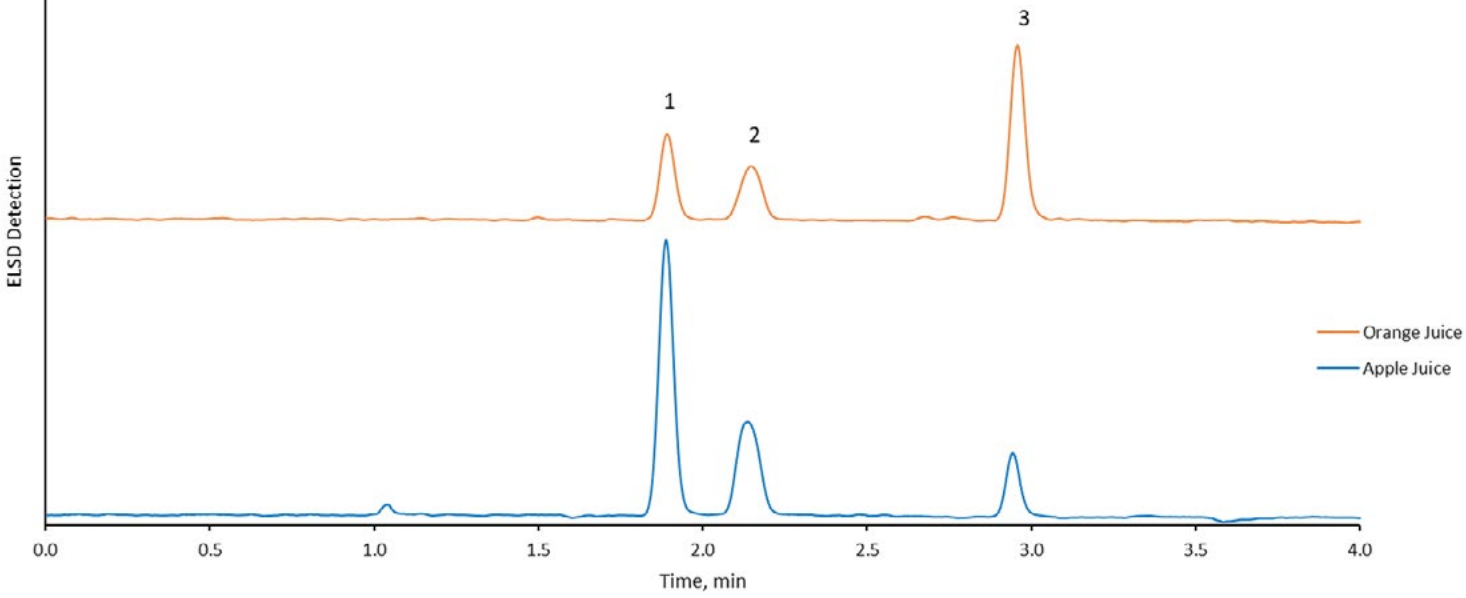
TEST CONDITIONS

Column:	HALO® Penta-HILIC 90 Å, 2.7 µm, 4.6 x 150 mm
Part Number:	92814-705
Mobile Phase A:	Water
Mobile Phase B:	Acetonitrile
Isocratic:	80 %B
Flow Rate:	1.4 mL/min
Pressure:	213 bar
Temperature:	65 °C
Detection:	ELSD, 40 °C, 3.3 bar
Injection Volume:	0.2 µL
Sample Solvent:	Water
Data Rate:	10 Hz
Response Time:	0.10 sec
Flow Cell:	1 µL
LC System:	Shimadzu Nexera

The main sugars in natural fruit juice are fructose, glucose, and sucrose. Each type of juice will contain different ratios of these sugars. Juices obtained from concentrate can also be found to have various amounts of artificial sweeteners. Analysis of sugars is performed on a HALO® Penta-HILIC column with excellent speed and resolution. A comparison of the different sugars in apple juice and orange juice is observed using an ELSD detector.

ANALYTES

1. Sucrose
2. Glucose
3. Fructose



Analysis of Sugars in Pure Honey — HPLC/UHPLC

TEST CONDITIONS

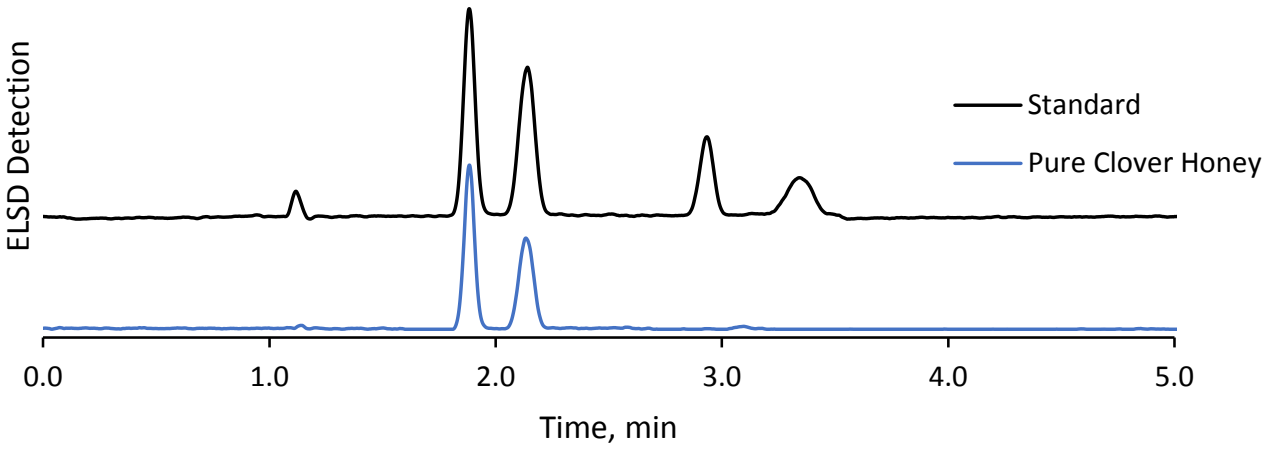
Column:	HALO® Penta-HILIC 90 Å, 2.7 µm, 4.6 x 150 mm
Part Number:	92814-705
Mobile Phase A:	Water
Mobile Phase B:	Acetonitrile
Isocratic:	80% B
Flow Rate:	1.4 mL/min
Initial Pressure:	213 bar
Temperature:	65 °C
Detection:	ELSD, 40 °C, 3.3 bar
Injection Volume:	15 µL
Sample Solvent:	80/20 ACN/ Water
Data Rate:	10 Hz
Response Time:	0.10 sec
LC System:	Shimadzu Nexera X2

Honey can significantly range in quality depending on its purity and levels of sucrose and maltose. Natural honey primarily consists of fructose and glucose, while adulterated honey can contain high levels of sucrose and maltose.

A HALO® Penta-HILIC column separates the primary monosaccharides in pure honey clover showing no signs of adulteration.

ANALYTES

1. D-(-) Fructose
2. D-(+) Glucose
3. Sucrose
4. D-(+) Maltose



Caffeine, Sweeteners and Preservatives — HPLC

TEST CONDITIONS

Column: Avantor® ACE® Excel C18-Amide, 100 Å, 3 µm, 4.6 x 150 mm
Part Number: EXL11121546U
Mobile Phase: A: 0.1% Phosphoric acid in H_2O
B: 0.1% Phosphoric acid in MeCN

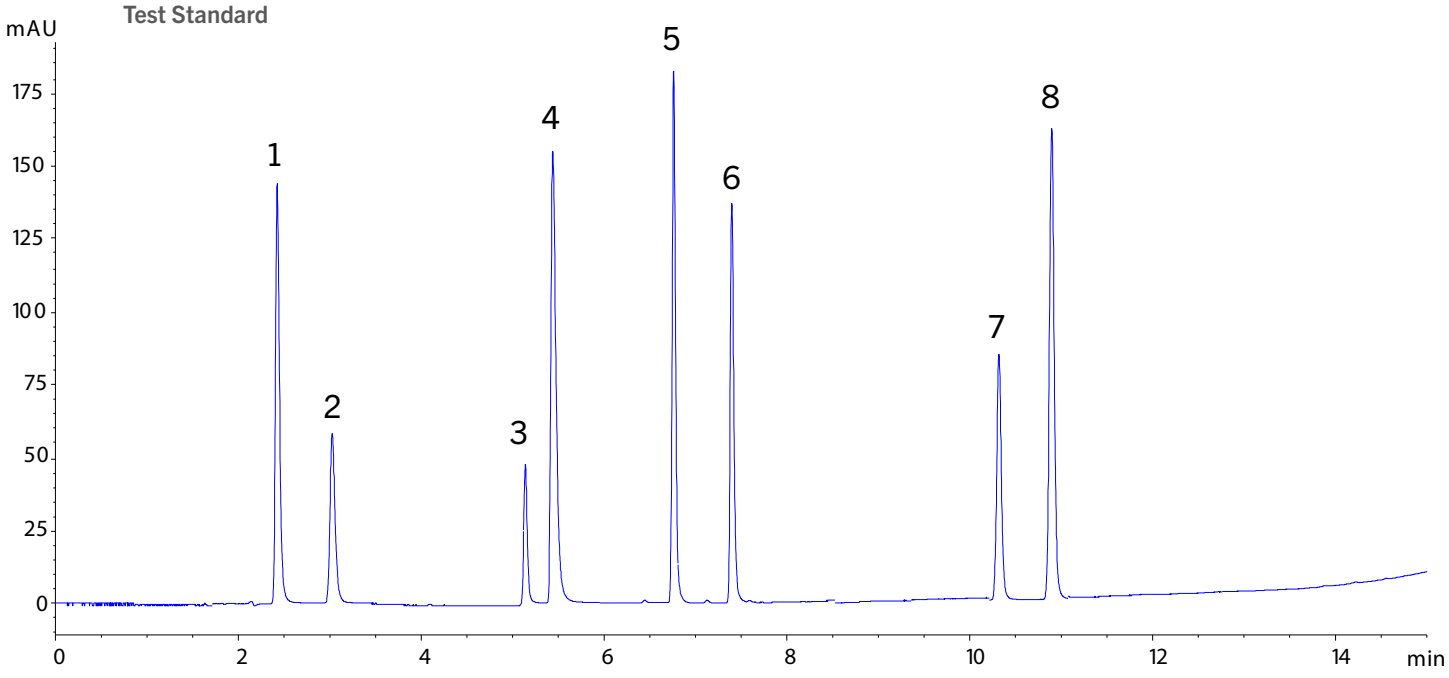
Gradient:

Time (min)	%B
0	5
12	65
15	95
16	95
16.5	5
30	5

Flow Rate: 1 mL/min
Temperature: 30 °C
Injection: 5 µL
Detection: UV, 214 nm
Instrument: VWR Hitachi Chromaster

