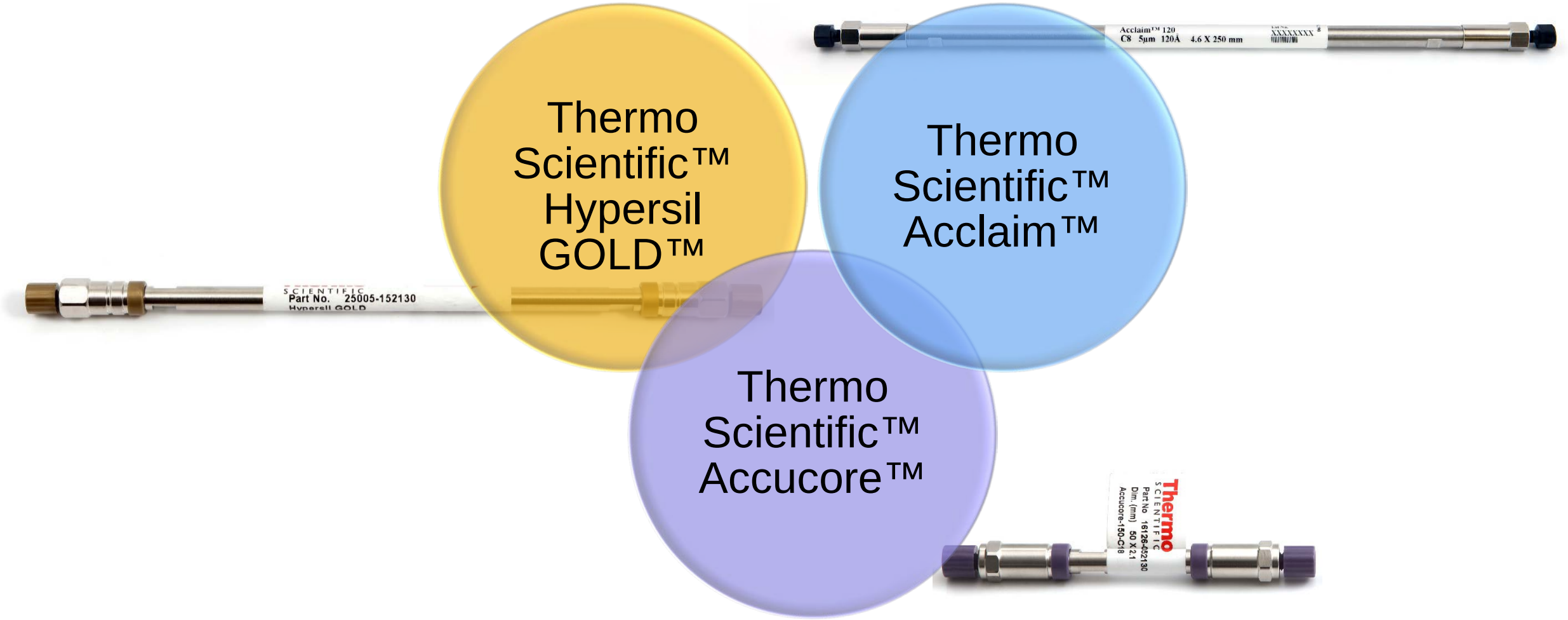
ThermoFisher SCIENTIFIC

Consider Column Variety for Effective Separations Biphenyl and Beyond

Tim Liddicoat
Senior Manager of Product Management – Chromatography Columns and Consumables

Thermo Scientific HPLC Column Families for Small Molecules



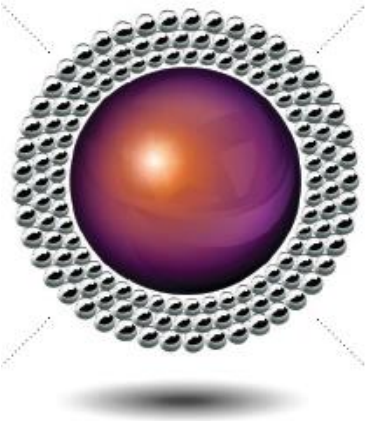
ThermoFisher SCIENTIFIC

Solid core, what is it?

Solid core (superficially porous) columns allow for highly efficient, fast separations with lower backpressures than fully porous sub-2µm particles.

They are used for the separation of small molecules, large molecules, and complex samples.

Switching to a solid core columns can offer your laboratory savings: lowering solvent consumption and decreasing sample run times.



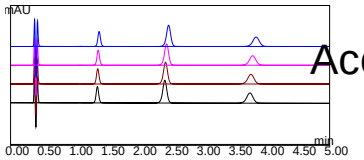
A solution for every chromatographer and every LC system

1.5µm particles- For UHPLC analysis where peak shape, resolution, and throughput are paramount

2.6µm particles – Rugged columns for customers with modern HPLC systems, offered in 14 chemistries

4µm particles – For modernization of methods using fully porous 5µm particles

Solid Core Resolution and Reproducibility

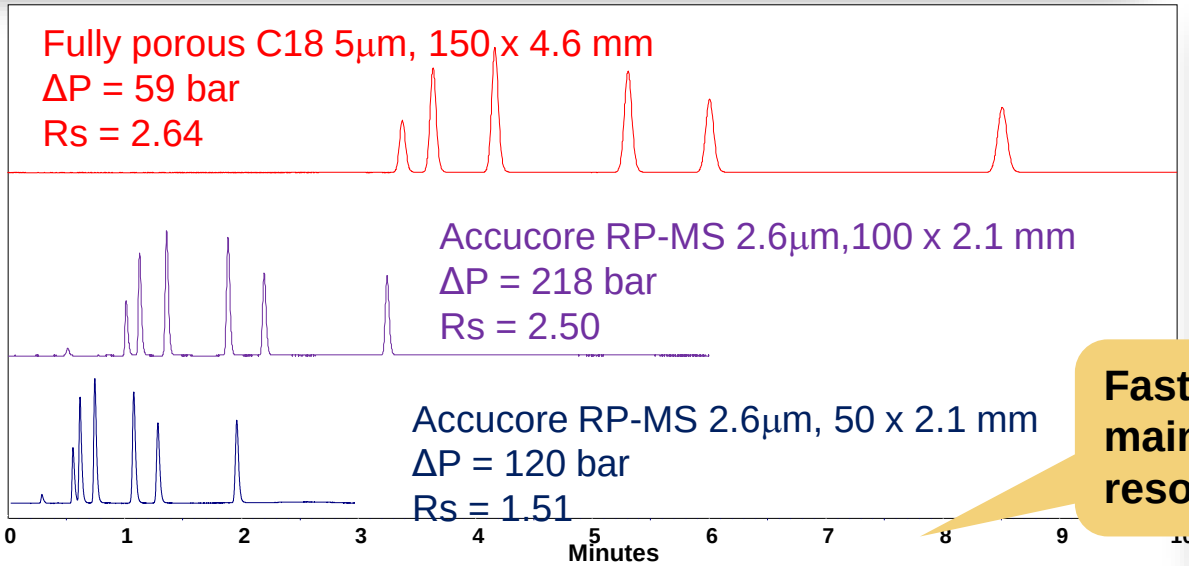


Accucore XL C8 4.0 µm 50 x 2.1 mm

Injection 2200
Injection 1500

Injection 750
Injection 1

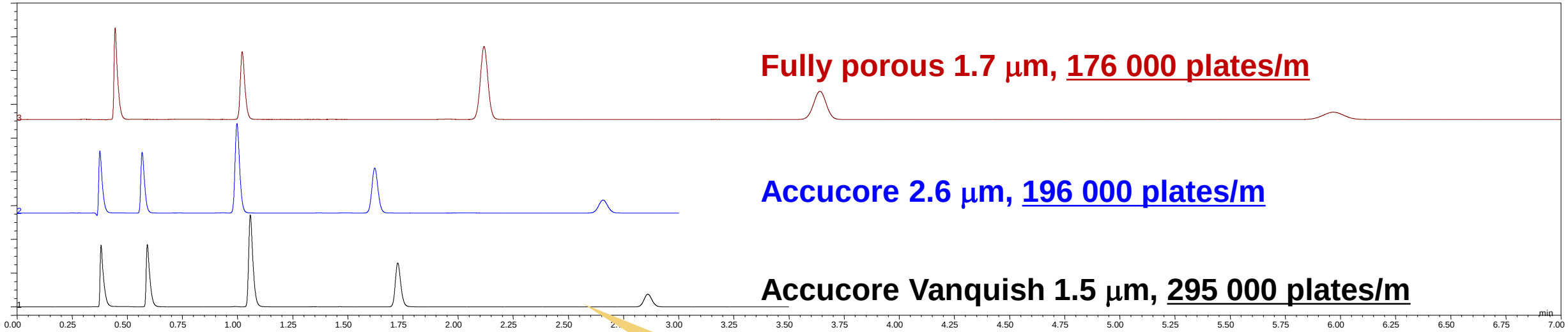
Separation of naproxen, fenoprofen and ibuprofen, with theophylline as a t_0 marker



Faster run maintaining resolution!

ThermoFisher SCIENTIFIC

What is Thermo Scientific Accucore Vanquish?



40% Efficiency Gain!

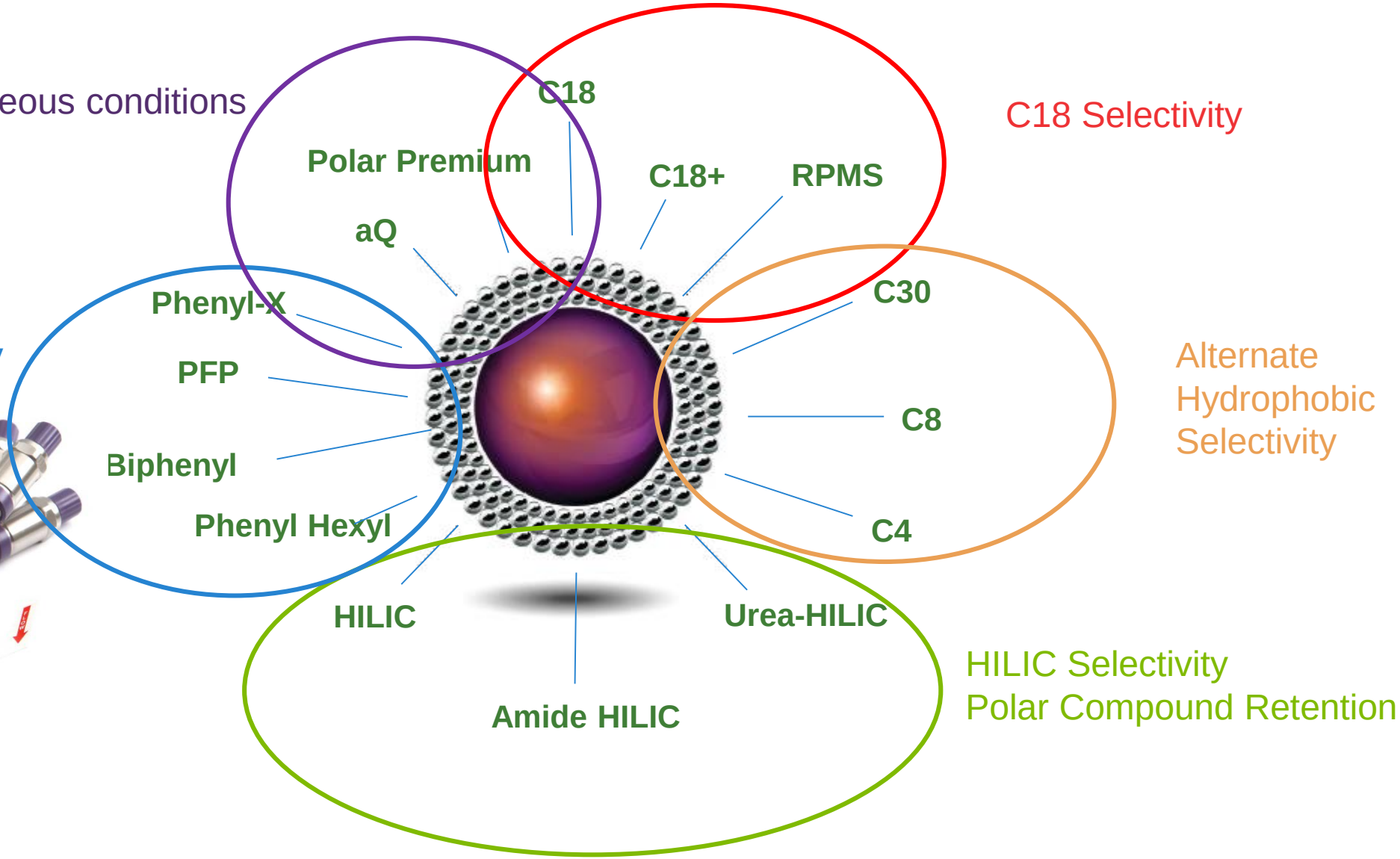
- **Next generation UHPLC column**
- **1.5 µm solid core particles**
- **Designed in synergy with the Thermo Scientific™ Vanquish™ UHPLC System to deliver:**
  - **Better Separations**: high efficiency enables the separation of very complex mixtures
  - **More Results:** High efficiency at high flow rates
  - **Easier Interactions:** Seamless workflow solution, for simple and easy separations

ThermoFisher SCIENTIFIC

15 Chemistry Phases Available

Polar Selectivity
Stability in 100% aqueous conditions

Aromatic Selectivity
Orthogonal to C18



C18 Selectivity

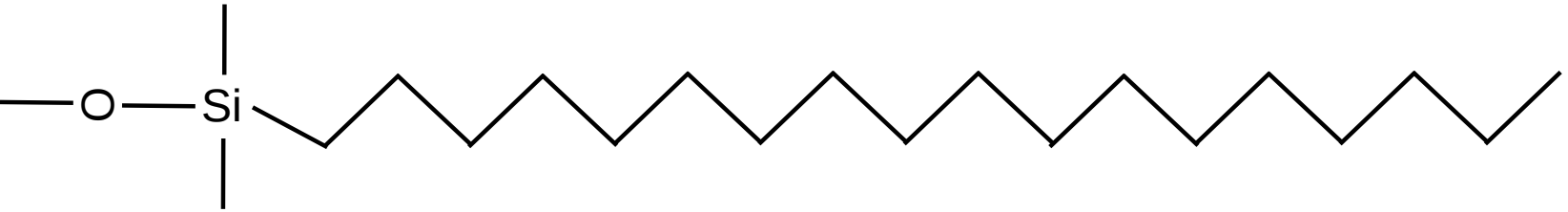
Alternate
Hydrophobic
Selectivity

HILIC Selectivity
Polar Compound Retention

ThermoFisher SCIENTIFIC

Chemistry

Column Chemistry



What is this?

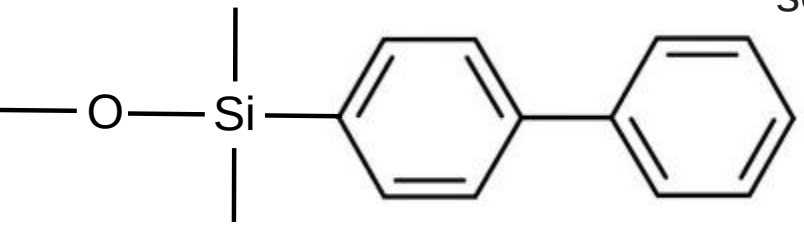
How does it work?

C18 Columns:

- Reversed phase (L1 Classification)
- Have 18 carbon chains bonded to the silica particles inside the column
- Phase is hydrophobic, so non-polar molecules will interact with it when they pass though the column
- **Hydrophobicity governs the separation**

When running samples, the least hydrophobic component elutes first, followed by more hydrophobic molecules

Column Chemistry

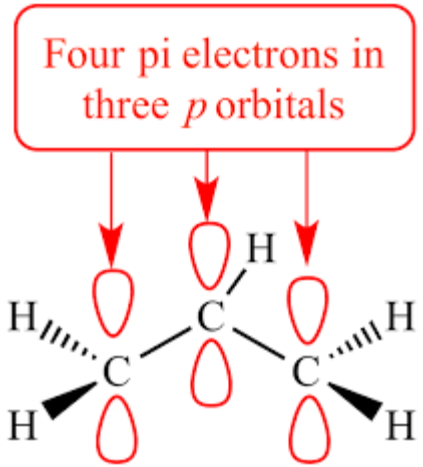


Phenyl type (L11) phases, such as a **biphenyl column** can be useful when separating a variety of analytes:

- Aromatic
- Polycyclic
- Unsaturated species

Why?

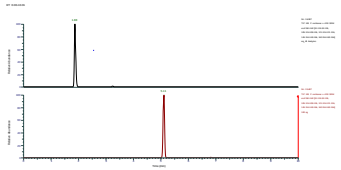
Due to the π-π interactions between the electron rich double bonds within the analyte and stationary phase

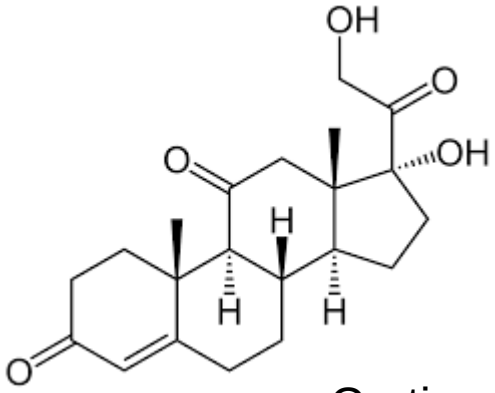


These phases also undergo **hydrophobic interactions** with analytes, which often **dominate the overall strength of interaction**.

Because there are so many pi electrons in conjugation on a biphenyl column, you get much better retention for small and polar analytes than on a phenyl-hexyl phase

Biphenyl and C18 Columns are Different

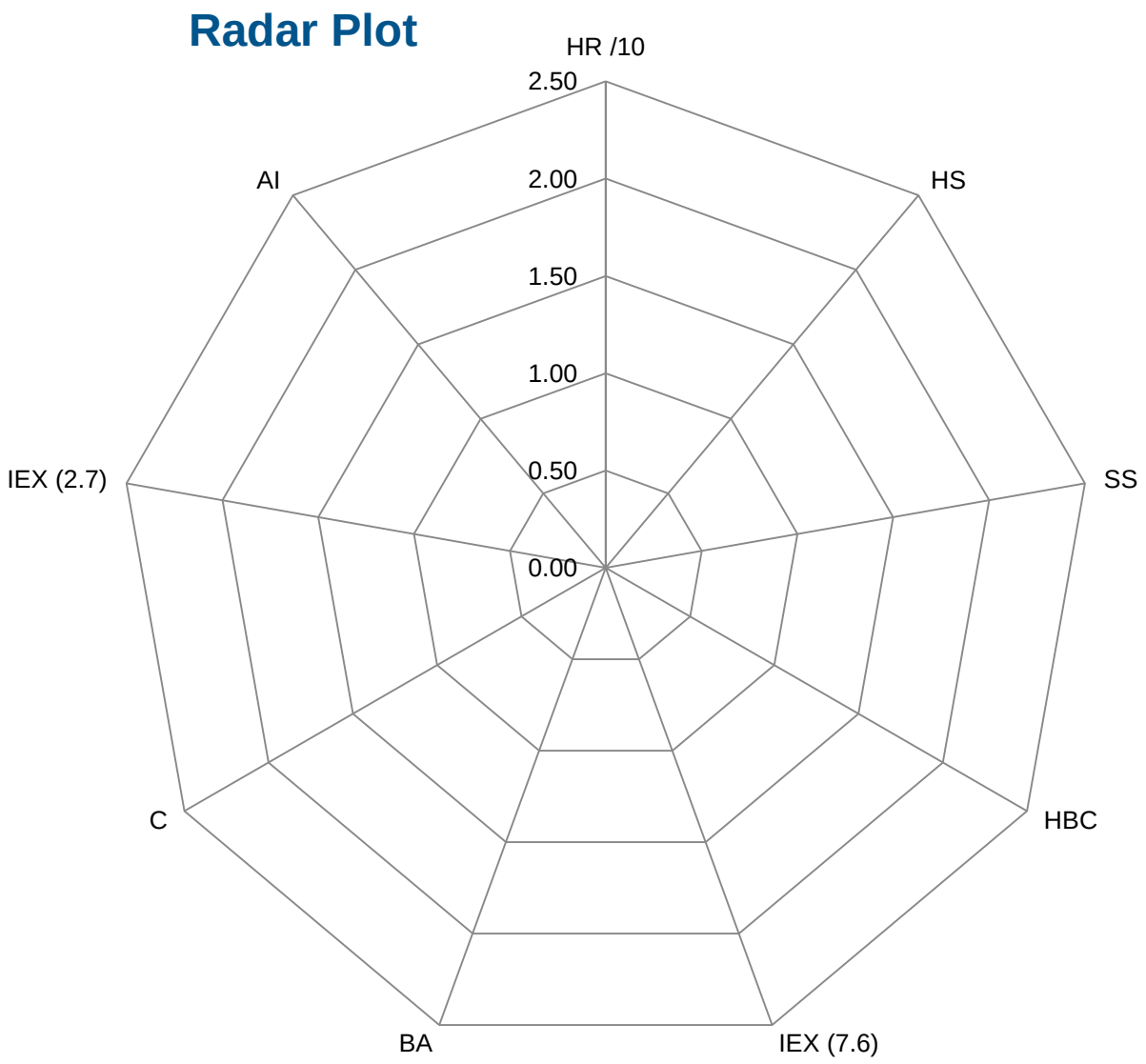




Cortisone

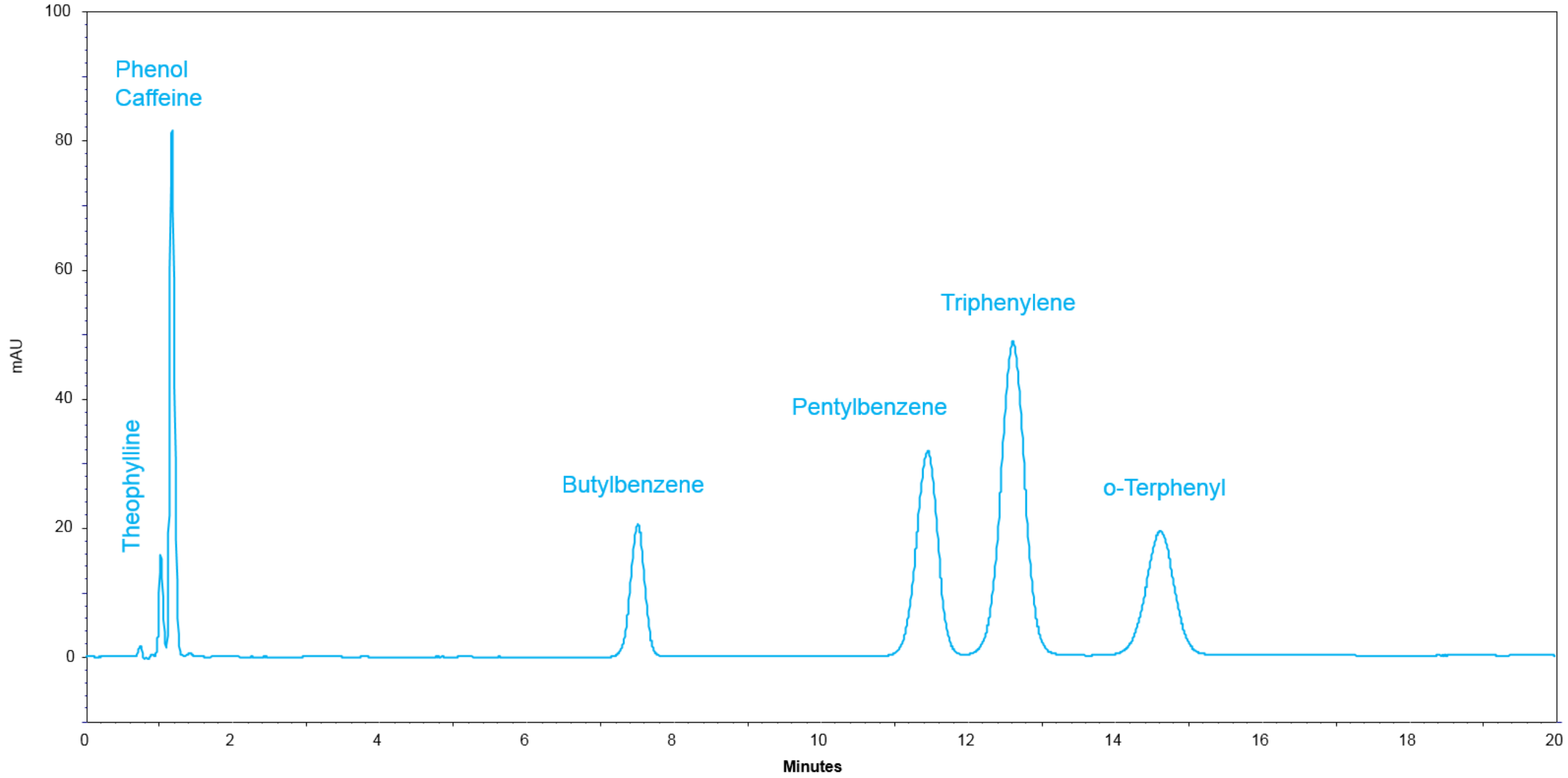
ThermoFisher SCIENTIFIC

Radar Plot of Tanaka Characteristics



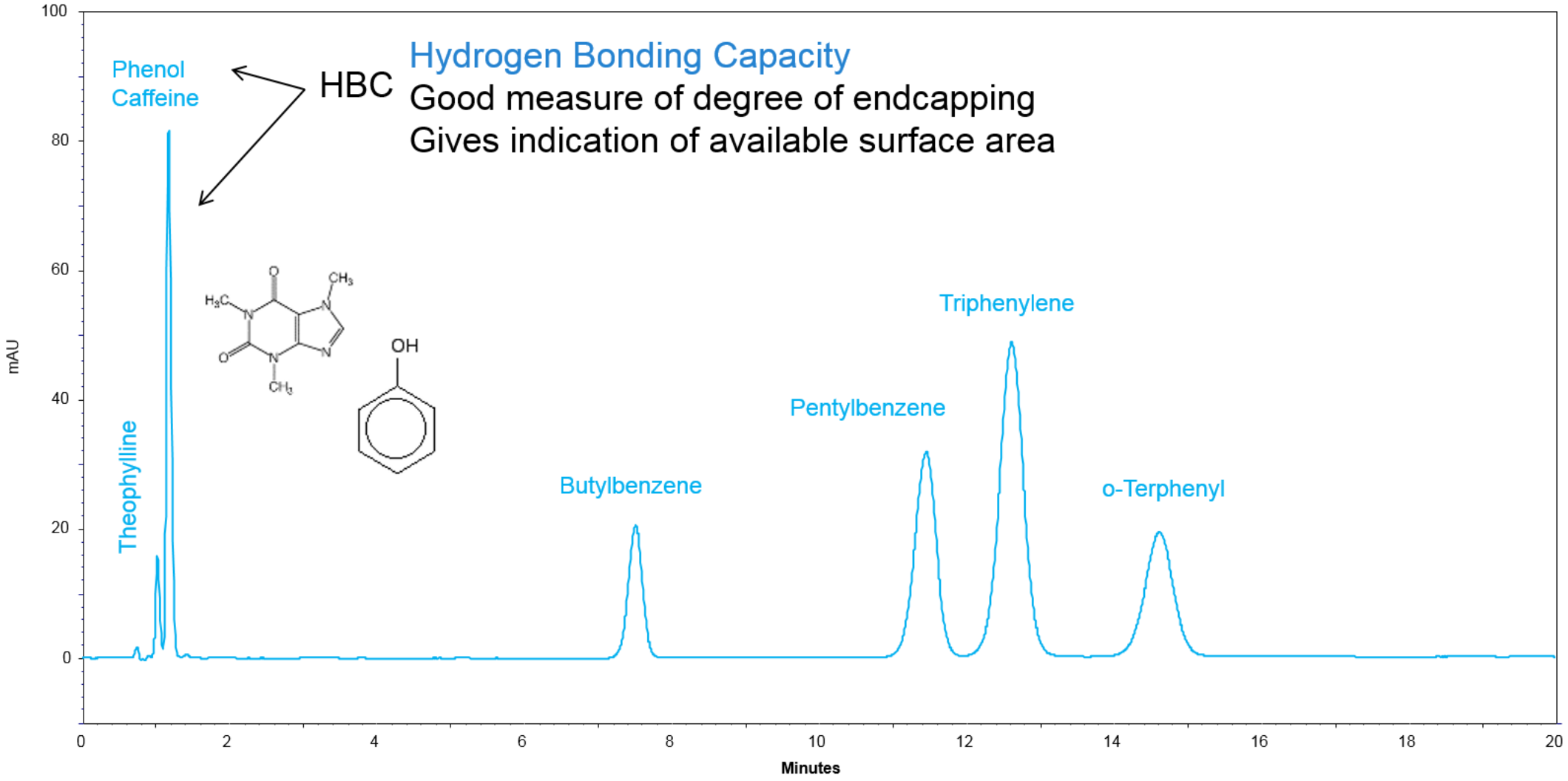
HR	Hydrophobic retention
HS	Hydrophobic selectivity
SS	Steric/aromatic selectivity
HBC	Hydrogen bonding capacity
BA	Base activity
C	Chelation
IEX (7.6)	Ion exchange capacity (pH 7.6)
AI	Acidic interaction
IEX (2.7)	Ion exchange capacity(pH 2.7)