ANALYTES

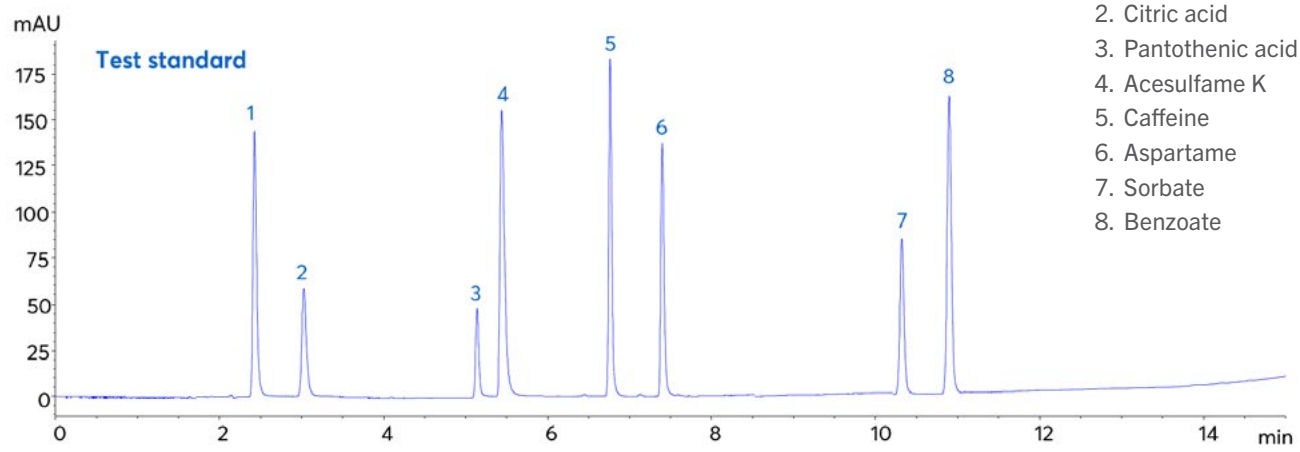
1. Pyridoxine (B6)
2. Citric acid
3. Pantothenic acid (B5)
4. Acesulfame K
5. Caffeine
6. Aspartame
7. Sorbate
8. Benzoate



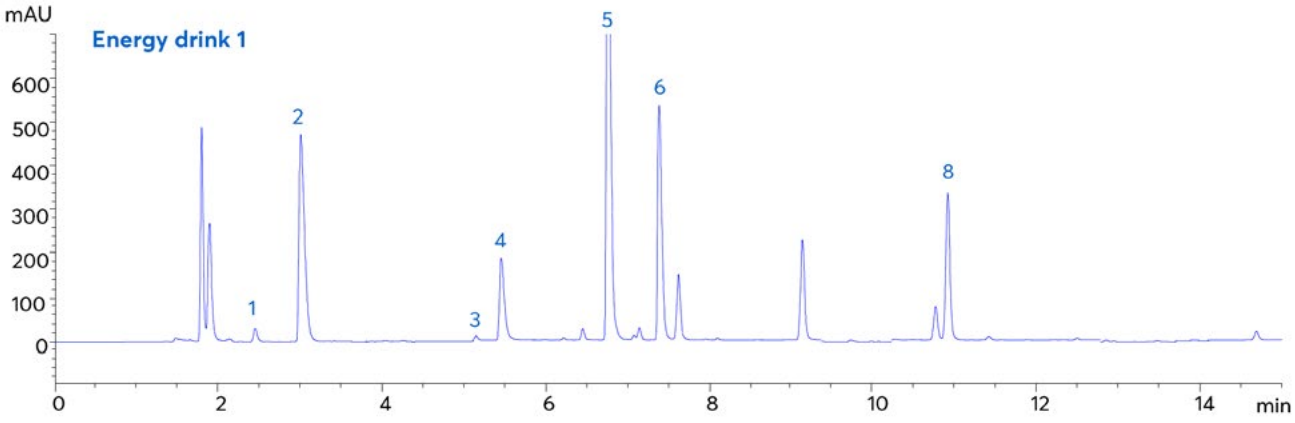
Caffeine, Sweeteners and Preservatives *(continued)*

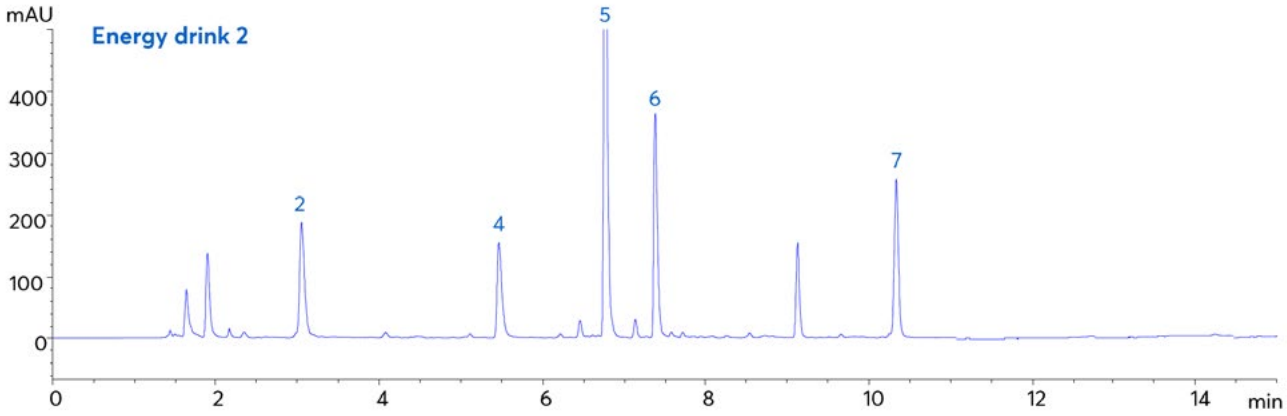
ANALYTES

1. Pyridoxine (B6)
2. Citric acid
3. Pantothenic acid (B5)
4. Acesulfame K
5. Caffeine
6. Aspartame
7. Sorbate
8. Benzoate



The method was used to test two brands of energy drink:





Fast Separation of Oligosaccharides — HPLC/UHPLC

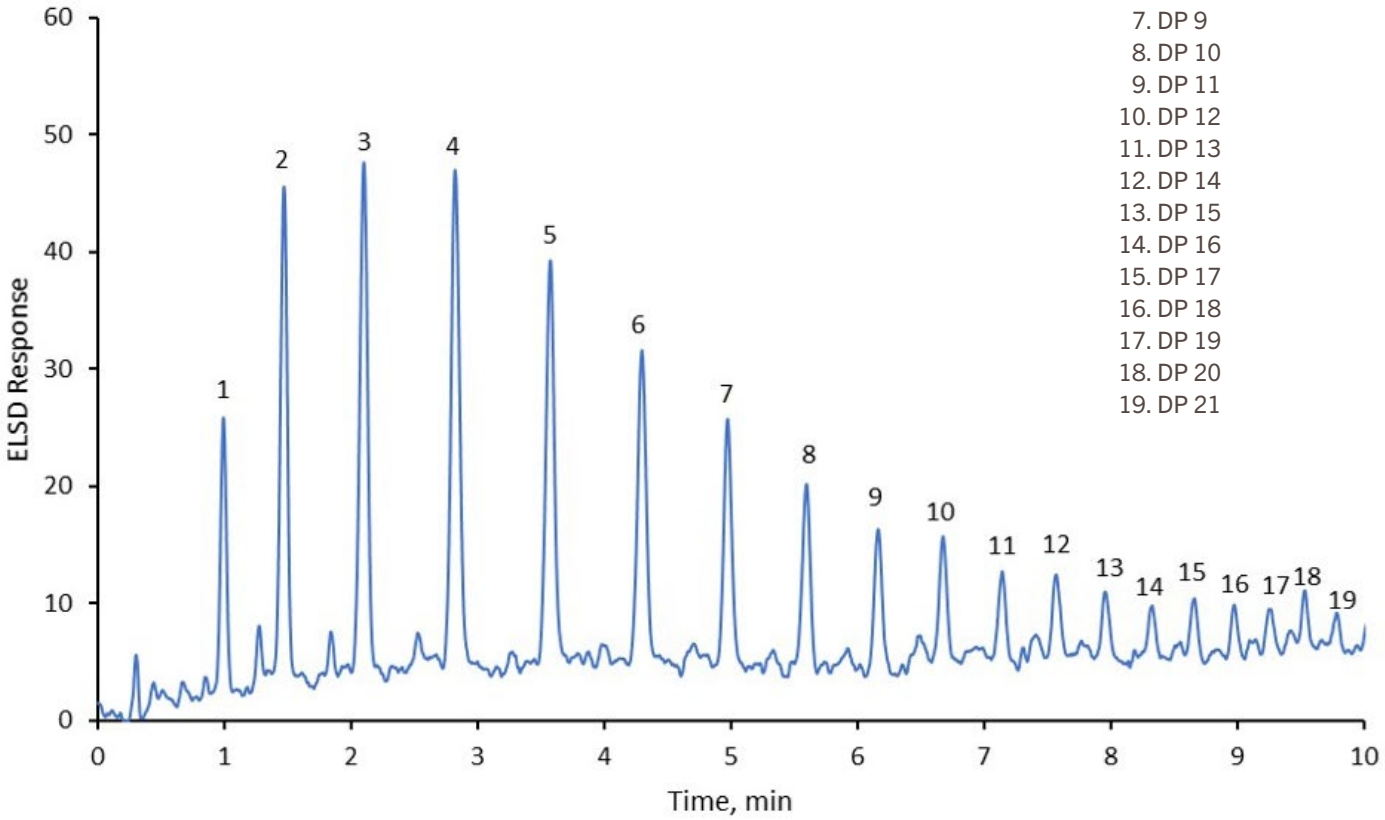
TEST CONDITIONS

Column:	HALO® Penta-HILIC 90 Å, 2.7 µm, 4.6 x 50 mm
Part Number:	92814-405
Mobile Phase A:	Water
Mobile Phase B:	ACN
Gradient:	75-55% B in 10 min
Flow Rate:	2.0 mL/min
Pressure:	105 bar
Temperature:	65 °C
Detection:	ELSD, 40 °C, 3.3 bar
Injection Volume:	20 µL
Sample Solvent:	70/30 ACN/Water
Data Rate:	10 Hz
Response Time:	0.10 sec
LC System:	Shimadzu Nexera X2

The combination of evaporative light scattering detection (ELSD) and a short 50 mm HALO® Penta-HILIC column enables a fast analysis of oligosaccharides in under 10 minutes whereas traditional columns could have analysis times as long as 30 minutes to more than an hour. Using ELSD eliminates the need to label the sugar with either a UV or fluorescent tag, which simplifies the analysis. Analytes are labeled by degree of polymerization (DP).

ANALYTES

1. DP 3
2. DP 4
3. DP 5
4. DP 6
5. DP 7
6. DP 8
7. DP 9
8. DP 10
9. DP 11
10. DP 12
11. DP 13
12. DP 14
13. DP 15
14. DP 16
15. DP 17
16. DP 18
17. DP 19
18. DP 20
19. DP 21