Tanaka Test Markers T1 Testing



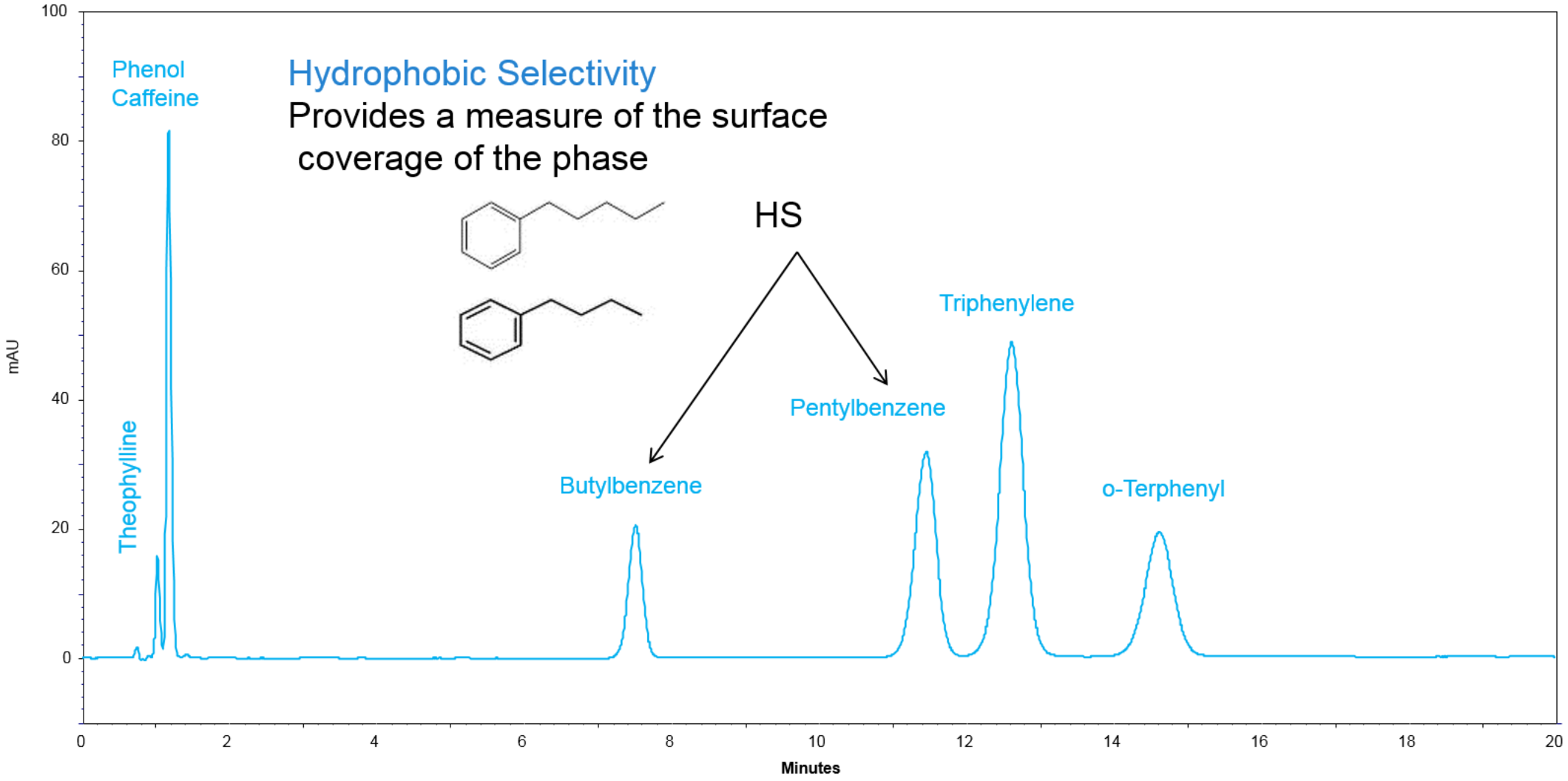
ThermoFisher SCIENTIFIC

Tanaka Test Markers T1 Testing



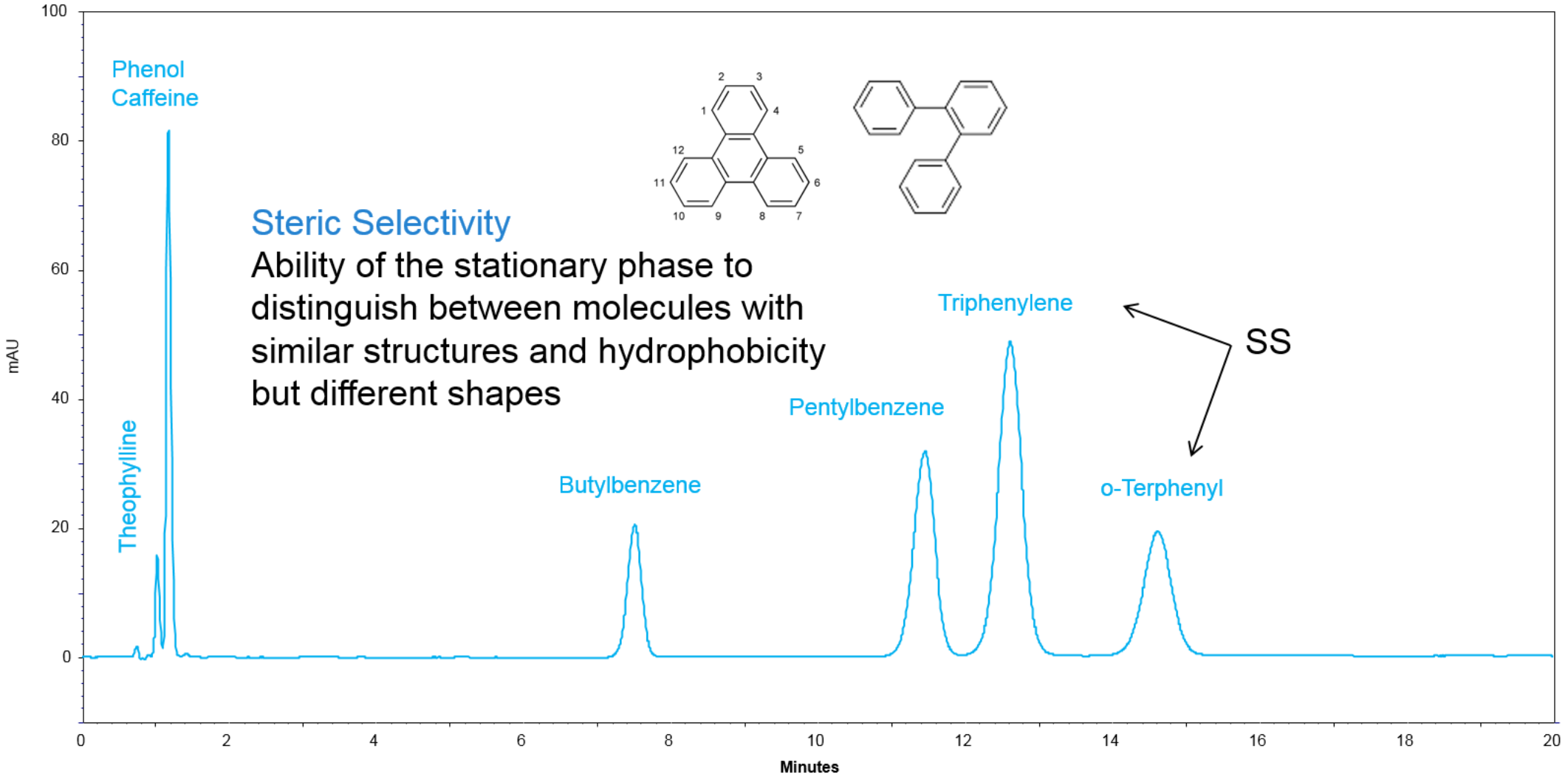
ThermoFisher SCIENTIFIC

Tanaka Test Markers T1 Testing



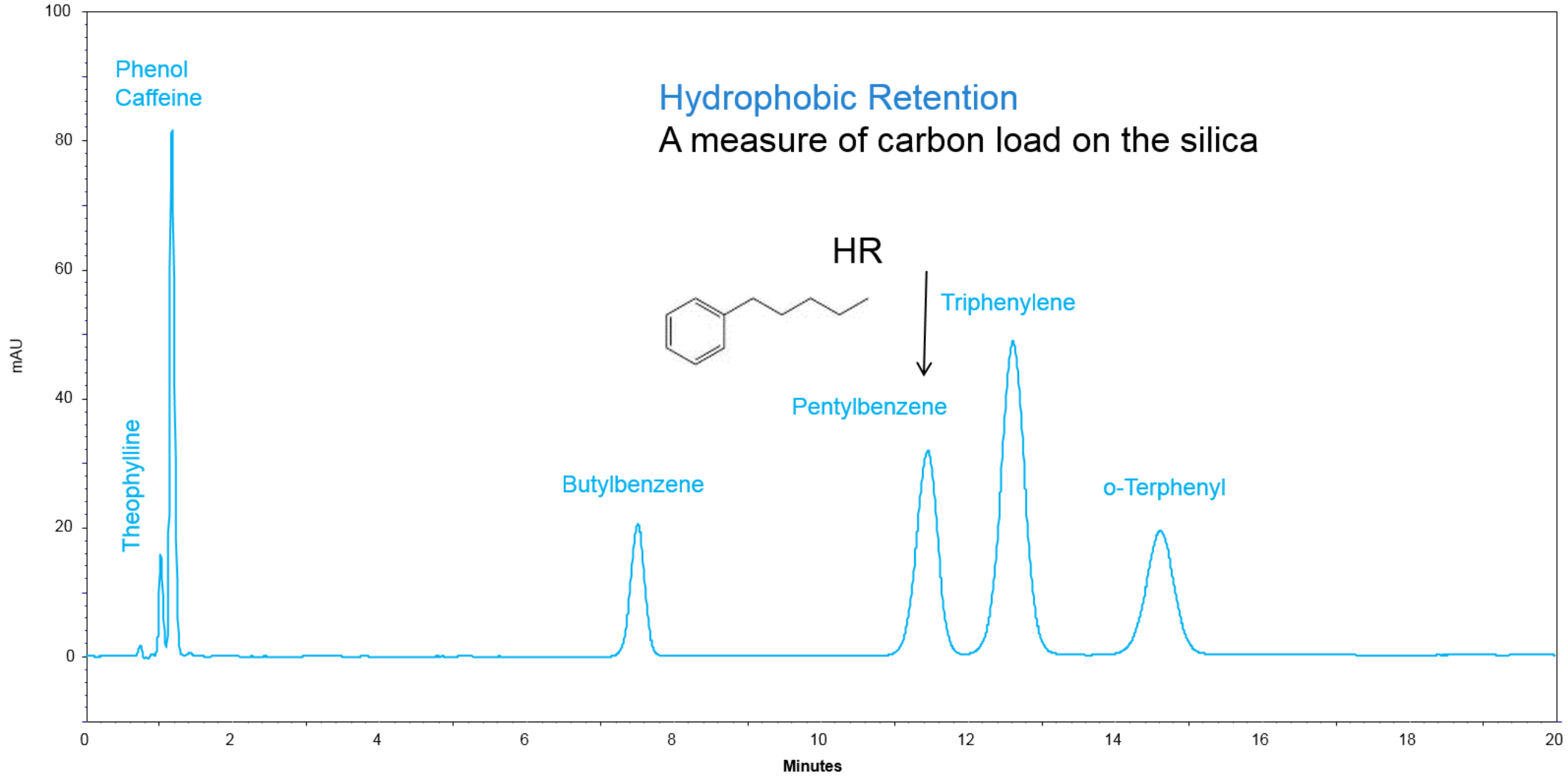
ThermoFisher SCIENTIFIC

Tanaka Test Markers T1 Testing



ThermoFisher SCIENTIFIC

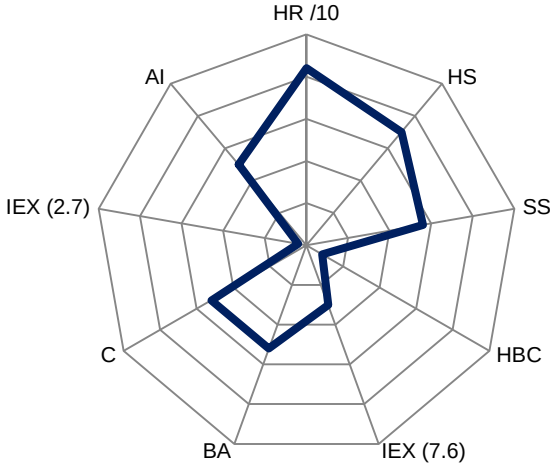
Tanaka Test Markers T1 Testing



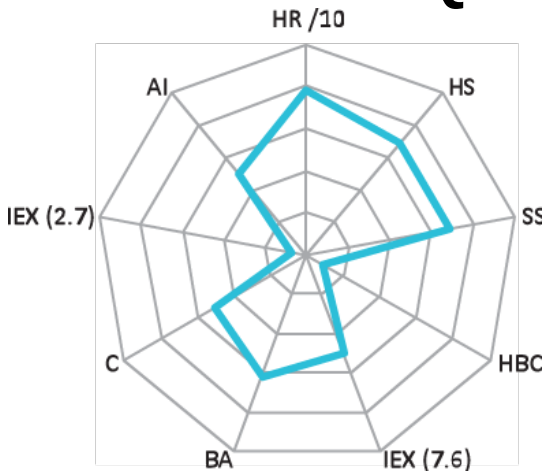
ThermoFisher SCIENTIFIC

Phase Characterization – Thermo Scientific Columns

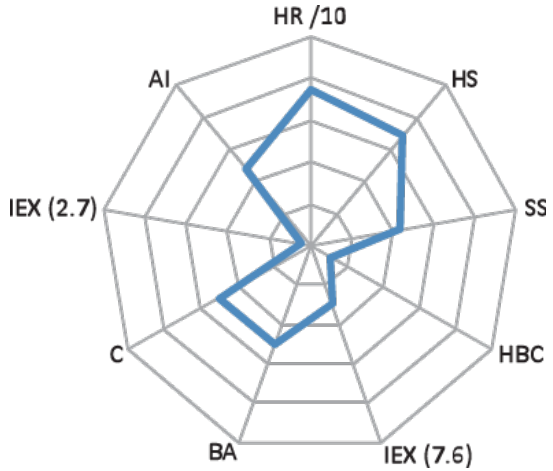
Accucore C18



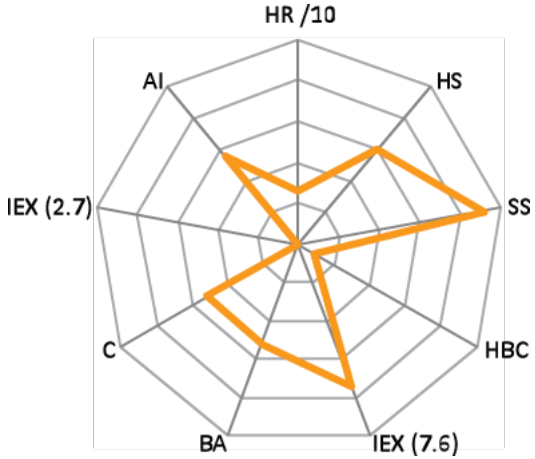
Accucore aQ



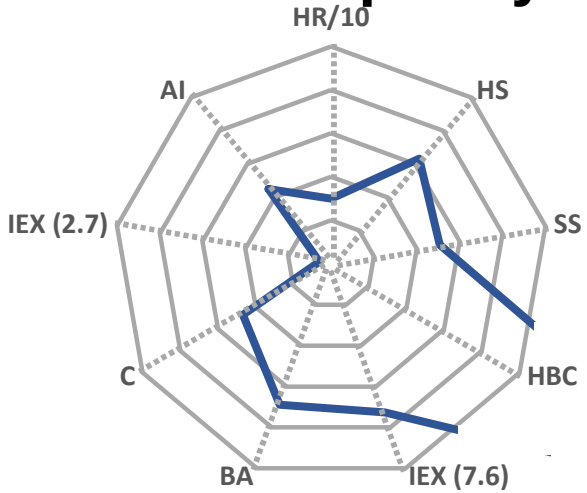
Accucore RPMS



Accucore PFP



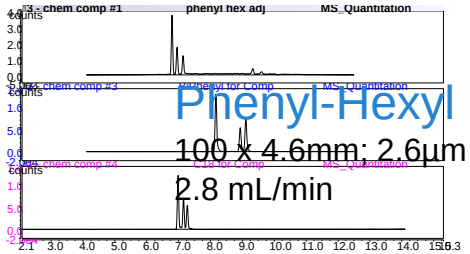
Accucore Biphenyl

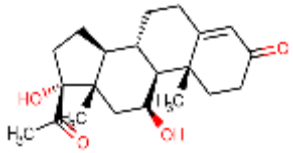


Accucore Phenyl-Hexyl

ThermoFisher SCIENTIFIC

Phase Comparisons - Phenyl Hexyl, Biphenyl, and C18





21-deoxycortisol

Phenyl-Hexyl

100 x 4.6mm; 2.6µm
2.8 mL/min

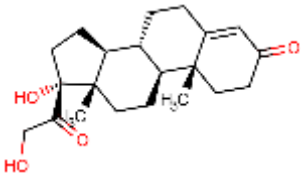
1.

3.

2.

$$F_2 = F_1 \times \frac{(d_{c2}^2)}{(d_{c1}^2)}$$

Flow rate adjustment



11-deoxycortisol

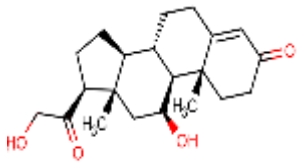
Biphenyl

100 x 2.1mm; 2.6µm
0.6 mL/min

1.

3.

2.



corticosterone

C18

100 x 2.1mm; 2.6µm
0.6 mL/min

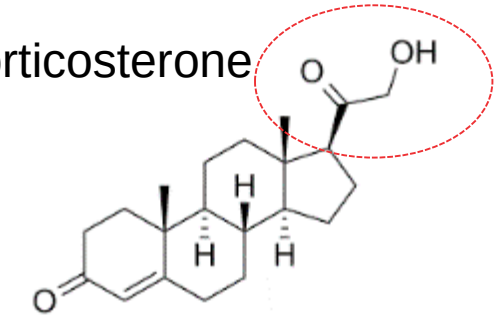
1.