High Resolution Separation of Oligosaccharides — HPLC/UHPLC

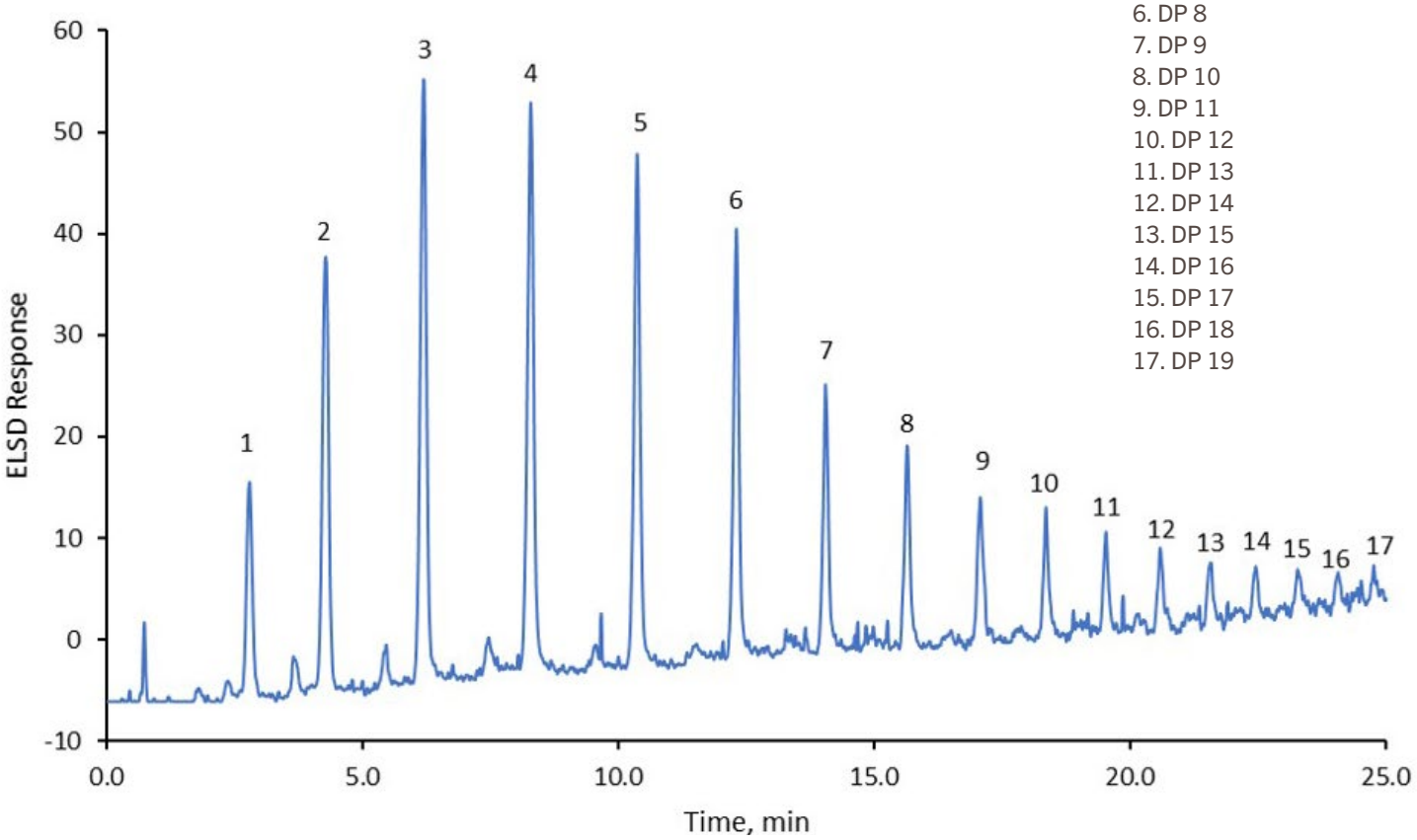
TEST CONDITIONS

Column:	HALO® Penta-HILIC 90 Å, 2.7 µm, 2.1 x 150 mm
Part Number:	92812-705
Mobile Phase A:	Water
Mobile Phase B:	ACN
Gradient:	75-55% B in 25 min
Flow Rate:	0.5 mL/min
Pressure:	168 bar
Temperature:	65 °C
Detection:	ELSD, 40 °C, 3.3 bar
Injection Volume:	20 µL
Sample Solvent:	70/30 ACN/Water
Data Rate:	10 Hz
Response Time:	0.10 sec
LC System:	Shimadzu Nexera X2

High resolution of oligosaccharides is demonstrated using a dextran ladder on a HALO® Penta-HILIC column with the simple mobile phases of acetonitrile and water. The use of the evaporative light scattering detector (ELSD) eliminates the need to label the sugars with either a UV or fluorescent tag, reducing the time required for sample preparation. Analytes are labeled by degree of polymerization (DP).

ANALYTES

1. DP 3
2. DP 4
3. DP 5
4. DP 6
5. DP 7
6. DP 8
7. DP 9
8. DP 10
9. DP 11
10. DP 12
11. DP 13
12. DP 14
13. DP 15
14. DP 16
15. DP 17
16. DP 18
17. DP 19



LC-MS Analysis of Stevia Extract — LC-MS

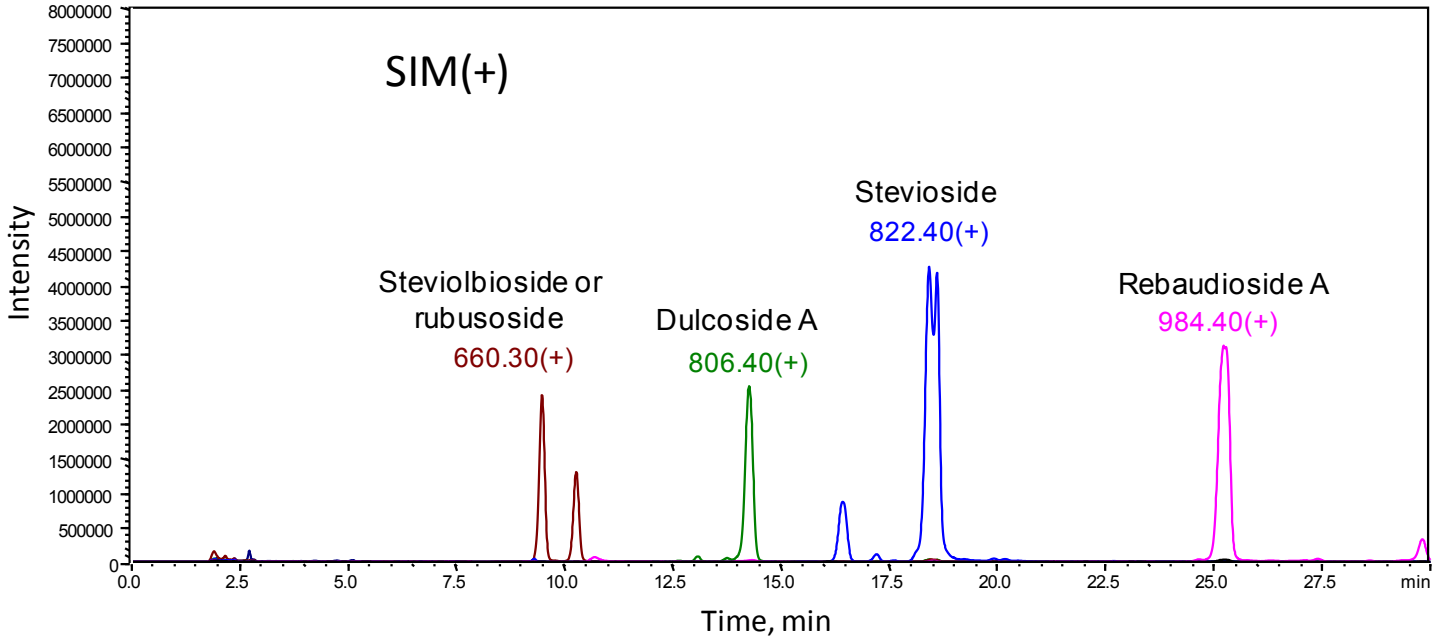
TEST CONDITIONS

Column:	HALO® Penta-HILIC 90 Å, 5 µm, 3.0 x 250 mm
Part Number:	95813-905
Mobile Phase:	A= 50/50 Water/Acetonitrile with 5 mM Ammonium formate, pH 3 B= 5/95 Water/Acetonitrile with 5 mM Ammonium formate, pH 3
Gradient :	90% B to 67% B over 30 min
Flow Rate:	0.5 mL/min
Pressure:	60 bar
Temperature:	Ambient
Injection Volume:	5 µL
Sample Solvent:	80/20 Acetonitrile/Water
LC System:	Shimadzu Nexera
MS:	Shimadzu LCMS 2020(single quadrupole)
ESI:	+4.5 kV,
Scan range:	200-1200 m/z
Scan rate:	2 pps
Capillary:	250 °C
Heat block:	350 °C
Nebulizing gas flow:	1.5 L/min
Drying gas flow:	15 L/min

EXTRACTION PROCEDURE

1. Weigh 400 mg of Stevia rebaudiana leaves (Sigma S5381)
2. Crush leaves with mortar and pestle and transfer to vial
3. Add 8 mL of 50/50 (v/v) acetonitrile/water
4. Sonicate vial contents for 15 minutes
5. Filter sample using 25 mm syringe filter having 0.2 µm PTFE membrane (VWR 28145-495)
6. Centrifuge @ 10K rpm (5 min) and collect supernate
7. Dilute 400 µL of extract in 600 µL of acetonitrile for overall concentration of 80/20 acetonitrile/water
8. Centrifuge diluted sample @ 10K (5 min) rpm and inject the supernate

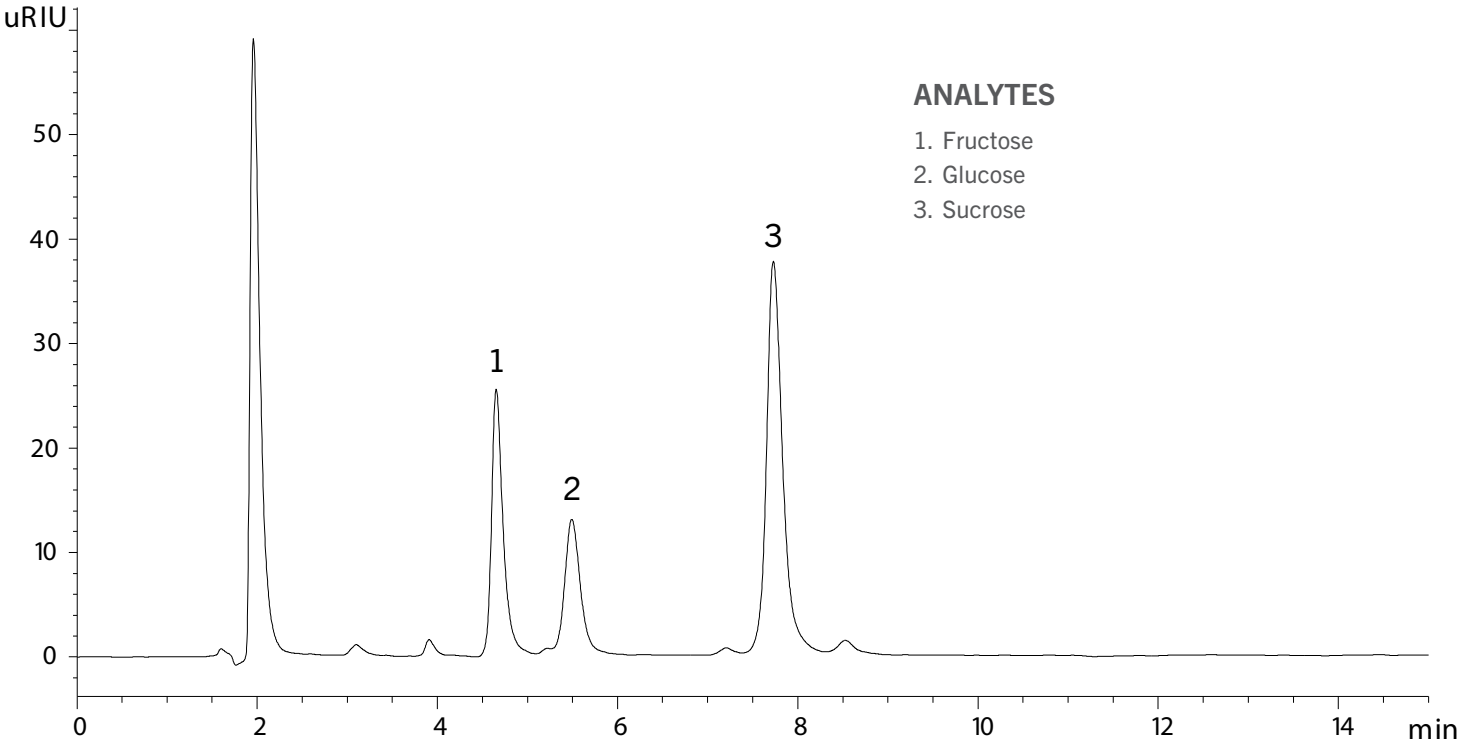
Stevia is a natural sweetener and is used as a substitute for sugar. LC/MS analysis of stevia glycosides from a Stevia extract is easily accomplished using HALO® Penta-HILIC column due to its unique bonded phase containing five OH groups and the high efficiency of the 5-micron Fused-Core particles.



Sugars – Orange Juice — HPLC

TEST CONDITIONS

Column:	Avantor® ACE® Excel NH_2, 100 Å, 3 µm, 4.6 x 150 mm
Part Number:	EXL11141546U
Mobile Phase:	MeCN/H_2O (75:25 v/v)
Flow Rate:	1 mL/min
Temperature:	35 °C
Injection Volume:	10 µL
Detection:	RI, 35 °C
Sample:	100 µL orange juice in 900 µL mobile phase
System:	Chromaster 600



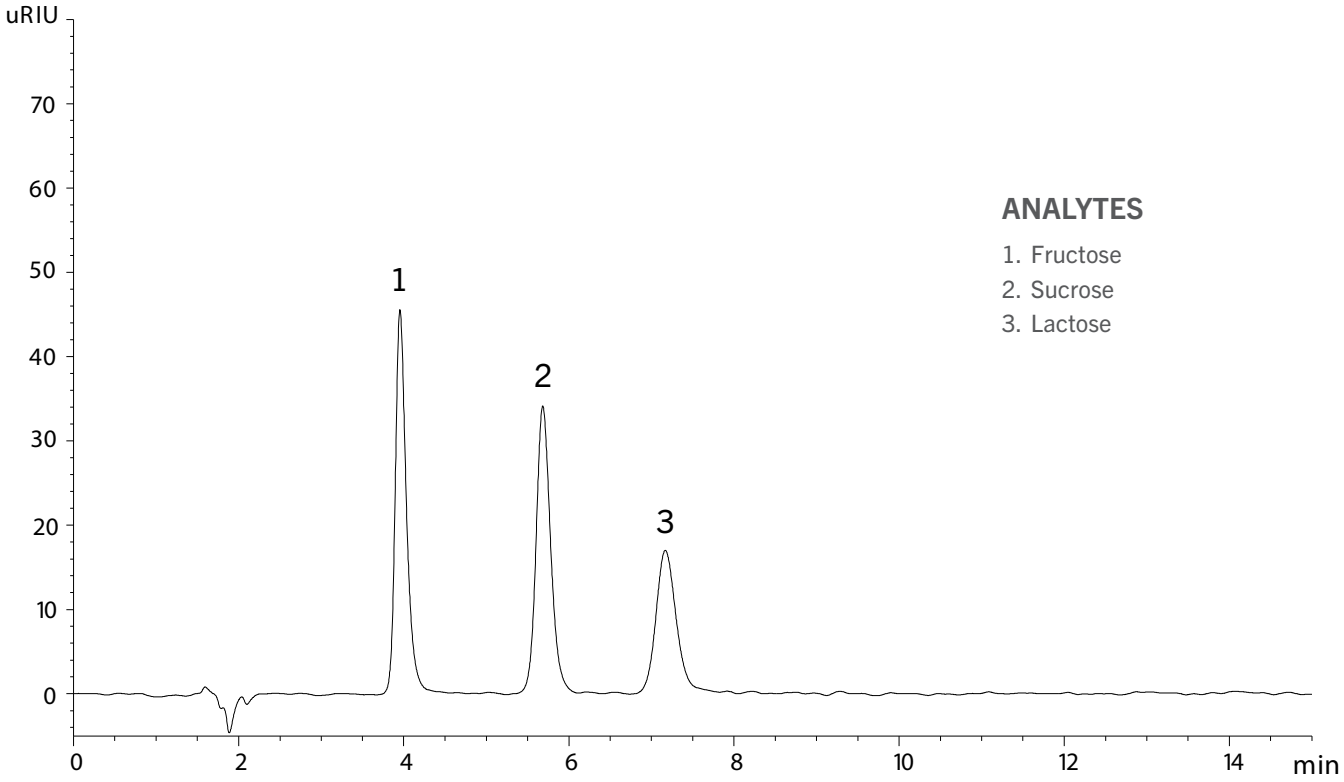
ANALYTES

1. Fructose
2. Glucose
3. Sucrose

Sugars Separation — HPLC

TEST CONDITIONS

Column:	Avantor® ACE® Excel NH_2, 100 Å, 5 µm, 4.6 x 150 mm
Part Number:	EXL12141546U
Mobile Phase:	MeCN/H_2O (70:30 v/v)
Flow Rate:	1 mL/min
Temperature:	35 °C
Injection Volume:	10 µL
Detection:	RI, 35 °C
Sample:	5 mg/mL in mobile phase
System:	Chromaster 600



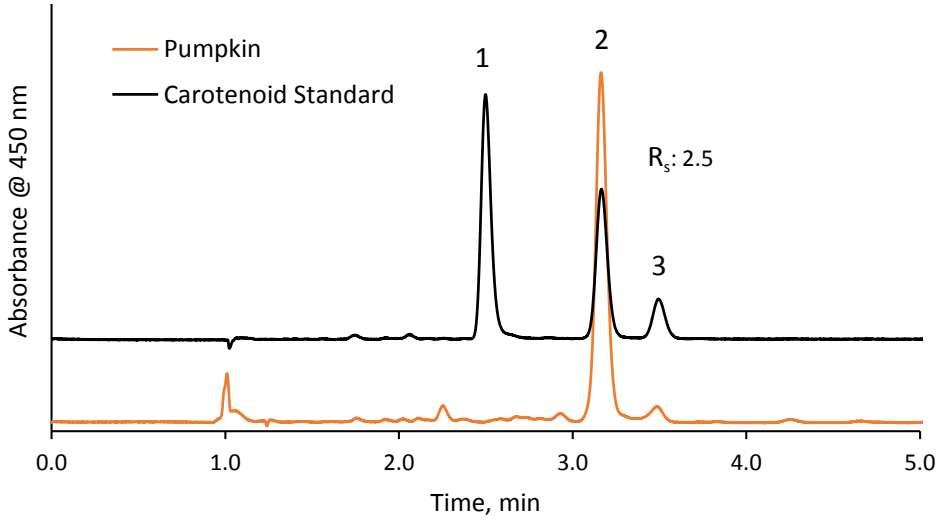
ANALYTES

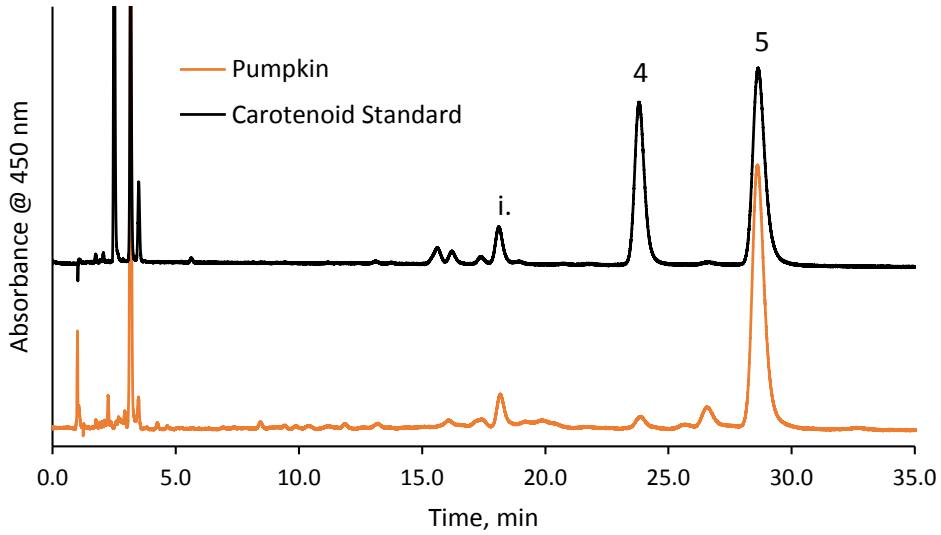
1. Fructose
2. Sucrose
3. Lactose

Carotenoid Analysis in Pumpkin — HPLC/UHPLC

TEST CONDITIONS

Column:	HALO® C30, 160 Å, 2.7 µm, 4.6 x 150 mm	Detection:	450 nm
Part Number:	92114-730	Injection Volume:	20.0 µL
Competitor:	FPP C30, 3.0 µm, 4.6 x 150 mm	Sample Solvent:	Methanol
Isocratic:	100% Methanol	Data Rate:	14 Hz
Flow Rate:	1.5 mL/min	Response Time:	0.12 sec
Initial HALO® Pressure:	277 bar	Flow Cell:	5 µL semi-micro
Temperature:	15 °C	LC System:	Agilent 1100





ANALYTES

1. Astaxanthin
2. Lutein
3. Zeaxanthin
4. α- Carotene
5. β-Carotene
   i. unidentified isomers

Pumpkins contain high amounts of carotenoids, especially beta carotene. Carotenoids are fat-soluble compounds that can be split into two main groups called xanthophylls and carotenes. These compounds both contain anti-oxidant properties and some can be converted into vitamin A when released into the body. A liquid-liquid extraction is performed with 0.2g of pumpkin pulp. Carotenoids are extracted from the pumpkin and analyzed on a HALO® C30 column. The HPLC oven set at sub-ambient temperature enables optimum resolution of early eluting peaks.

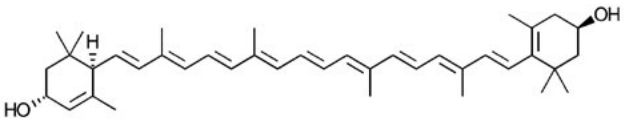
Carotenoids Extracted From Carrot Juice — HPLC/UHPLC

TEST CONDITIONS

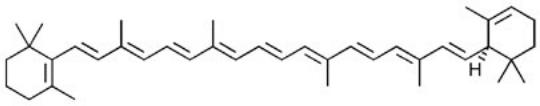
Column:	HALO® C30, 160 Å, 2.7 µm, 2.1 x 50 mm
Part Number:	92112-430
Isocratic:	100 % Methanol
Flow Rate:	0.4 mL/min
Pressure:	100 bar
Temperature:	30 °C
Detection:	UV 450 nm, PDA
Injection Volume:	2.5 µL
Sample Solvent:	Methanol/ Isopropyl alcohol
Data Rate:	40 Hz
Response Time:	0.025 sec
Flow Cell:	1 µL
LC System:	Shimadzu Nexera X2

The carotenoids lutein, α- carotene, and β- carotene were isolated from a commercially available carrot juice using liquid liquid extraction. Carotenes are responsible for the orange color in vegetables such as carrots and are considered antioxidants. The separation was performed on a HALO® C30 column with high resolution between the α- and β-carotene peaks.