3.

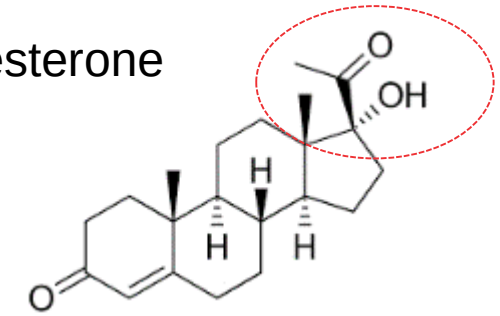
2.

Biphenyl and C18 Columns are Different Continued

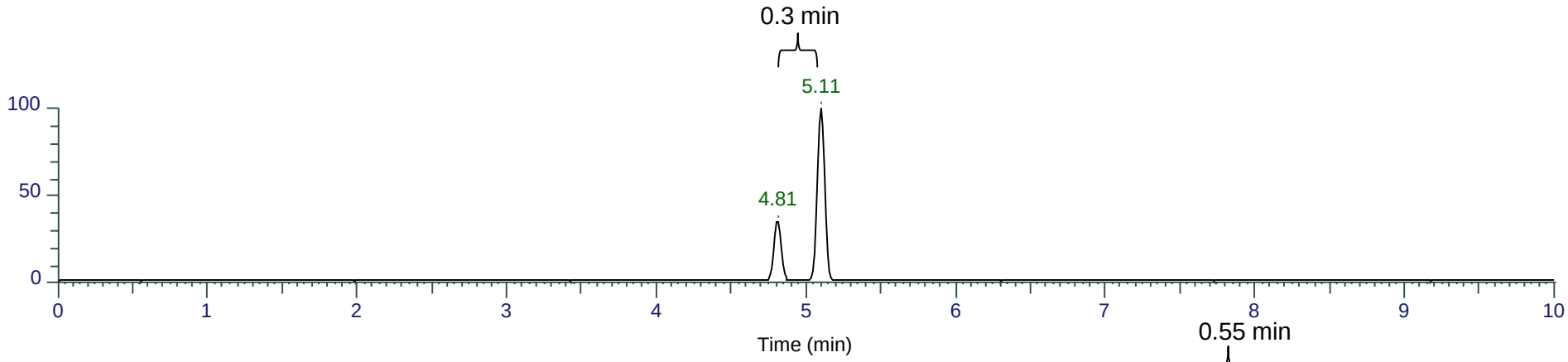
11-deoxycorticosterone



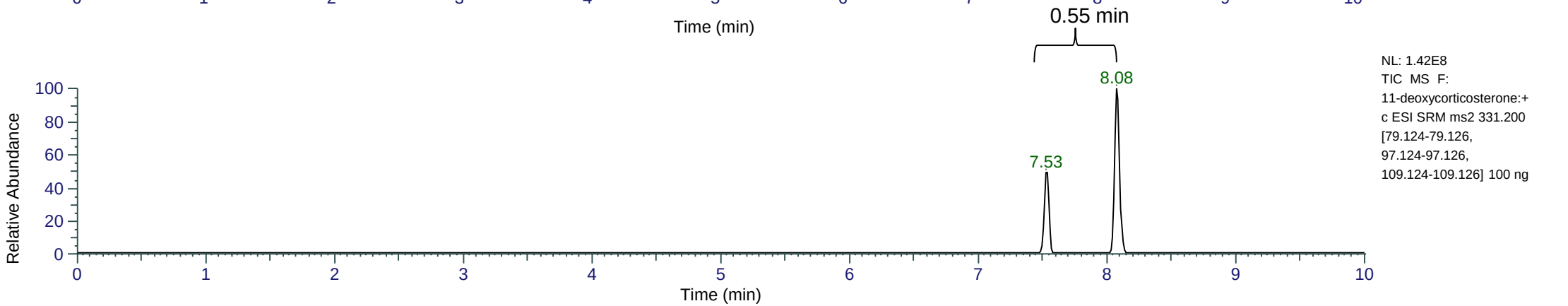
17-α-hydroxyprogesterone



Accucore aQ

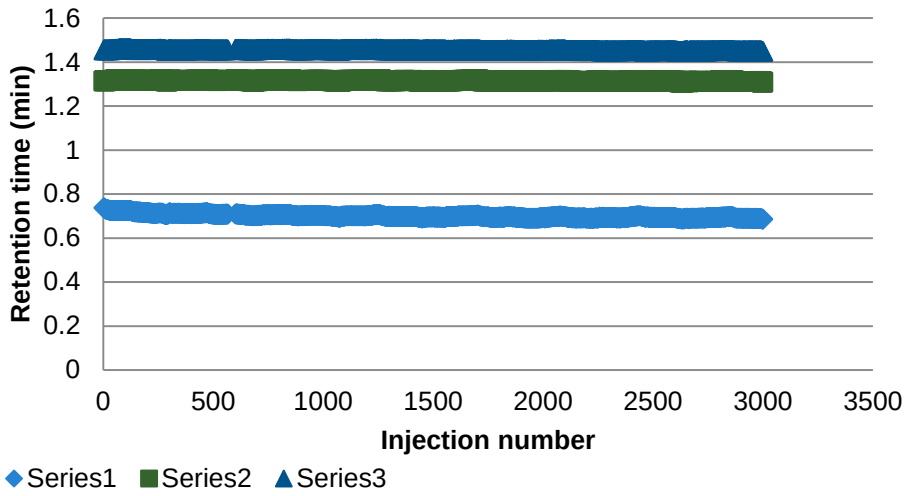


Accucore Biphenyl

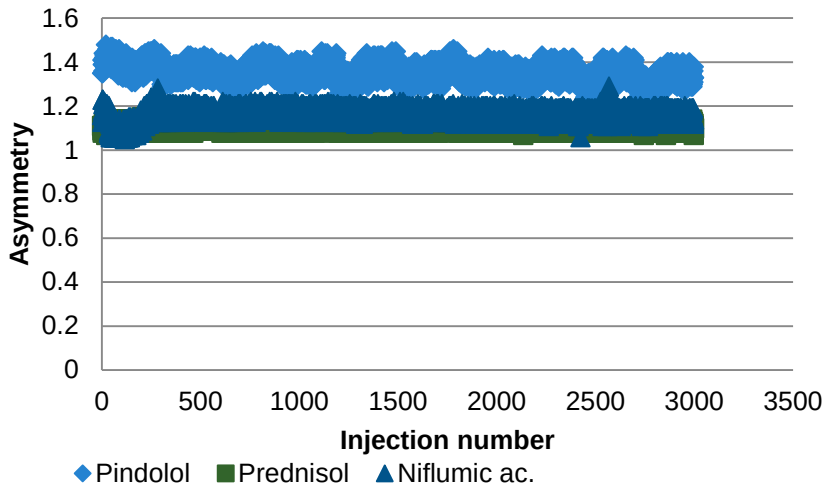


3000 Injection Ruggedness 2.1 x 50mm Column

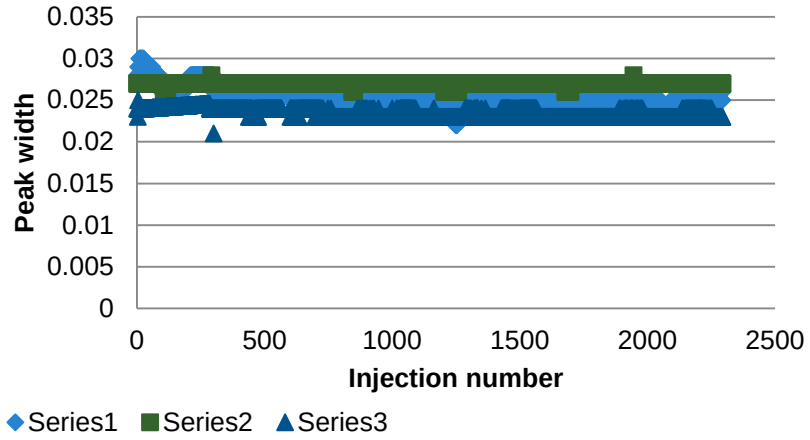
Retention time (min)



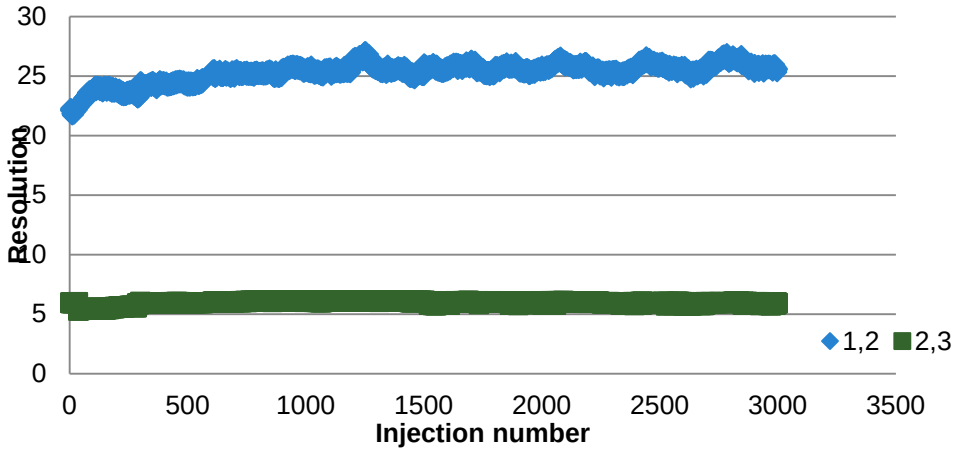
Peak Width 10%



Asymmetry 10%

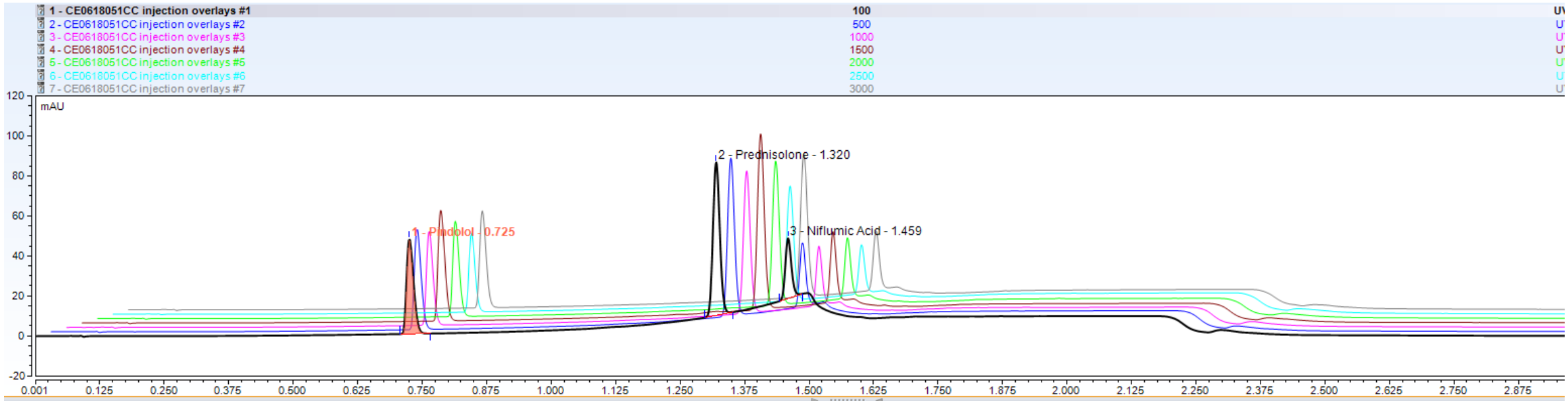


Resolution

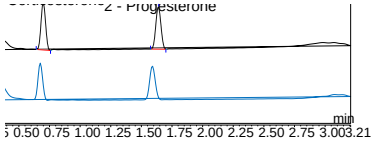


ThermoFisher SCIENTIFIC

3000 Injection Ruggedness 2.1 x 50mm Column



Lifetime in 1:3 Diluted Urine*

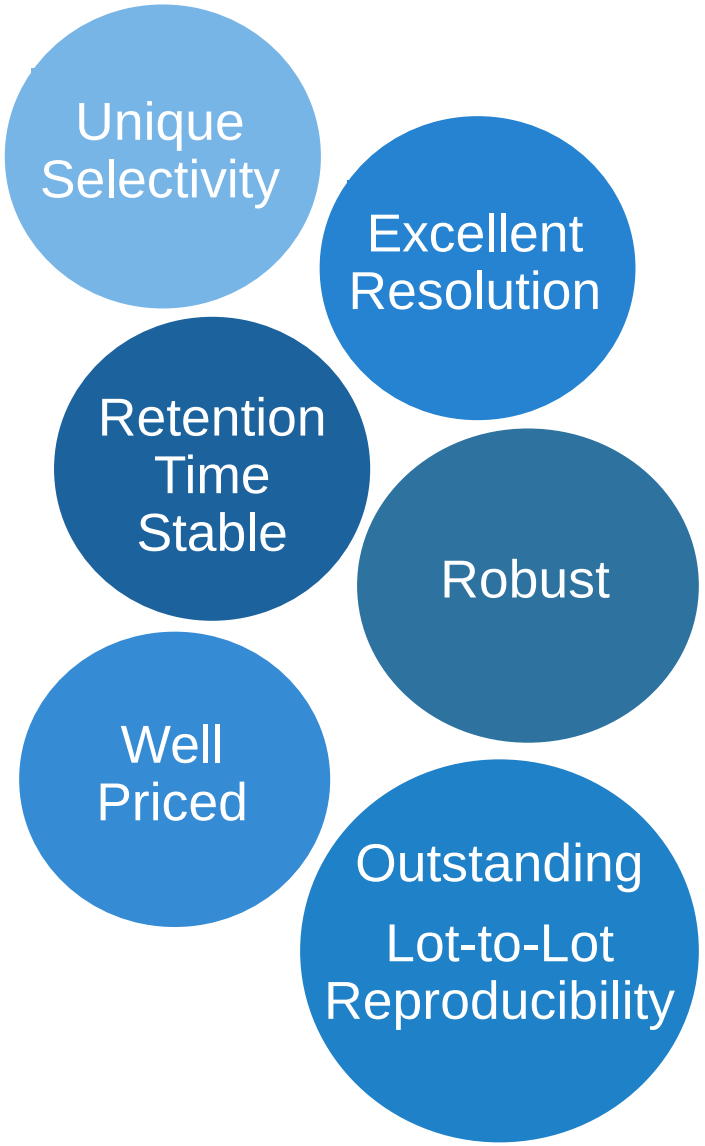


Accucore Biphenyl Injection 1

Accucore Biphenyl Injection 6050

 *For research use only

Biphenyl Summary

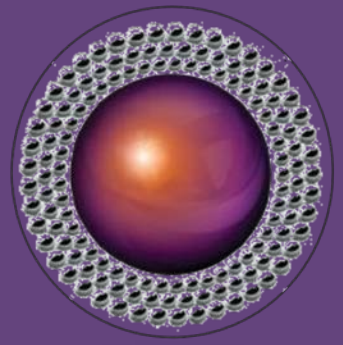


Not just another column…

Accucore offers the widest range of selectivity to meet the needs of your diverse range of compounds. Our solid core columns offer **enhanced resolution** separations on a wide variety of chemistries and particle sizes.

Designed around voice of customer…

The Thermo Scientific Accucore Biphenyl column offers a robust platform for separations requiring alternate selectivity to a C18 column. Customers should expect excellent resolution, retention time stability and a rugged column, durable for their challenging separations



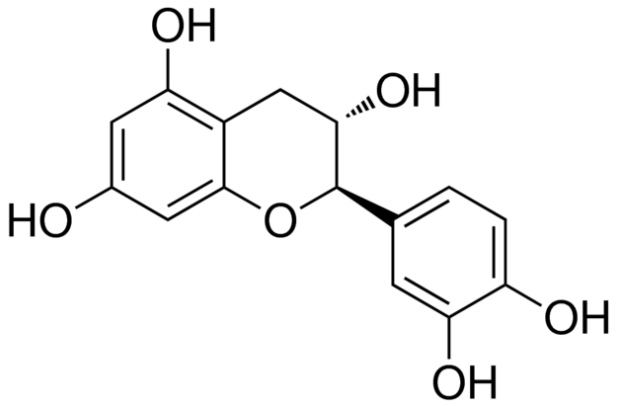
Food For Thought

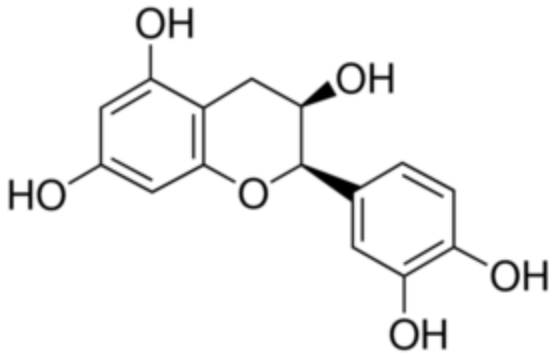
-Applications-

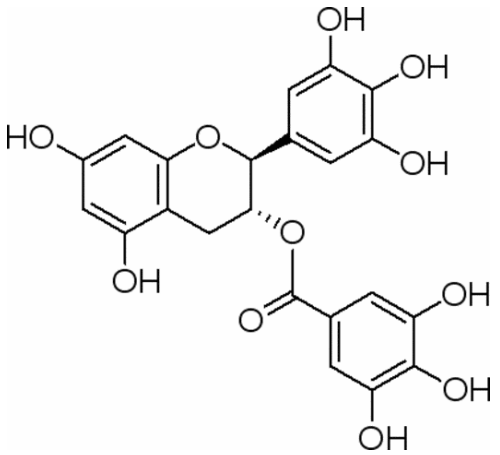
Wine - Accucore XL C8

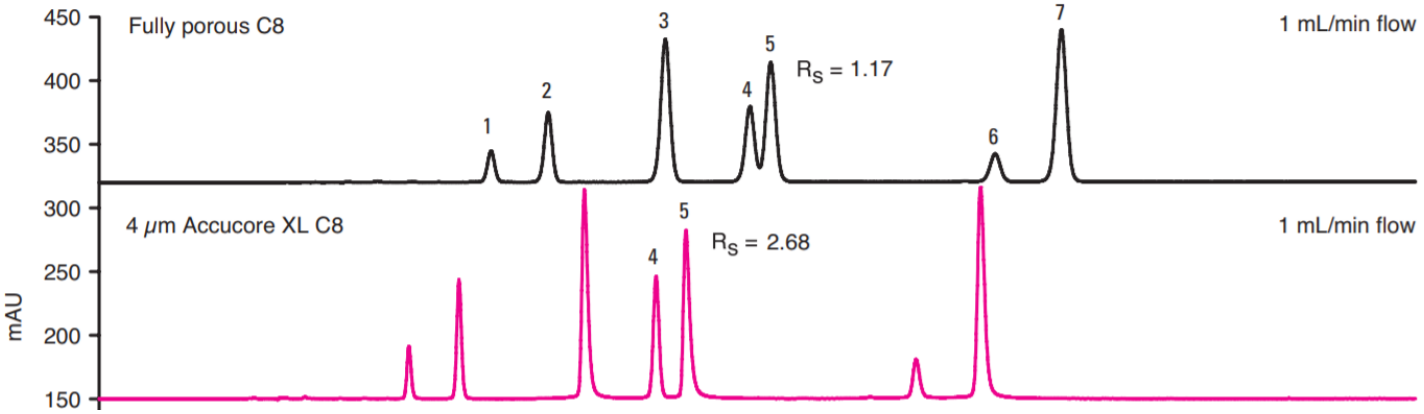
Wine - Accucore XL C8

Catechins in Wine:
Application Note 20583 and 20536





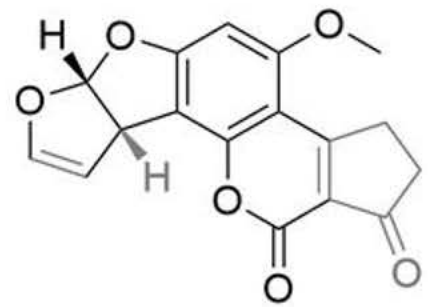




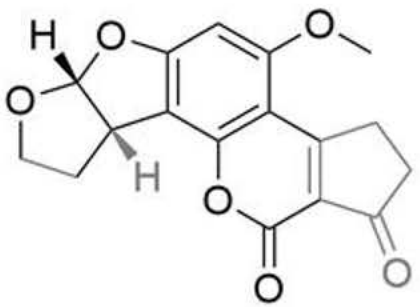
Compound	Peak Width		
	Fully Porous	Accucore XL C8 4 µm HPLC column	
	1 mL/min flow rate	1 mL/min flow rate	1.5 mL/min flow rate
1. Epigallocatechin	0.157	0.094	0.075
2. Catechin	0.173	0.101	0.080
3. Epigallocatechin gallate	0.201	0.132	0.108
4. Epicatechin	Partial co-elution	0.124	0.106
5. Gallocatechin gallate		0.149	0.124
6. Epicatechin gallate	0.228	0.151	0.136
7. Catechin gallate	0.227	0.158	0.139

Bread- Accucore aQ

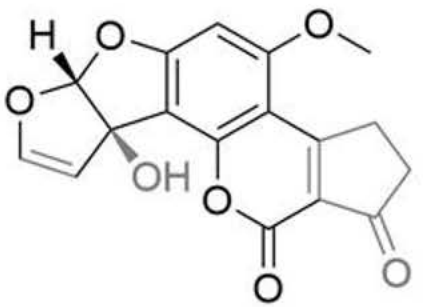
Bread- Accucore aQ



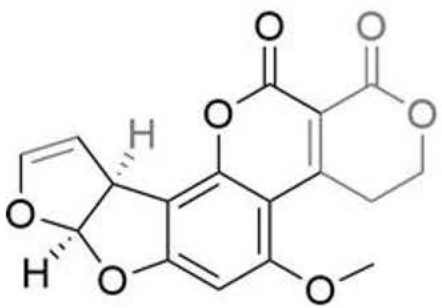
AFB_1



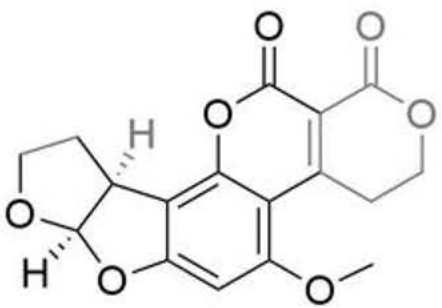
AFB_2



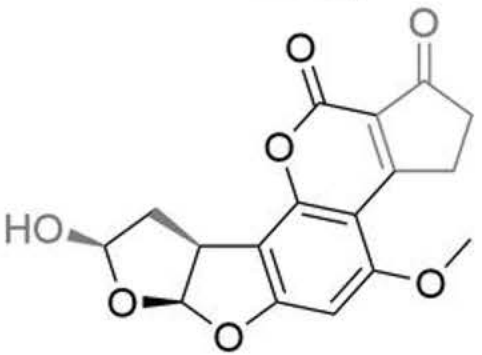
AFM_1



AFG_1



AFG_2



AFB_{2a}

Bread- Accucore aQ

Mycotoxins in Grain and Dairy
Application Notes 21121 and 64596

Application Note 21121

Determination of Multiple Mycotoxins in Grain Using a QuEChERS Sample Preparation Approach and LC-MS/MS Detection

Jon Bardsley, Mike Oliver, Thermo Fisher Scientific, Runcorn, UK

Key Words
Mycotoxins, food, HyperSep, QuEChERS, dispersive SPE, Accucore aQ, TSQ Vantage

Goal
To demonstrate a fast, easy, and cost-effective approach for the determination of 16 mycotoxin residues in grain-based food using QuEChERS sample preparation with a Thermo Scientific™ Accucore™ aQ HPLC column and a Thermo Scientific™ TSQ™ Vantage™ triple quadrupole mass spectrometer for HPLC separation.

Introduction

17 Mycotoxins in Grain on Accucore aQ

Application Brief 64596

Simultaneous Analysis of Mycotoxins in Dairy Products by Liquid Chromatography - Quadrupole Orbitrap Mass Spectrometry

Complete method: Jia, W. et al, Multi-Mycotoxin Analysis in Dairy Products by Liquid Chromatography Coupled to Quadrupole Orbitrap Mass Spectrometry, *J. Chrom. A.*, **2014**, 1345, pp 107-114, DOI: 10.1016/j.chroma.2014.04.021

Highlights
- Validated method can simultaneously analyze 58 mycotoxins in dairy products at low concentration levels.
- Extraction recoveries ranged between 86.6 and 113.7%, with the coefficient of variation < 6.2%.
- Limits of detection were 0.001–0.92 µg/kg.
- Sample preparation is simple, robust, and inexpensive

Introduction
Mycotoxins are a diverse group of highly toxic secondary metabolic products from various fungal species. Their analysis can be challenging because the physicochemical

LC-MS Conditions
UHPLC analysis was performed using the Thermo Scientific™ Accela™ 1250 LC pump and an open autosampler with a Thermo Scientific™ Accucore™ aQ column (100 × 2.1 mm, 2.6 µm) connected to an Accucore aQ guard column (10 × 2.1 mm). Mobile phases were (A) water and (B) methanol, each containing 4 mM ammonium formate + 0.10% formic acid. The injection volume was 5 µL, and the flow rate was 300 µL/min. A Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap mass spectrometer with a heated electrospray ionization probe was used for analysis. All quantitative data were acquired in full MS scan mode. If a targeted compound was present, its precursor ion scan triggered

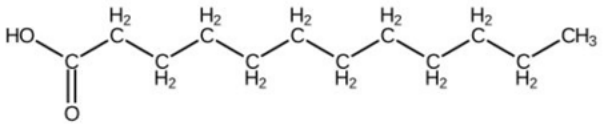
58 Mycotoxins in Dairy on Accucore aQ

ThermoFisher SCIENTIFIC

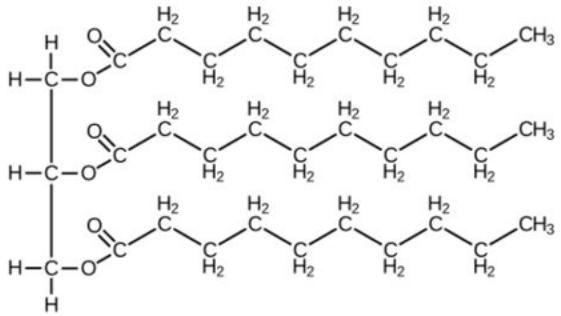
Lipids, Triglycerides, Fat Soluble Vitamins Accucore C30 and Accucore C18