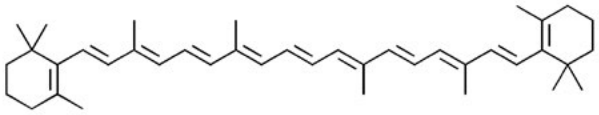
STRUCTURES



Lutein



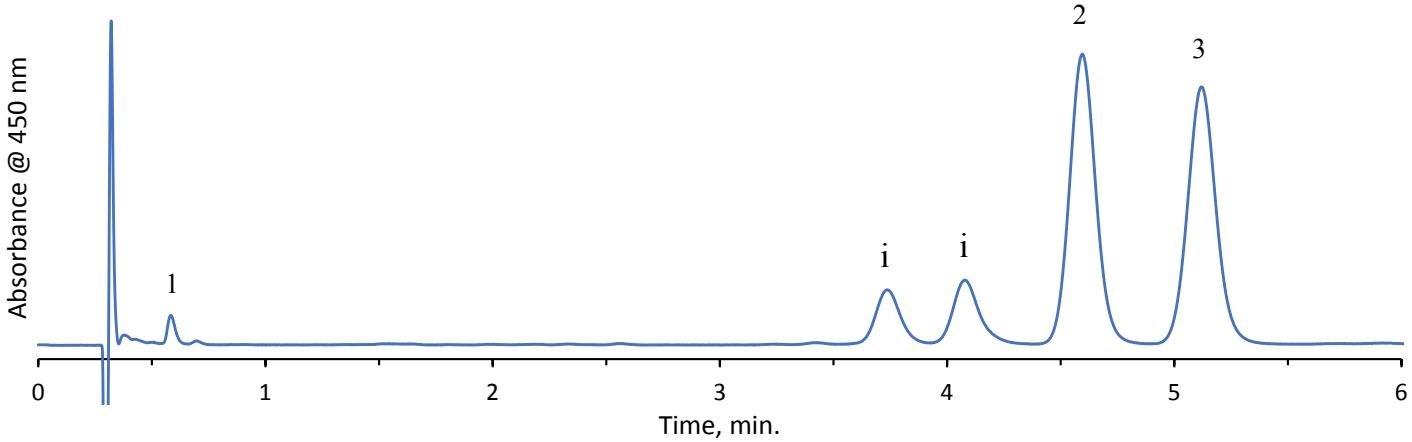
Alpha carotene



Beta carotene

ANALYTES

1. Lutein
2. α-carotene
3. β-carotene
   i. unidentified isomers



Carotenoids on a C30 Column — HPLC/UHPLC

TEST CONDITIONS

Column:	HALO® C30, 160 Å, 2.7 µm, 3.0 x 150 mm
Part Number:	92113-730
Mobile Phase A:	Methanol
Mobile Phase B:	Ethanol
Gradient:	

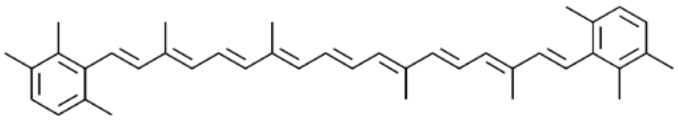
Time (min)	%B
0.0	0
20.0	40

Flow Rate:	0.65 mL/min
Temperature:	38 °C
Detection:	UV 471 nm, PDA
Injection Volume:	0.6 µL
Data Rate:	2.5 Hz
Response Time:	2 sec
Flow Cell:	13 µL
LC System:	Agilent 1100

Data Courtesy of Nature's Sunshine Products

Carotenoids can be split into two main classes called xanthophylls and carotenes. They are responsible for absorbing light for photosynthesis and protecting chlorophyll from photodamage. A separation done by Nature's Sunshine Products shows excellent resolution of carotenoids on a HALO® C30 column.

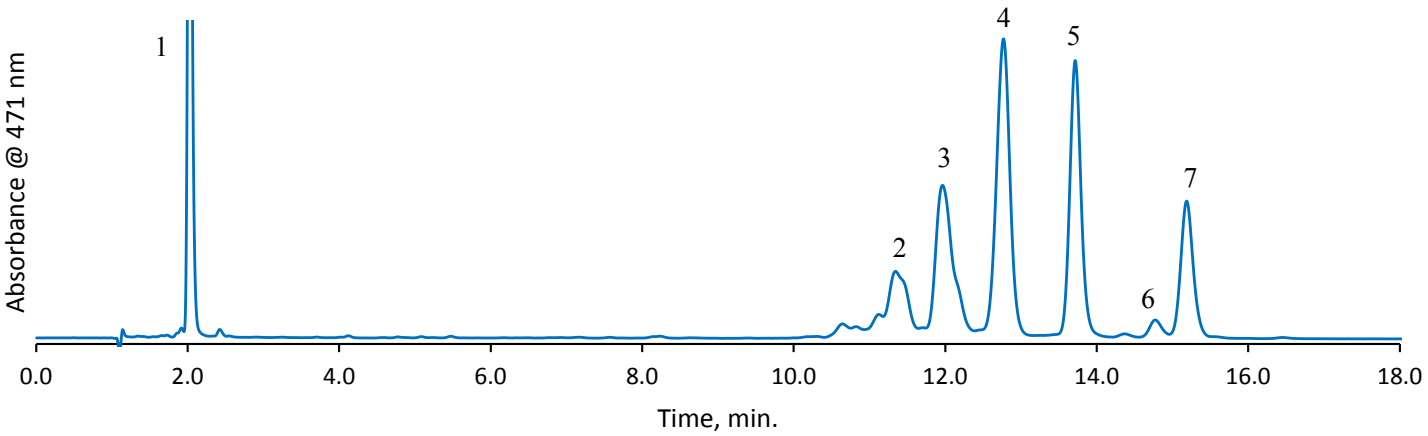
STRUCTURES



General structure of a carotenoid

ANALYTES

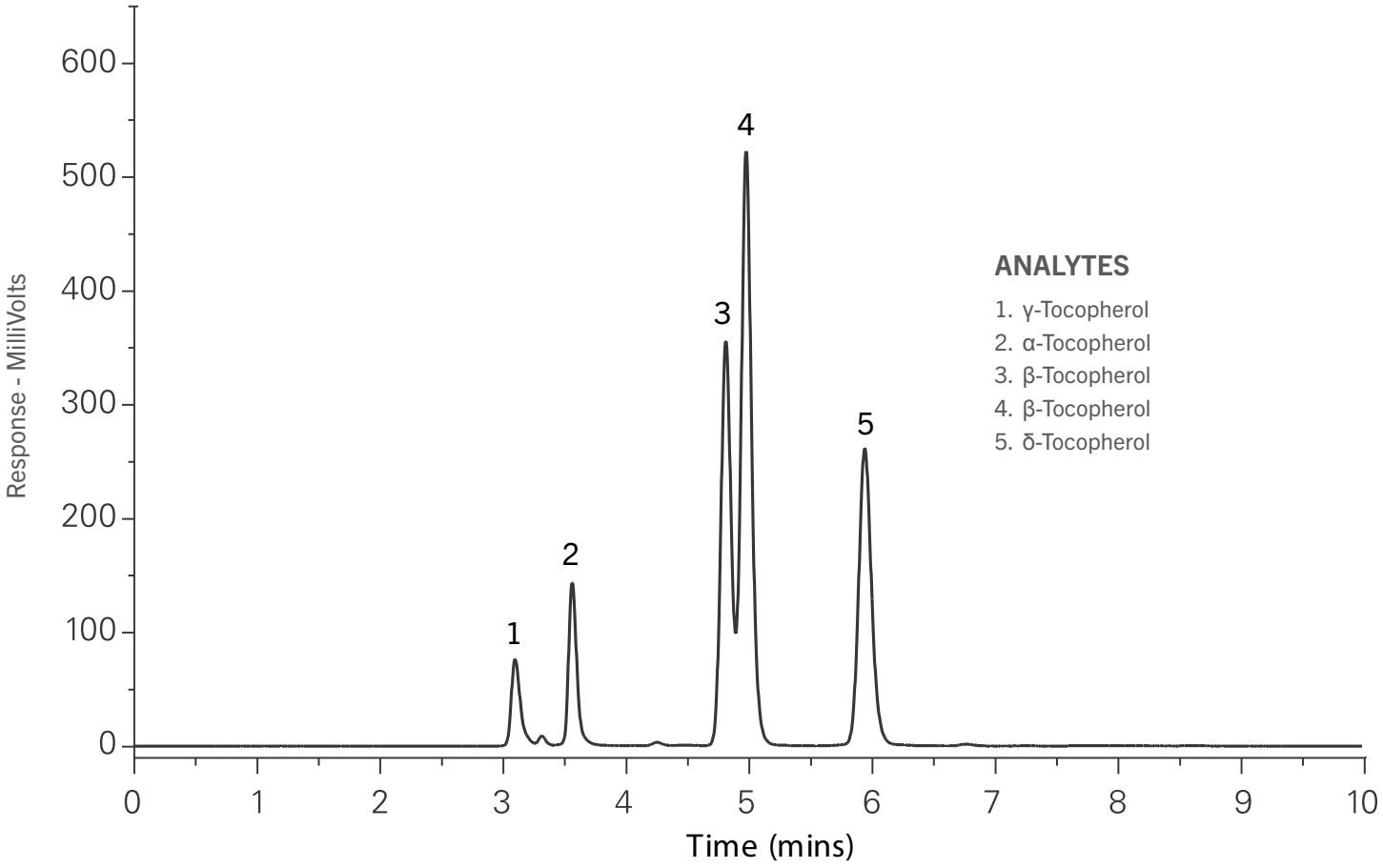
1. Lutein
2. *cis*- carotenoid 1
3. *cis*- carotenoid 2
4. α- Carotene
5. β- Carotene
6. *cis*- Lycopene
7. Lycopene



Tocopherols — HPLC

TEST CONDITIONS

Column:	Avantor® ACE® SIL, 100 Å, 5 µm, 4.6 x 250 mm
Part Number:	ACE1272546
Mobile Phase:	Hexane/IPA (98:2 v/v)
Flow Rate:	1 mL/min
Temperature:	Ambient
Injection Volume:	1 µL
Detection:	UV-Vis, 450 nm

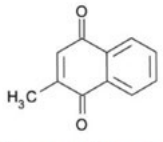


Vitamin K — UHPLC

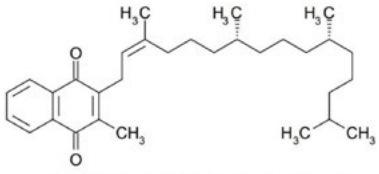
TEST CONDITIONS

Column:	Avantor® ACE® Excel CN-ES, 100 Å, 2 µm, 3.0 x 100 mm
Part Number:	EXL10131003U
Mobile Phase:	95:5 MeOH/H_2O
Flow Rate:	1 mL/min
Temperature:	30 °C
Injection:	5 µL
Detection:	UV, 270 nm

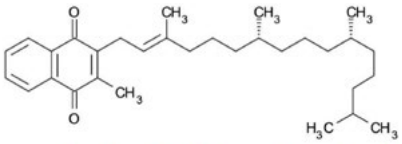
STRUCTURES



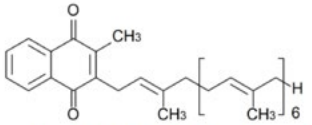
Vitamin K3 (Menadione)



cis-Vitamin K1 (2,3-*cis*-phylloquinone)



trans-Vitamin K1 (2,3-*trans*-phylloquinone)



Vitamin K2 (MK-7) – Menaquinone 7

ANALYTES

1. Vitamin K3 (Menadione)
2. *cis*-Vitamin K1 (2,3-*cis*-phylloquinone)
3. *trans*-Vitamin K1 (2,3-*trans*-phylloquinone)
4. Vitamin K2 (MK-7) - Menaquinone 7

Vitamins - Water Soluble (Gradient) C18 — HPLC

TEST CONDITIONS

Column: Avantor® ACE® Excel C18, 100 Å, 5 µm, 4.6 x 250 mm
Part Number: EXL1212546U
Mobile Phase: A: 50 mM KH_2PO_4 pH 3.0 in H_2O
B: MeOH