Lipids, Triglycerides, Fat Soluble Vitamins Accucore C30 and Accucore C18

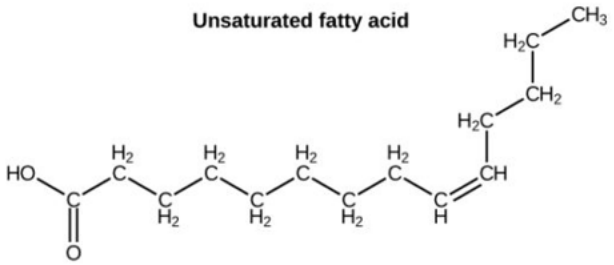
Saturated fatty acid



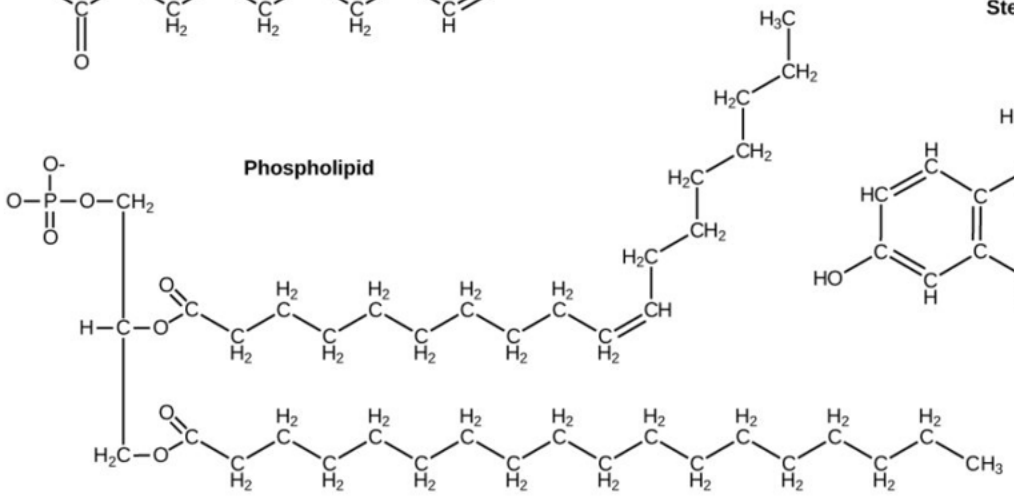
Triglyceride



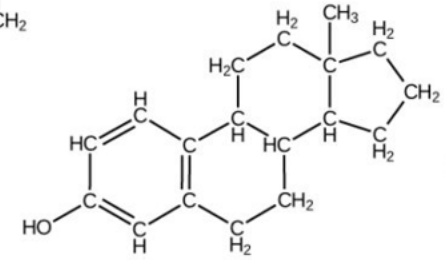
Unsaturated fatty acid



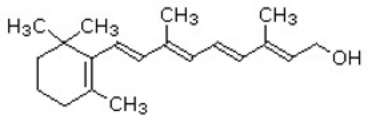
Phospholipid



Steroid

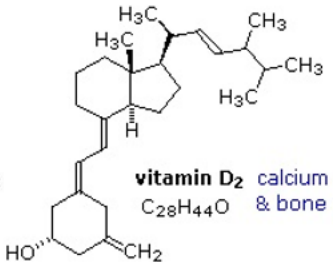


Lipid Soluble Vitamins



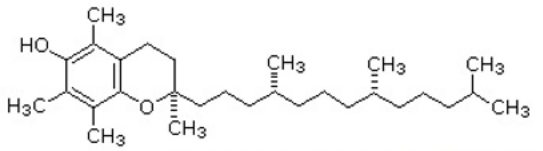
vitamin A

$C_{20}H_{30}O$ part of the visual pigment

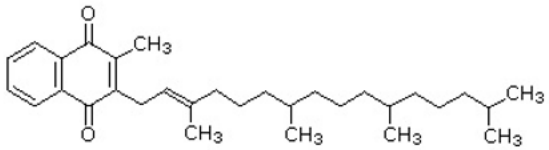


vitamin D_2 calcium metabolism

$C_{28}H_{44}O$ & bone growth



$C_{29}H_{50}O_2$ **vitamin E** an antioxidant



vitamin K_1 a blood clotting factor

$C_{31}H_{46}O_2$

Lipids, Triglycerides, Fat Soluble Vitamins Accucore C30 and Accucore C18

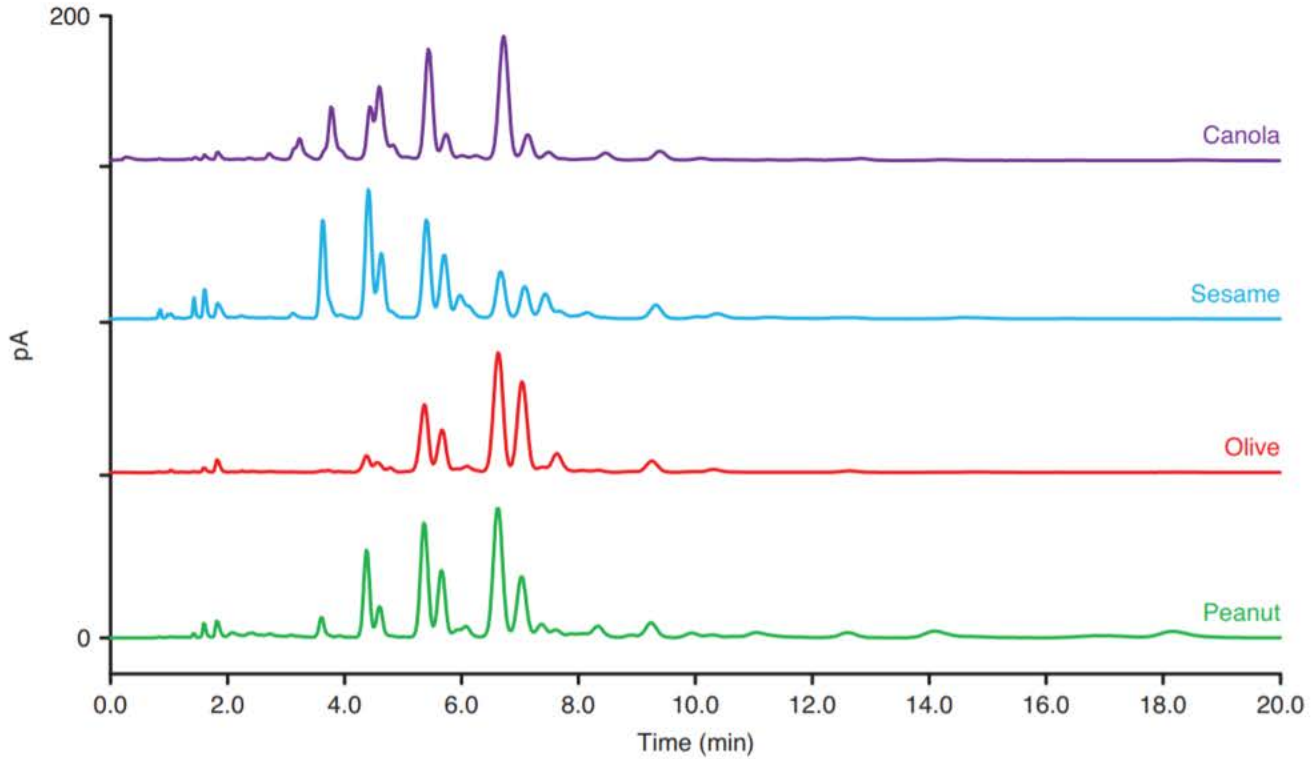


Figure 1: Comparison chromatograms showing the differences in constituents amongst four types of cooking oil using an Accucore C30 HPLC column and aerosol detection

Lipids and Cooking Oils:
Accucore C30
Application 20663

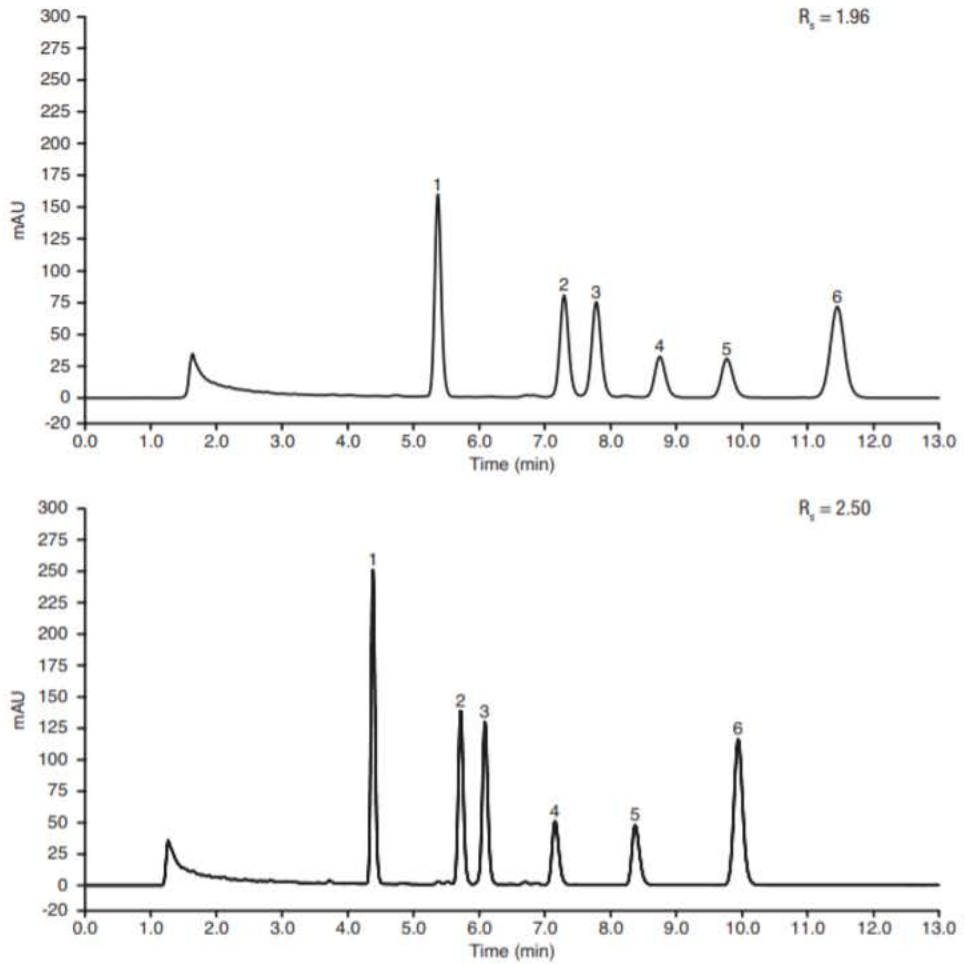
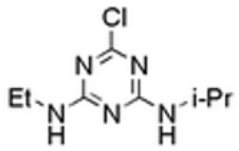


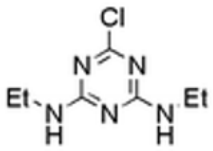
Figure 1: Chromatograms for 5 µm fully porous C18 (top) and 4 µm Accucore XL C18 (bottom)

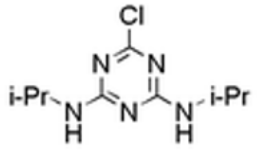
ThermoFisher SCIENTIFIC

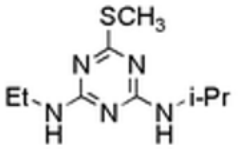
Salad Course- Accucore aQ

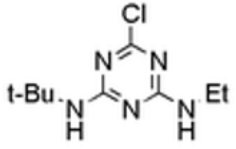
Salad Course- Accucore aQ

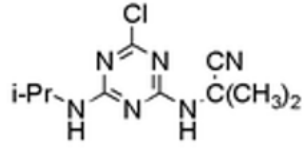

atrazine

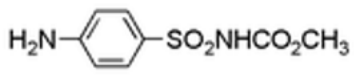

simazine

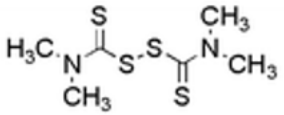

propazine

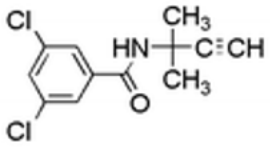

ametryn

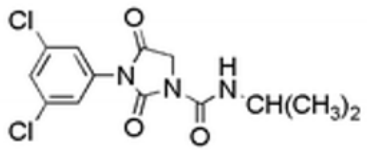

terbutylazine

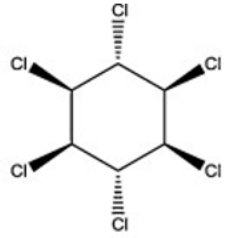

cyanazine

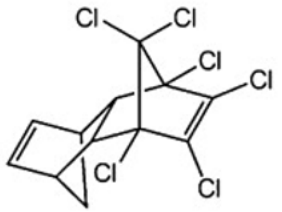

asulam

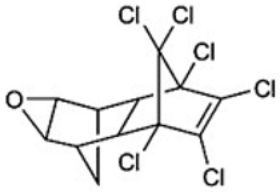

thiuram

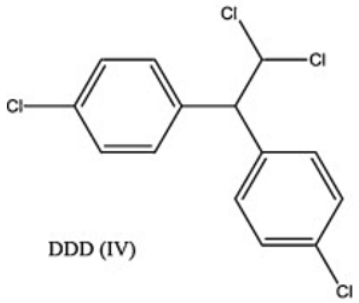

propyzamide

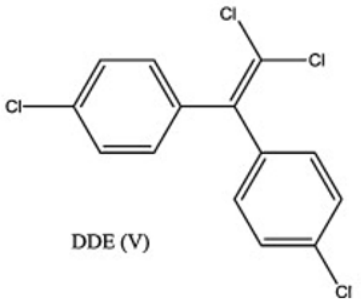

iprodion

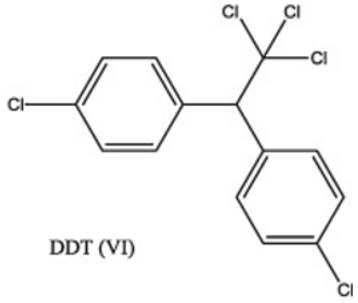

lindane (I)

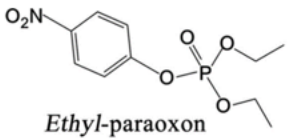

aldrin (II)

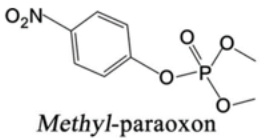

dieldrin (III)

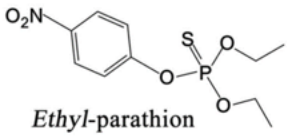

DDD (IV)

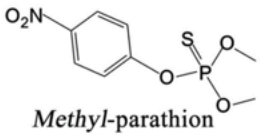

DDE (V)

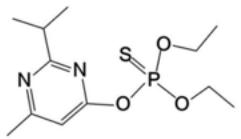

DDT (VI)

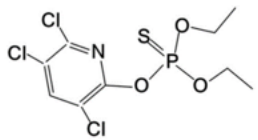

Ethyl-paraoxon

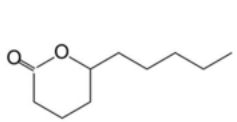

Methyl-paraoxon


Ethyl-parathion

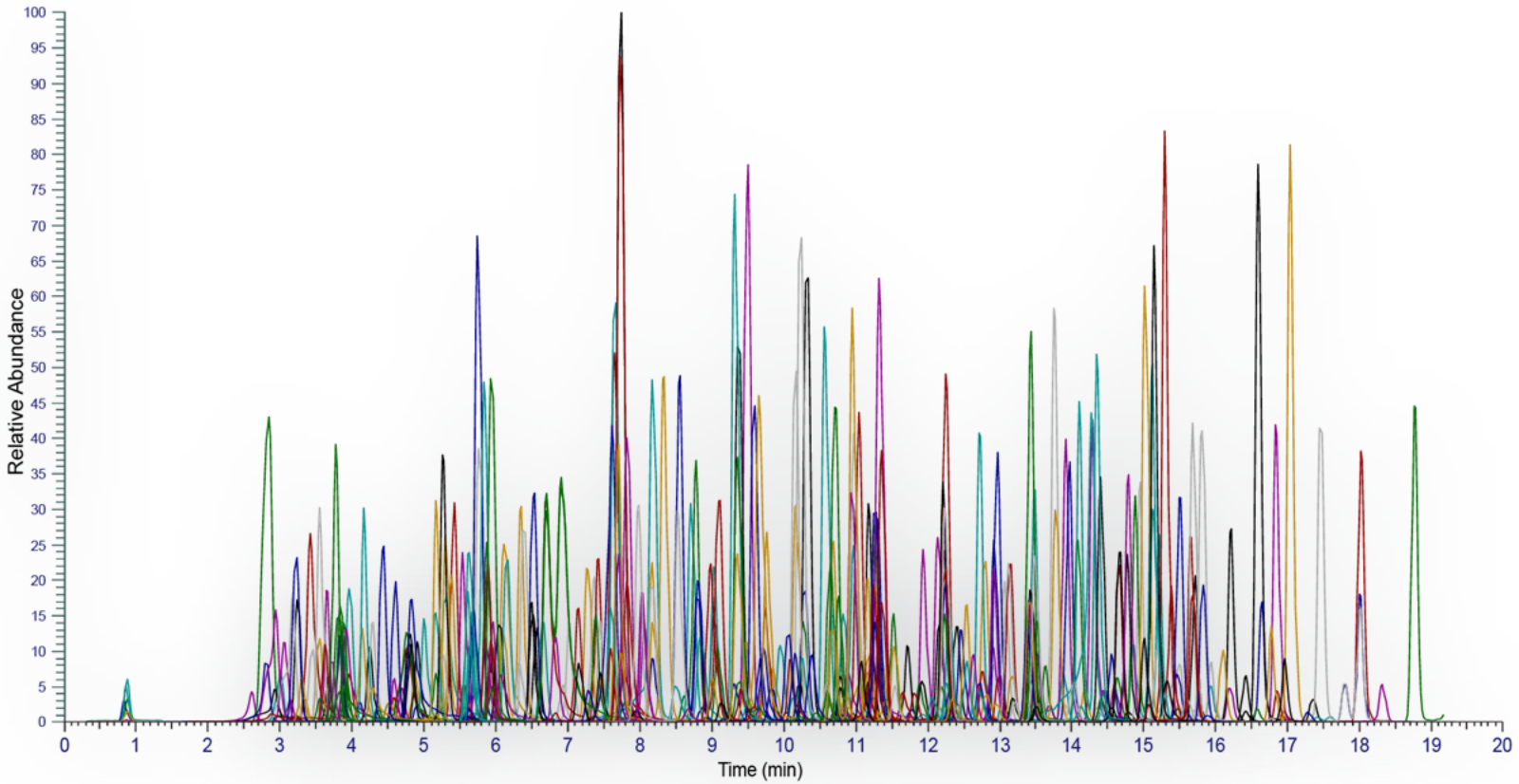

Methyl-parathion


Diazinon


Chlorpyrifos


δ-Decanolactone

Salad Course- Accucore aQ



440 Pesticides in **25 minutes** (5µL injection) on a TSQ Endura

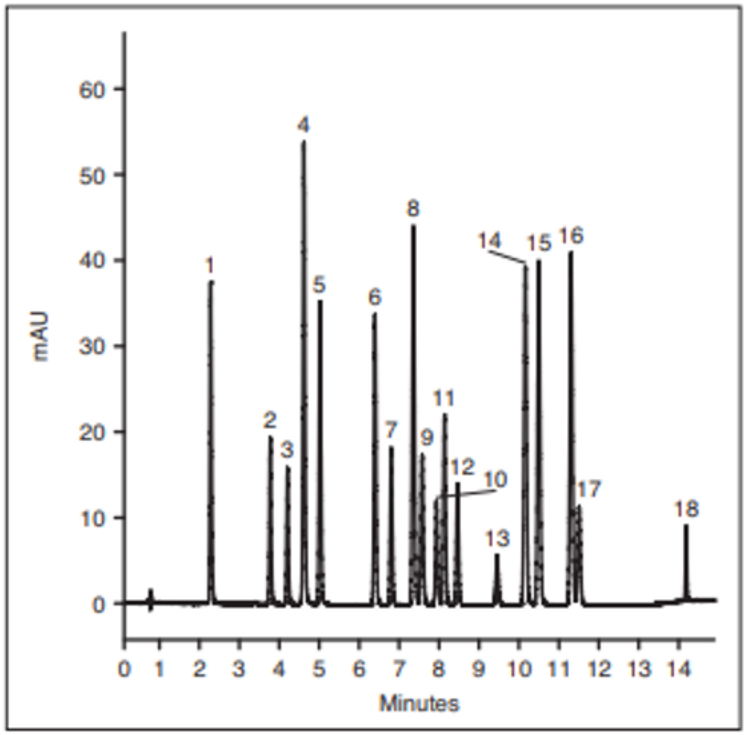


Figure 1: Example chromatography – analysis of eighteen pesticide mixture at 400 µL/min
1. desethylatrazine 2. metoxuron 3. hexazinone 4. simazine 5. cyanazine 6. methabenzthiazuron 7. Chlorotoluron 8. Atrazine 9. monolinuron 10. diuron 11. isoproturon 12. metobromuron 13. metazachlor 14. sebuthylazin15. propazine 16. terbuthylazine 17. linuron 18. metolachlor