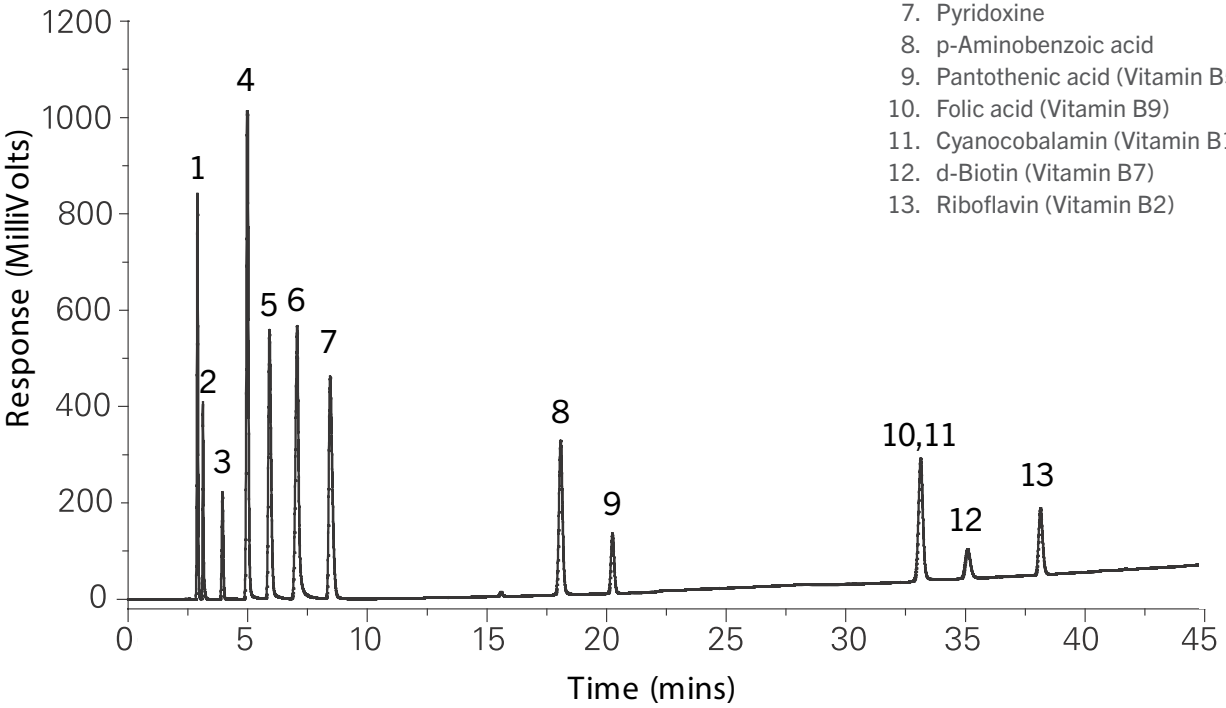
Gradient:

Time (min)	%B
0	3
5	3
45	45
50	80

Flow Rate: 1 mL/min
Temperature: Ambient
Injection Volume: 1 µL
Detection: UV, 205 nm

ANALYTES

1. Pyridoxamine
2. Thiamine (Vitamin B1)
3. L-Ascorbic acid (Vitamin C)
4. Nicotinic acid
5. Pyridoxal
6. Impurity
7. Pyridoxine
8. p-Aminobenzoic acid
9. Pantothenic acid (Vitamin B5)
10. Folic acid (Vitamin B9)
11. Cyanocobalamin (Vitamin B12)
12. d-Biotin (Vitamin B7)
13. Riboflavin (Vitamin B2)



Vitamins - Water Soluble (Gradient) C8 — HPLC

TEST CONDITIONS

Column: Avantor® ACE® C8, 100 Å, 5 µm, 4.6 x 250 mm
Part Number: ACE1222546
Mobile Phase: A: 50 mM KH_2PO_4 pH 2.5 in H_2O
B: MeOH

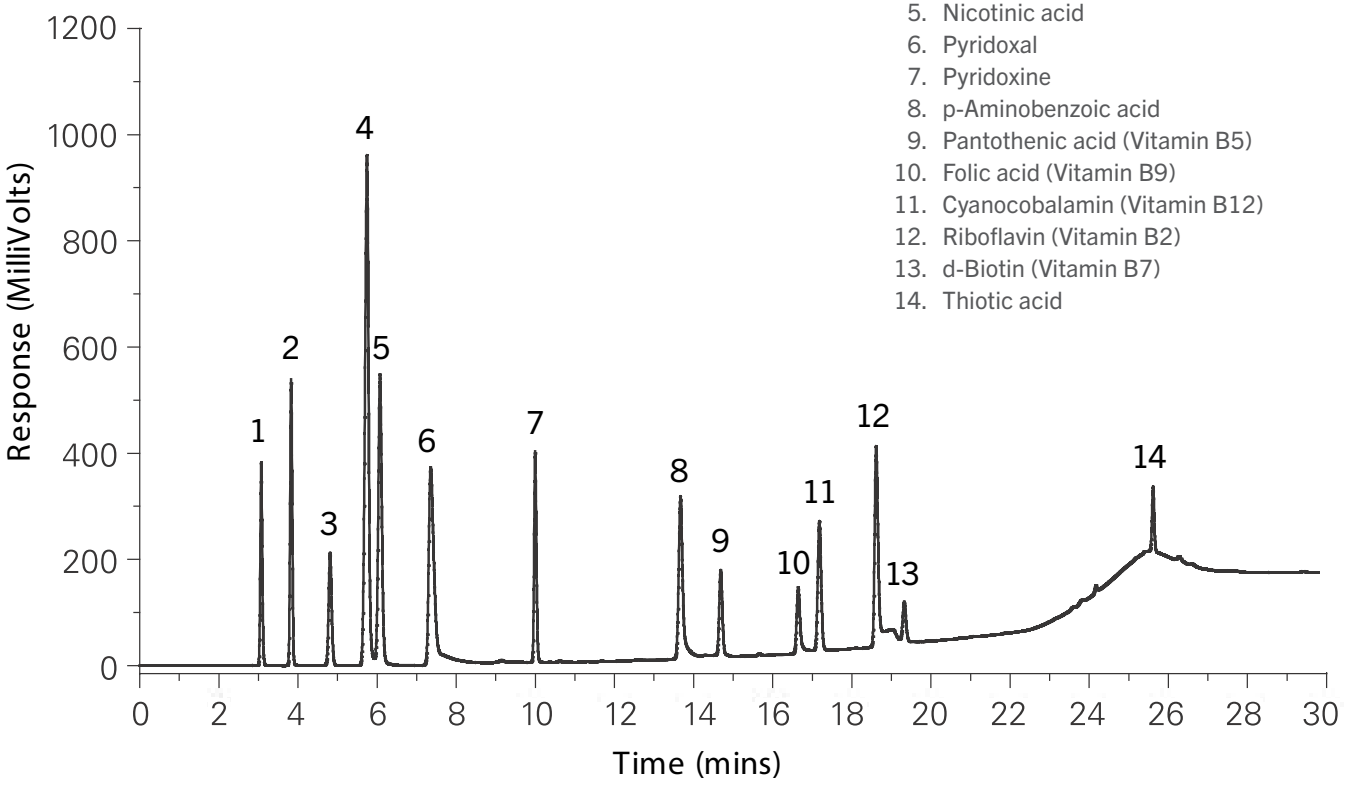
Gradient:

Time (min)	%B
0	0
3	0
16.5	45
19.5	80

Flow Rate: 1 mL/min
Temperature: Ambient
Detection: UV, 205 nm

ANALYTES

1. Pyridoxamine
2. Thiamine (Vitamin B1)
3. L-Ascorbic acid (Vitamin C)
4. Niacinamide (Vitamin B3)
5. Nicotinic acid
6. Pyridoxal
7. Pyridoxine
8. p-Aminobenzoic acid
9. Pantothenic acid (Vitamin B5)
10. Folic acid (Vitamin B9)
11. Cyanocobalamin (Vitamin B12)
12. Riboflavin (Vitamin B2)
13. d-Biotin (Vitamin B7)
14. Thiotic acid



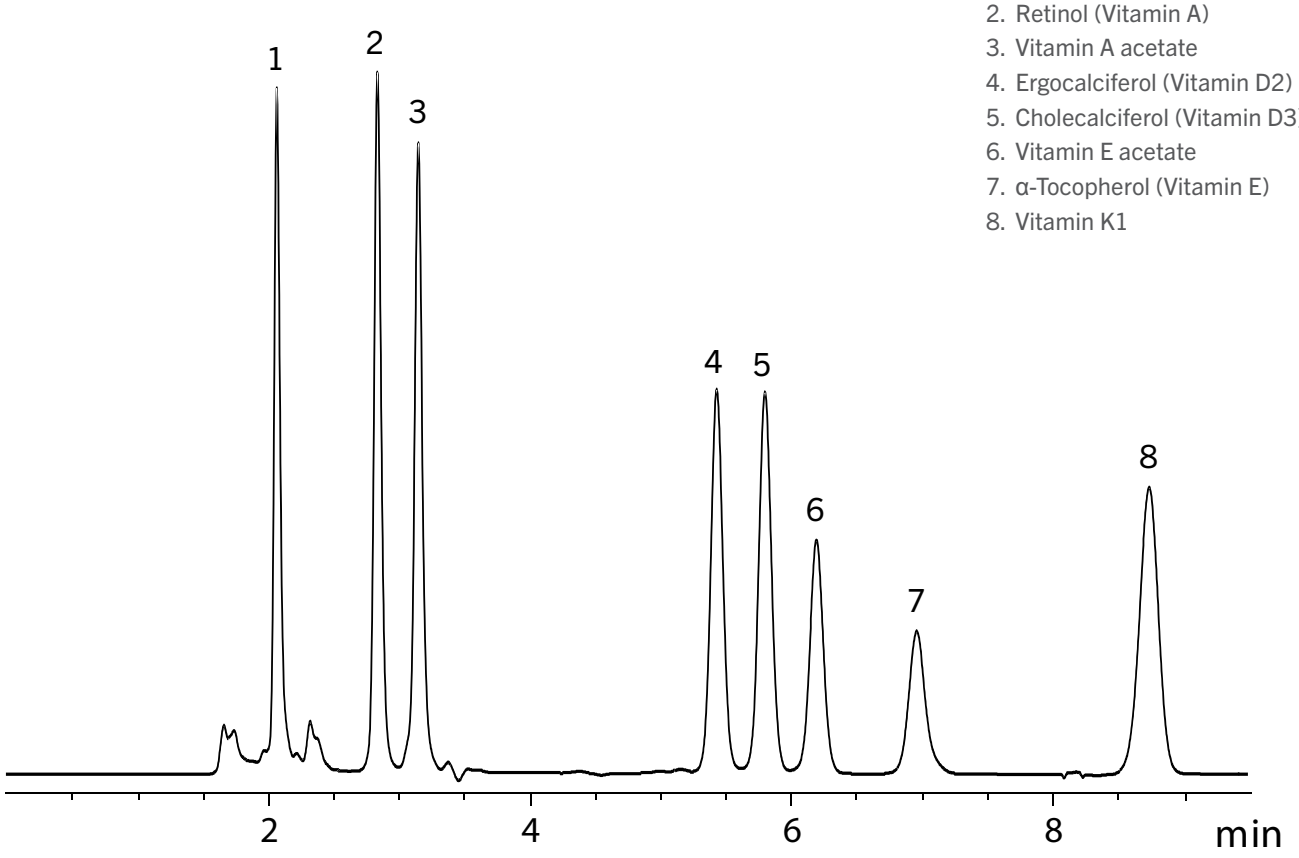
Vitamins – Fat Soluble — HPLC

TEST CONDITIONS

Column:	Avantor® ACE® Excel C18-Amide, 100 Å, 3 µm, 4.6 x 150 mm
Part Number:	EXL11121546U
Mobile Phase:	MeOH/MeCN (90:10 v/v)
Flow Rate:	1 mL/min
Temperature:	20 °C
Detection:	UV, 280 nm

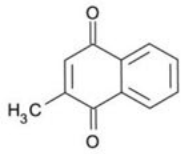
ANALYTES

1. Menadione (Vitamin K3)
2. Retinol (Vitamin A)
3. Vitamin A acetate
4. Ergocalciferol (Vitamin D2)
5. Cholecalciferol (Vitamin D3)
6. Vitamin E acetate
7. α-Tocopherol (Vitamin E)
8. Vitamin K1