Pesticides:
Application Note 643

ThermoFisher SCIENTIFIC

Main Course- Chicken, Beef, Pork- Accucore Vanquish C18+

Veterinary Antibiotics:
Application Note 21000

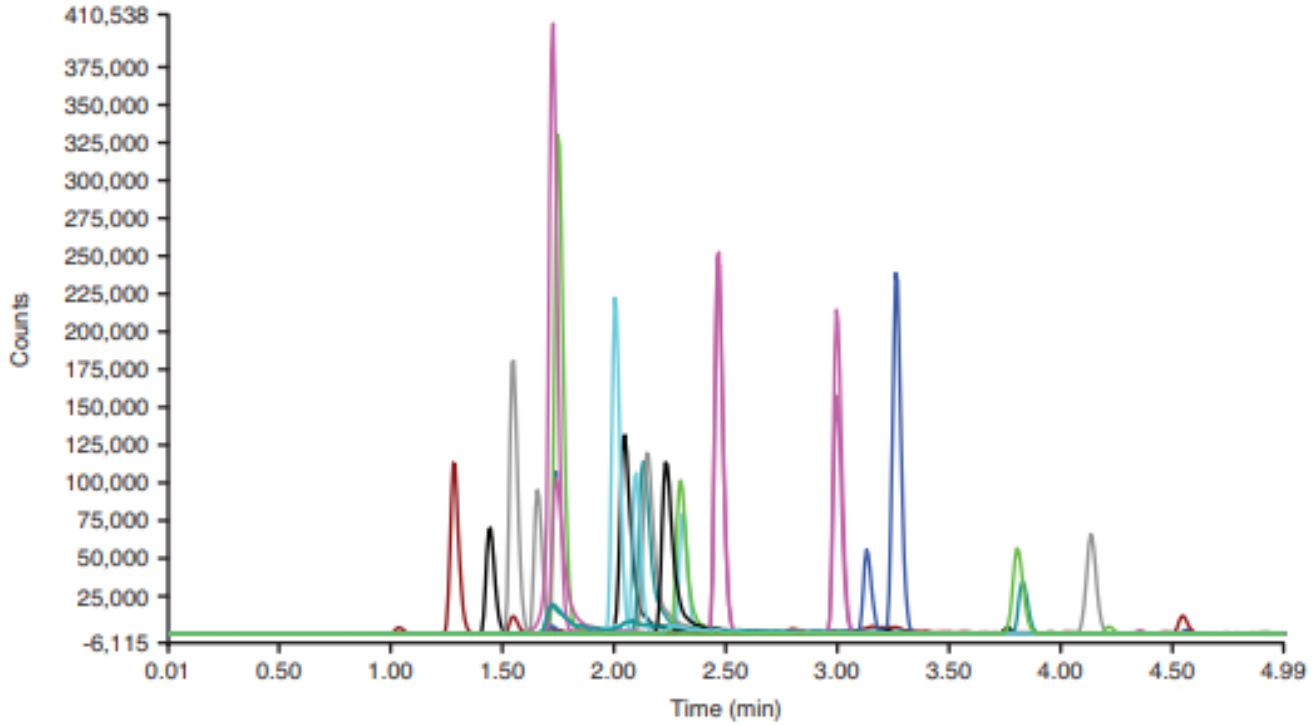


Figure 1: Overlaid selected-reaction monitoring chromatograms showing detection of 36 compounds within a 5 minute detection window

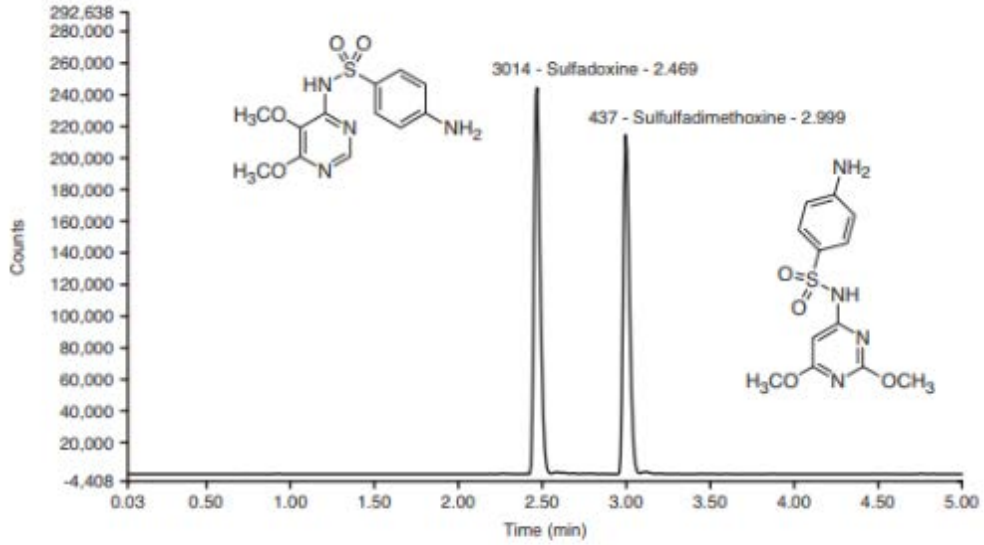
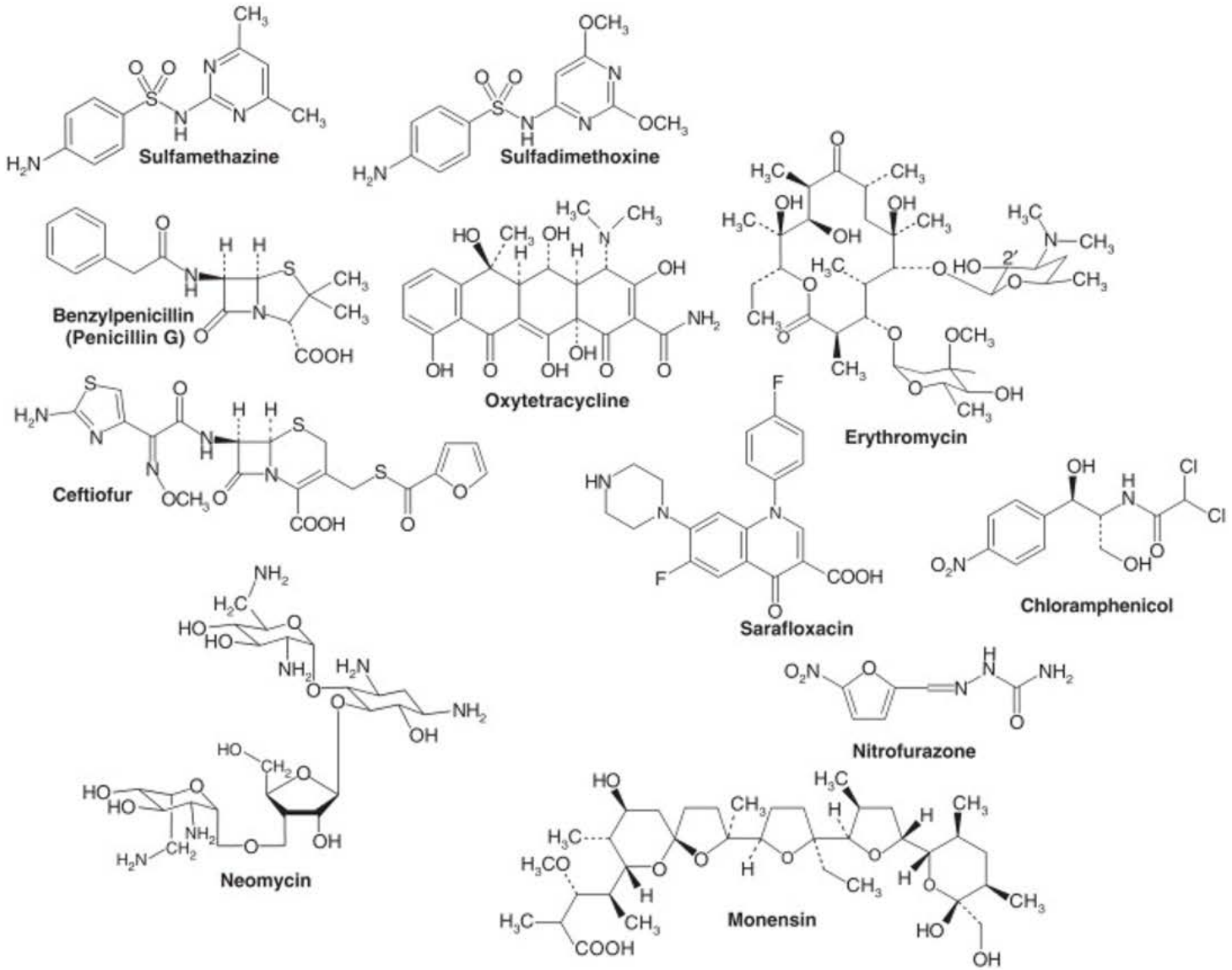


Figure 5: Selected-reaction monitoring chromatogram showing the chromatographic separation of structural isomers

Main Course- Chicken, Beef, Pork- Accucore Vanquish C18+

Main Course- Chicken, Beef, Pork- Accucore Vanquish C18+



Main Course- Chicken, Beef, Pork- Accucore Vanquish C18+

Veterinary Antibiotics: Application Note 21000

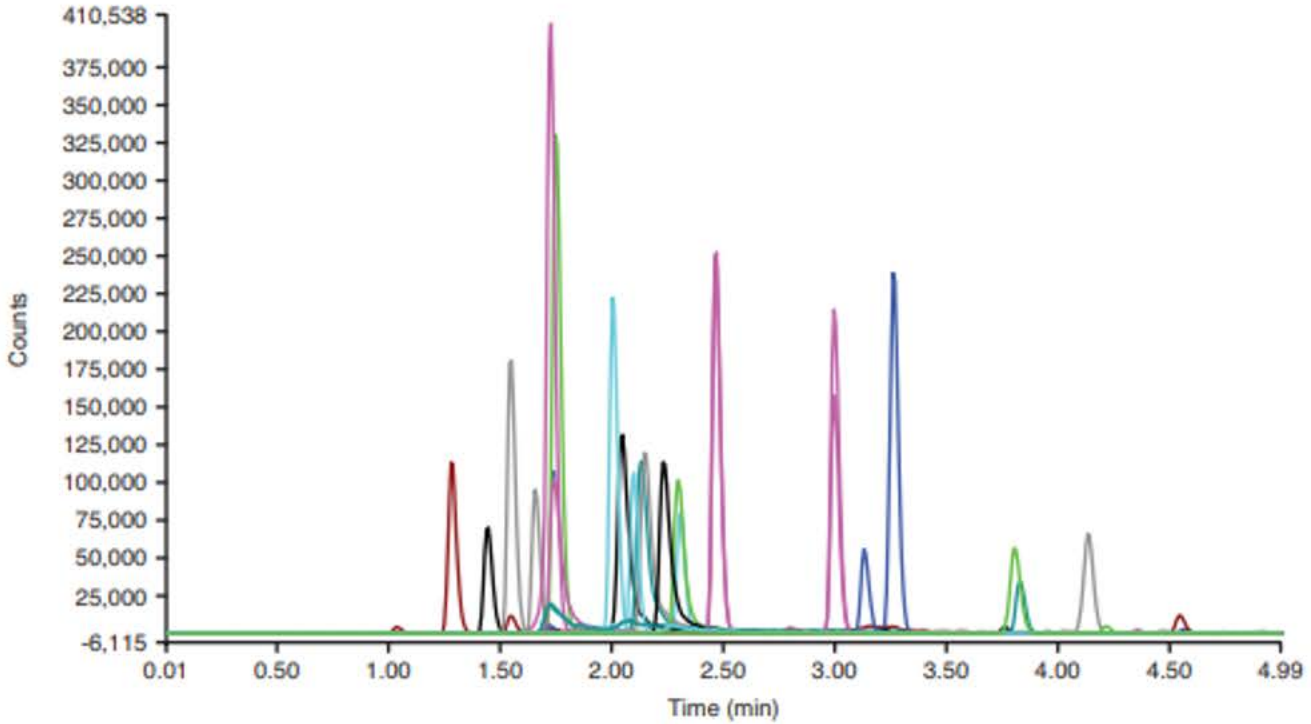


Figure 1: Overlaid selected-reaction monitoring chromatograms showing detection of 36 compounds within a 5 minute detection window

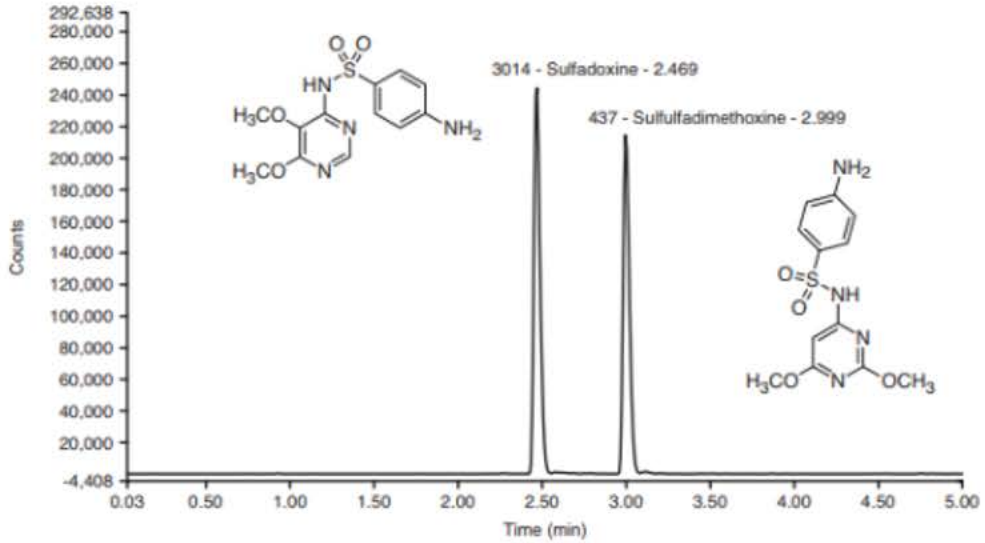
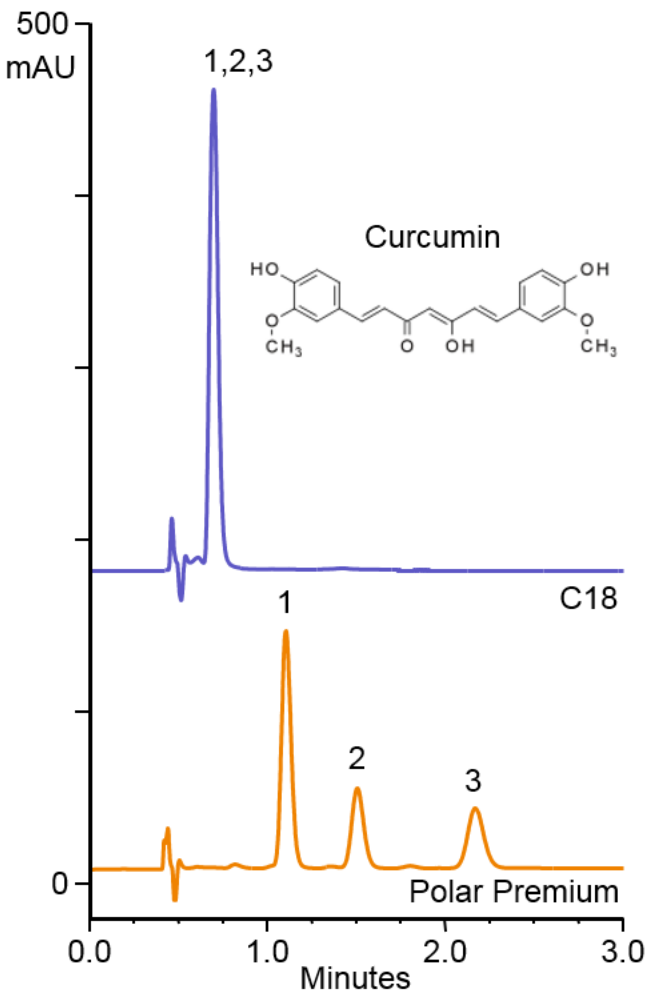