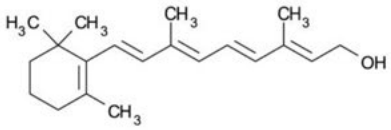


Vitamins – Fat Soluble *(continued)*

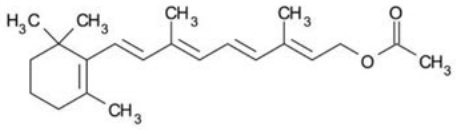
STRUCTURES



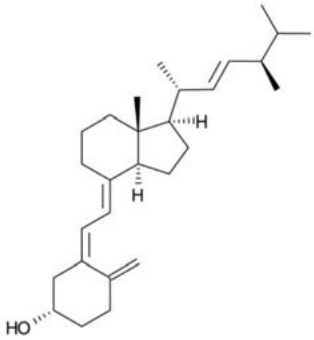
Menadione (Vitamin K3)



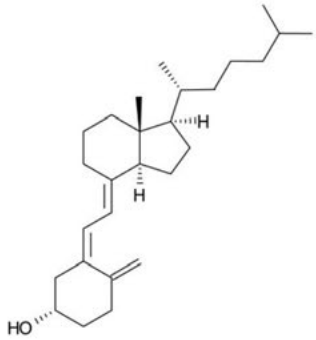
Retinol (Vitamin A)



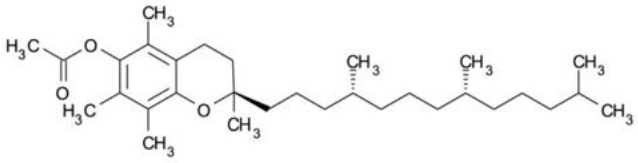
Vitamin A acetate



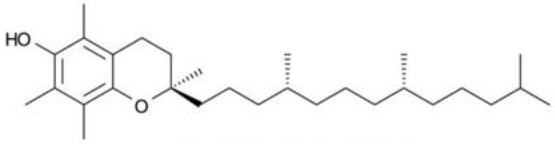
Ergocalciferol (Vitamin D2)



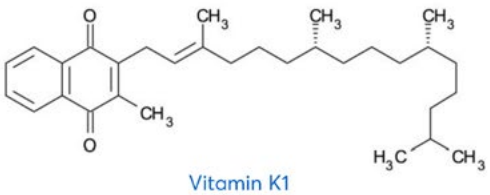
Cholecalciferol (Vitamin D3)



Vitamin E acetate



α-Tocopherol (Vitamin E)



Vitamin K1

Vitamins D2 and D3 — UHPLC

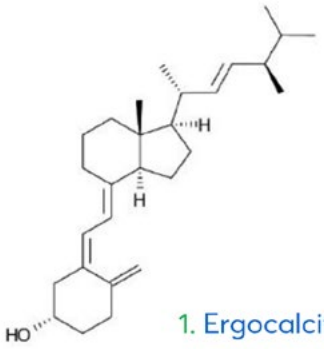
TEST CONDITIONS

Column:	Avantor® ACE® Excel C18-Amide, 100 Å, 2 µm, 3.0 x 50 mm
Part Number:	EXL10120503U
Mobile Phase:	100% MeCN
Flow Rate:	0.43 mL/min
Temperature:	20 °C
Injection:	2 µL
Detection:	UV, 265 nm

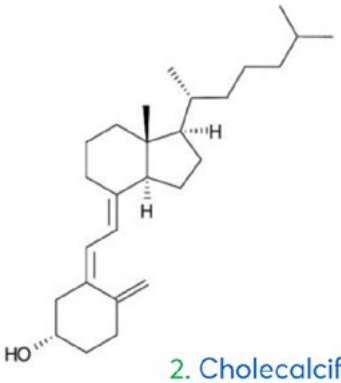
ANALYTES

1. Ergocalciferol (D2)
2. Cholecalciferol (D3)

STRUCTURES



1. Ergocalciferol (D2)



2. Cholecalciferol (D3)

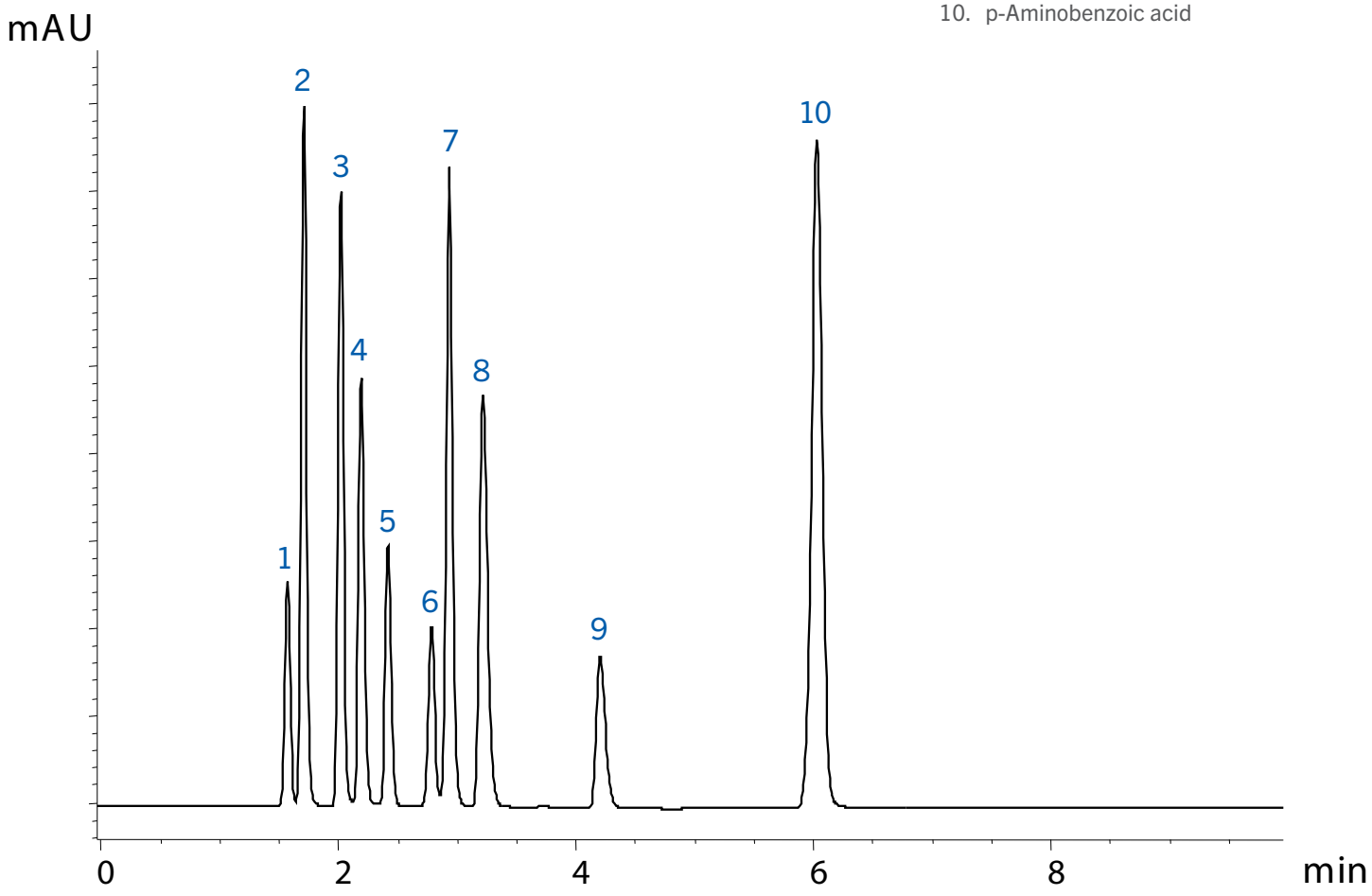
Water Soluble Vitamins (Polar Molecules) — HPLC

TEST CONDITIONS

Column:	Avantor® ACE® C18-AR, 100 Å, 3 µm, 4.6 x 150 mm
Part Number:	ACE1191546
Mobile Phase:	0.1% Phosphoric acid in H_2O/MeOH (96.5:3.5 v/v)
Flow Rate:	1 mL/min
Temperature:	22 °C
Injection:	2 µL
Detection:	UV, 260 nm

ANALYTES

1. Pyridoxamine (Vitamin B6)
2. Thiamine (Vitamin B1)
3. Isonicotinamide
4. Nicotinamide
5. L-Ascorbic acid (Vitamin C)
6. Orotic acid
7. Hypoxanthine
8. Pyridoxal (Vitamin B6)
9. Pyridoxine (Vitamin B6)
10. p-Aminobenzoic acid



Caffeine and Metabolites — HPLC

TEST CONDITIONS

Column: Avantor® ACE® Excel SuperC18, 100 Å, 5 µm, 4.6 x 150 mm
Part Number: EXL12111546U
Mobile Phase: A: 20 mM Ammonium acetate pH 7.0 in H_2O
B: 20 mM Ammonium acetate pH 7.0 in MeCN/H_2O (90:10 v/v)

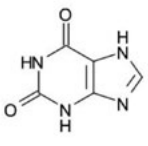
Gradient:

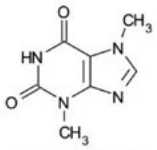
Time (min)	%B
0	2
45	15
48	15
49	2

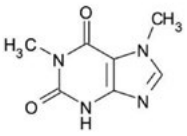
Post time 10 minutes

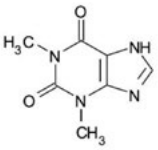
Flow Rate: 1 mL/min
Temperature: 60 °C
Injection: 1 µL
Detection: UV, 273 nm

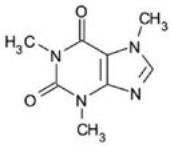
STRUCTURES

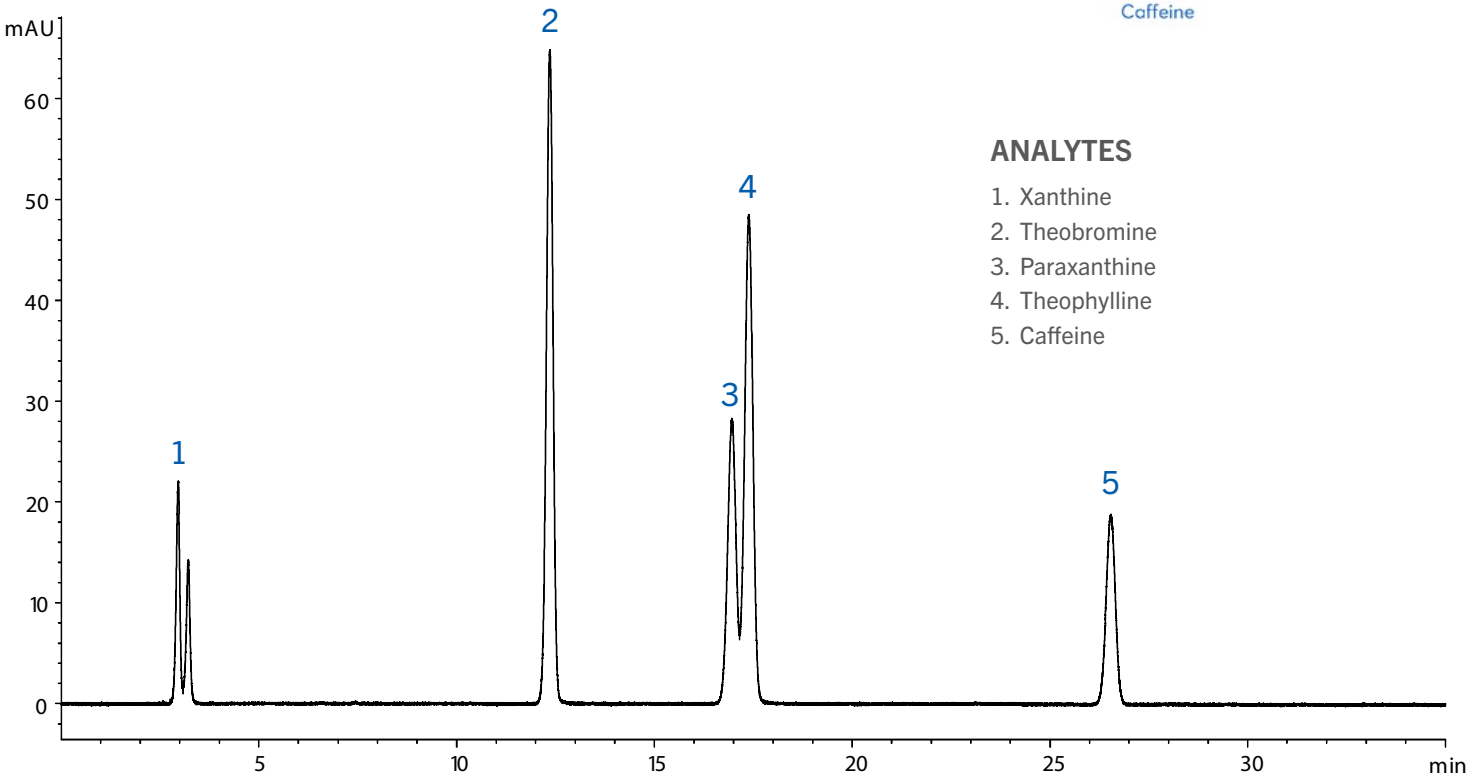

Xanthine


Theobromine


Paraxanthine


Theophylline


Caffeine



ANALYTES

1. Xanthine
2. Theobromine
3. Paraxanthine
4. Theophylline
5. Caffeine

Caffeine and Related Compounds Using HILIC Mode — HPLC

TEST CONDITIONS

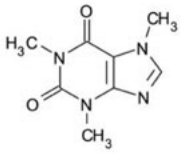
Column: Avantor® ACE® HILIC-N, 100 Å, 5 µm, 4.6 x 150 mm
Part Number: HILN51546U
Mobile Phase: A: 10 mM Ammonium formate pH 3.0 in MeCN/H_2O (96:4 v/v)
B: 10 mM Ammonium formate pH 3.0 in MeCN/H_2O (50:50 v/v)

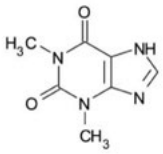
Gradient:

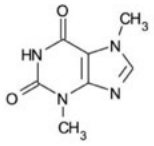
Time (min)	%B
0	0
15	100

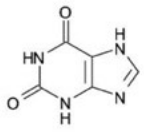
Flow Rate: 1.5 mL/min
Temperature: 15 °C
Injection: 2 µL
Detection: UV, 275 nm
Sample: Caffeine (25 mg/mL) with related substances theophylline, theobromine, xanthine and hypoxanthine at 0.5% w/w dissolved in MeCN/H_2O (90:10 v/v)

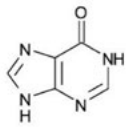
STRUCTURES

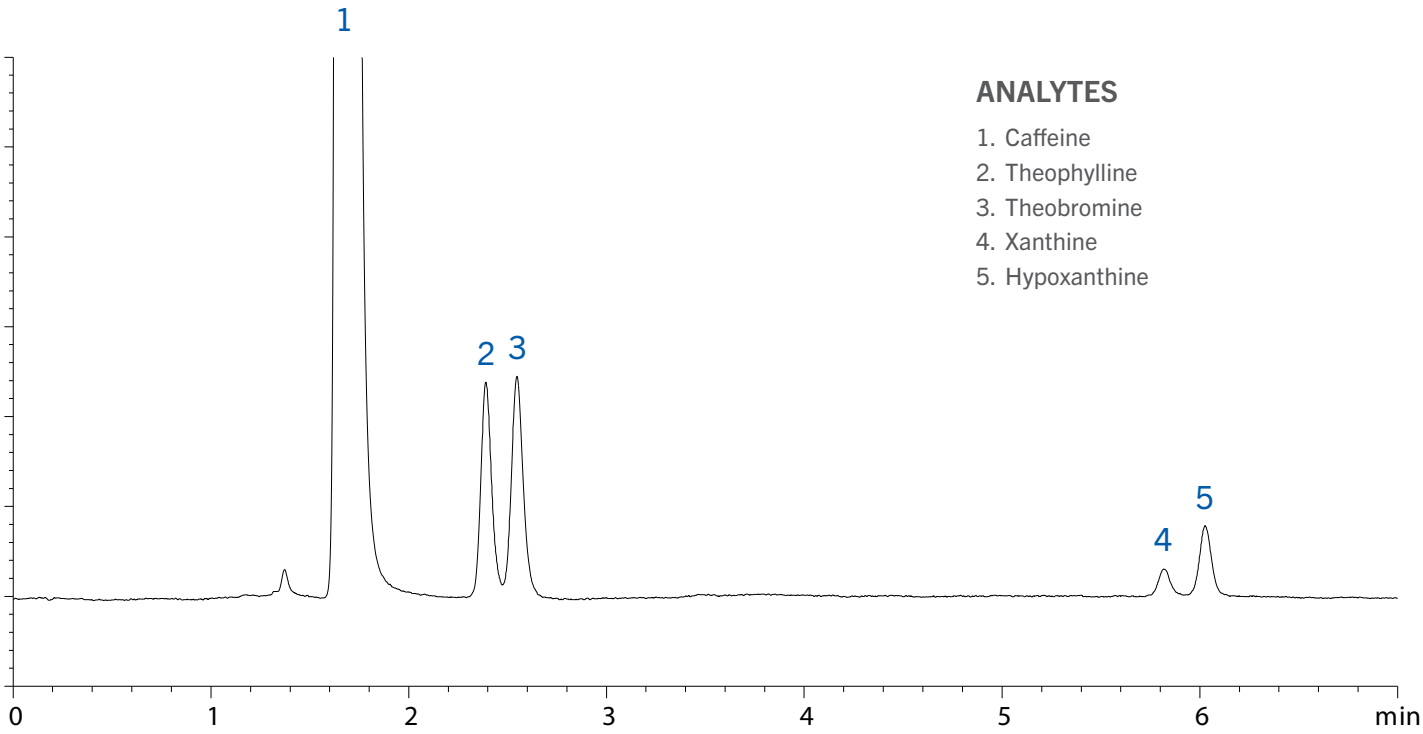

Caffeine


Theophylline


Theobromine


Xanthine


Hypoxanthine



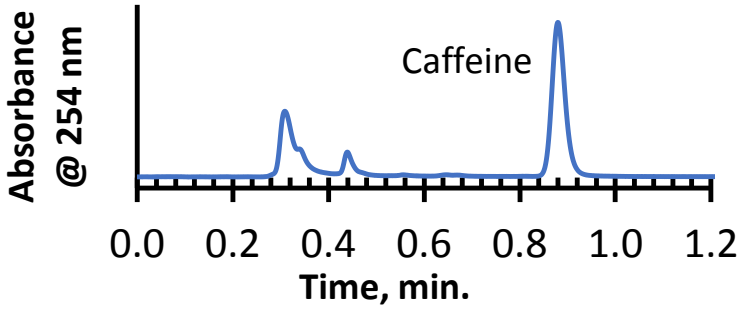
ANALYTES

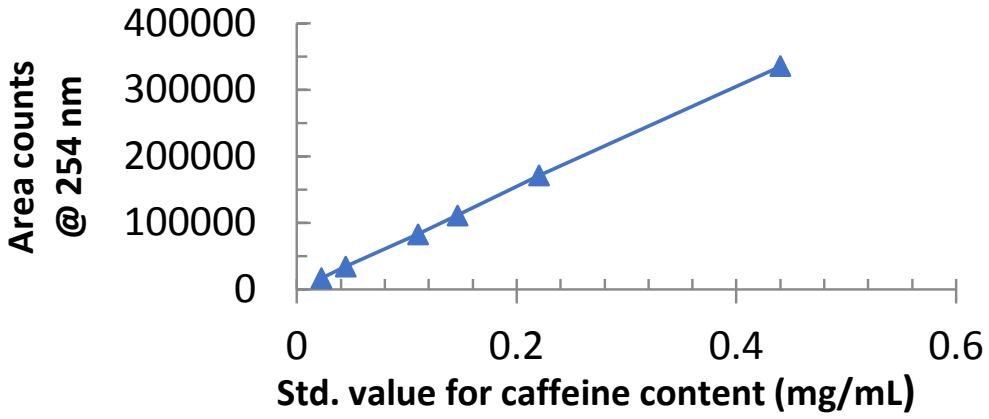
1. Caffeine
2. Theophylline
3. Theobromine
4. Xanthine
5. Hypoxanthine

Caffeine in Soda — HPLC

TEST CONDITIONS

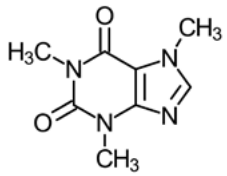
Column:	HALO® C18, 90 Å, 5 µm, 3.0 x 50 mm with guard column
Part Number:	95813-402
GC Part Number:	95813-102
Mobile Phase:	75/25: A/B A= 0.1% Formic acid in water B= Methanol
Flow Rate:	0.8 mL/min
Pressure:	120 Bar
Temperature:	30 °C
Injection Volume:	1.0 µL
Sample Solvent:	(caffeine std.) mobile phase
Detection:	UV 254 nm, VWD
Response Time:	0.02 sec
Date rate:	25 Hz
Flow Cell:	2.5 µL semi-micro
LC System:	Shimadzu Prominence UFLC XR
ECV:	~14 µL





Caffeine in Soda *(continued)*

STRUCTURE:



Caffeine

Sample	Caffeine tested mg/(355 mL)	Can value mg/(355 mL)
Store brand cola 1	12	N/A
Cola 2	53	54
Cola 3	43	43
Cola 4	36	38
Cola 5	38	38
Store brand diet cola 1	12	N/A
Diet cola 2	45	46
Diet cola 3	34	34
Diet cola 4	36	35
Energy drink 1*	160	160
Energy drink 2**	79	80
Diet Energy drink**	79	80
Non-cola drink 1	53.3	54
Non-cola drink 2	22	22
Diet non-cola drink	43	41
Diet cola 1 non caffeinated	0	N/A
Diet cola 2 non-caffeinated	0	N/A
Diet cola 3 non-caffeinated	0	N/A

355 mL = 12 oz
*amount in 16 oz (473 mL) cans
**amount in 8.4 oz (248 mL) cans

DISCUSSION:

A selection of sodas was purchased along with several energy drinks at a local grocery store. An approximate 10 mL sample from each soda was placed in a separate 20 mL scintillation vial and capped. The vials were shaken several times and then the cap was loosened and the vial sonicated for 10 minutes to remove CO_2. Then a 1 mL aliquot was placed into a 1.5 mL HPLC sample vial. A one microliter quantity was injected into the HPLC under conditions tabulated elsewhere on this page. A guard column was used to prevent the buildup of a brown material on the analytical column packing. The material was likely caramel coloring.

The chromatogram shown is from a regular cola drink.

A standard curve of peak area vs. caffeine concentration was made over the range of 0.11-0.44 mg/mL. These values were used to calculate the line fit of Y=mX + b. From the peak response the concentration of (mg of caffeine)/mL was calculated and then multiplied by the number of mL in the beverage can (usually 355 mL). Results are shown on the table to the left.

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