Figure 5: Selected-reaction monitoring chromatogram showing the chromatographic separation of structural isomers

Spices: Accucore Polar Premium

Spices: Accucore Polar Premium

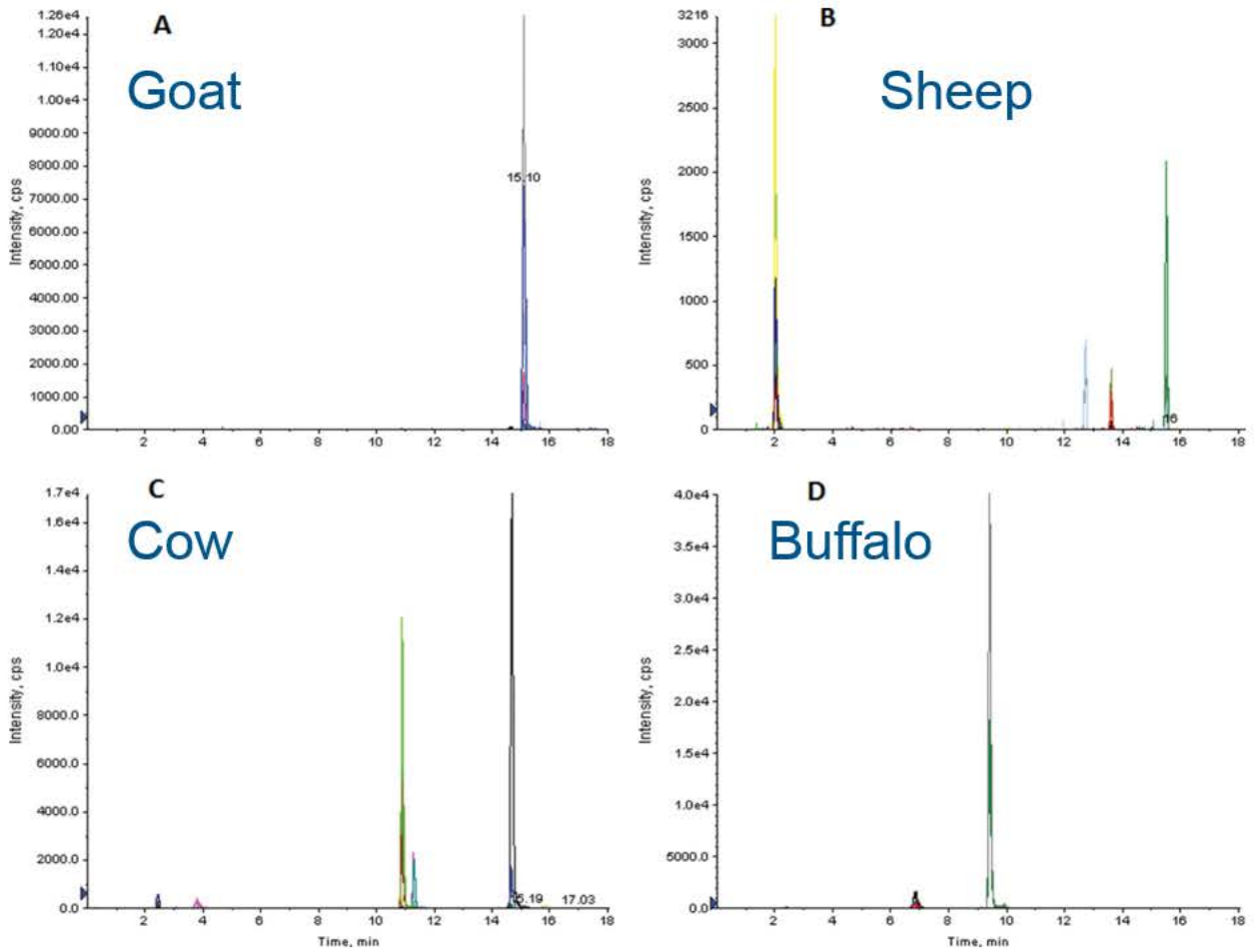


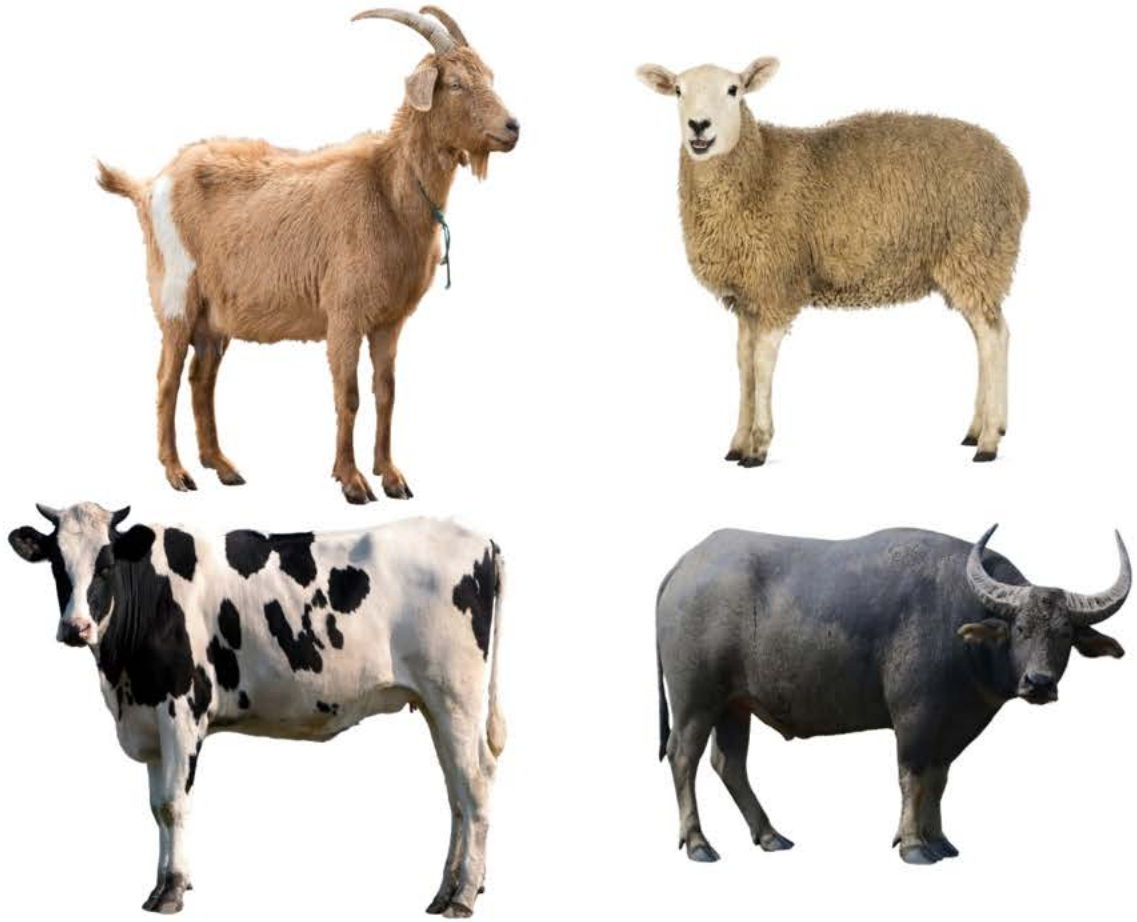
Columns:	Thermo Scientific Accucore Polar Premium Phenomenex Kinetex C18
Dimensions:	2.6 µm, 3 x 100 mm
LC System:	Thermo Scientific Dionex UltiMate 3000
Mobile Phases:	A: Methanol B: 10 mM Phosphoric acid
Isocratic:	80% A, 20% B
Flow:	0.800 mL/min
Temperature:	40 °C
Injection:	6 µL
Detector:	Diode array, UV 428 nm
Sample:	Extract 100 mg ground turmeric with 20 mL of hot 70% ethanol; centrifuge, filter and dilute 10x in methanol.
Peaks:	1. Curcumin 2. Desmethoxycurcumin 3. Bis-desmethoxycurcumin

Curcuminoid in Tumeric: Application Note 20853

Cheese Authenticity- Accucore C18

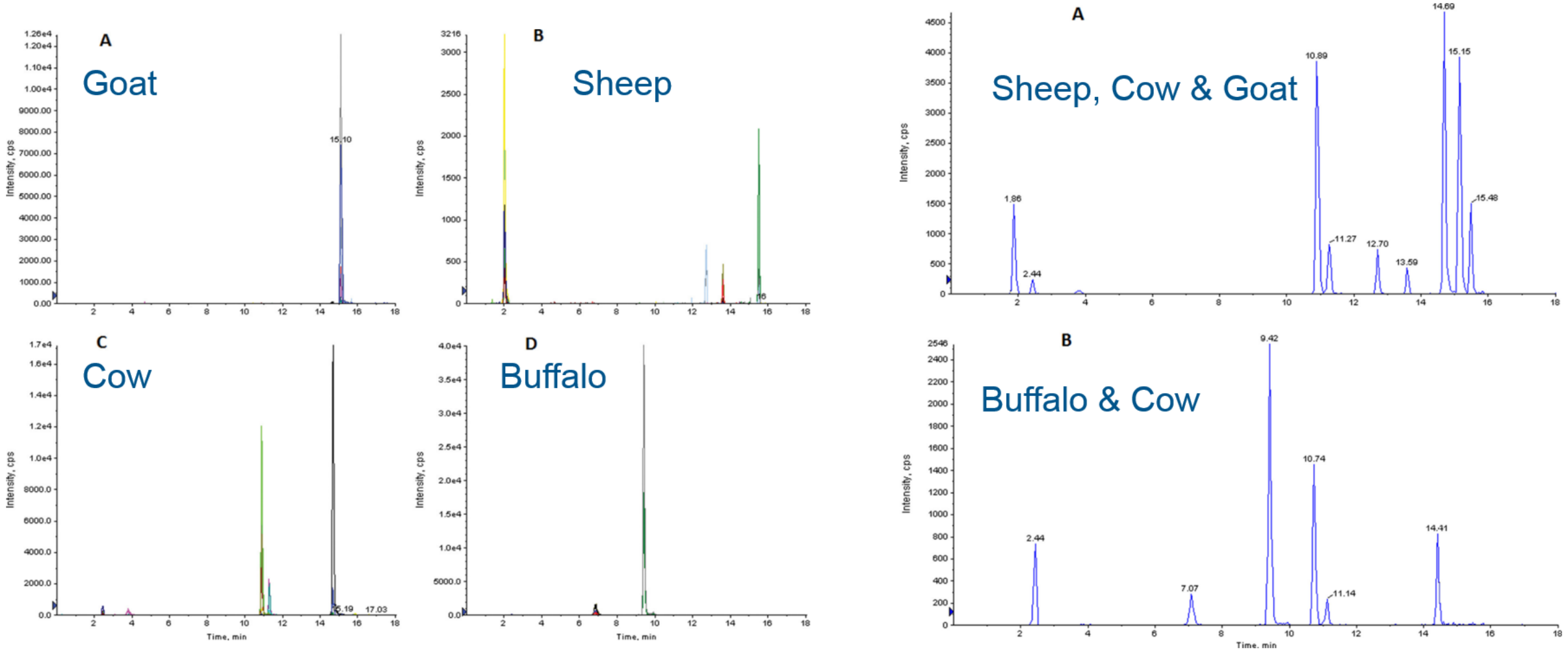
Cheese Authenticity- Accucore C18





ThermoFisher SCIENTIFIC

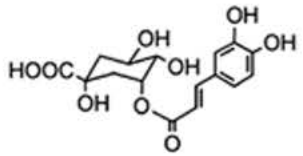
Cheese Authenticity- Accucore C18



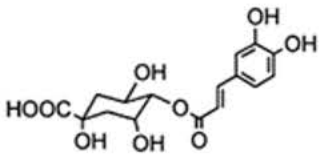
A rapid high-performance liquid chromatography-tandem mass spectrometry assay for unambiguous detection of different milk species employed in cheese manufacturing- Bernardi, N. et al. Journal of Dairy Science Volume 98, Issue 12, December 2015, Pages 8405-8413

Coffee Bean Extracts- Accucore RP-MS

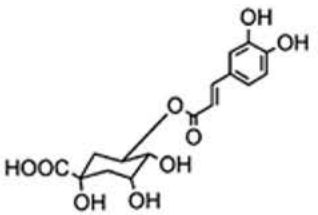
Coffee Bean Extracts- Accucore RP-MS



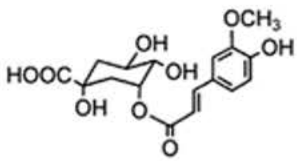
3-*O*-Caffeoylquinic acid



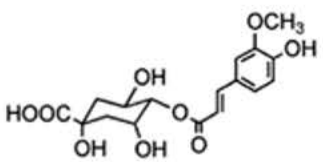
4-*O*-Caffeoylquinic acid



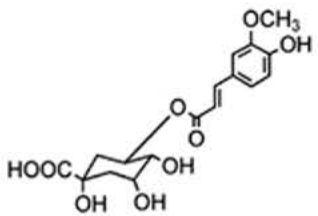
5-*O*-Caffeoylquinic acid



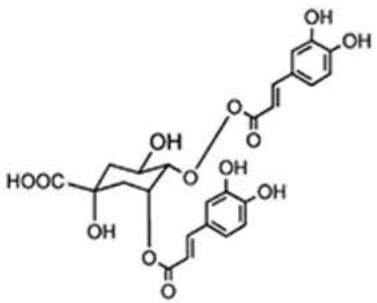
3-*O*-Feruloylquinic acid



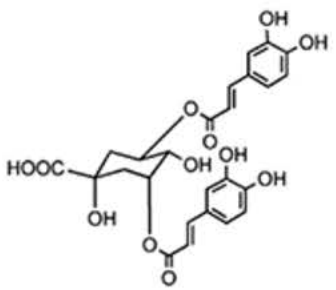
4-*O*-Feruloylquinic acid



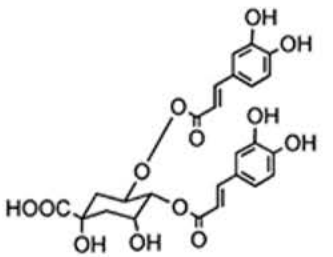
5-*O*-Feruloylquinic acid



3,4-*O*-Dicaffeoylquinic acid



3,5-*O*-Dicaffeoylquinic acid



4,5-*O*-Dicaffeoylquinic acid

Coffee Bean Extracts- Accucore RP-MS

Phenols, Polyphenols in Coffee: Application Note 20610

Peak number	Compound	Peak number	Compound
1	3-O-Caffeoylquinic acid	**7**	3,4-O-Dicaffeoylquinic acid
2	4-O-Caffeoylquinic acid	**8**	3,5-O-Dicaffeoylquinic acid
3	5-O-Caffeoylquinic acid	**9**	4,5-O-Dicaffeoylquinic acid
4	3-O-Feruloylquinic acid	**10**	3-O-Feruloyl-4-O-caffeoylquinic acid
5	4-O-Feruloylquinic acid	**11**	3-O-Caffeoyl-5-O-feruloylquinic acid
6	5-O-Feruloylquinic acid	**12**	4-O-Caffeoyl-5-O-feruloylquinic acid

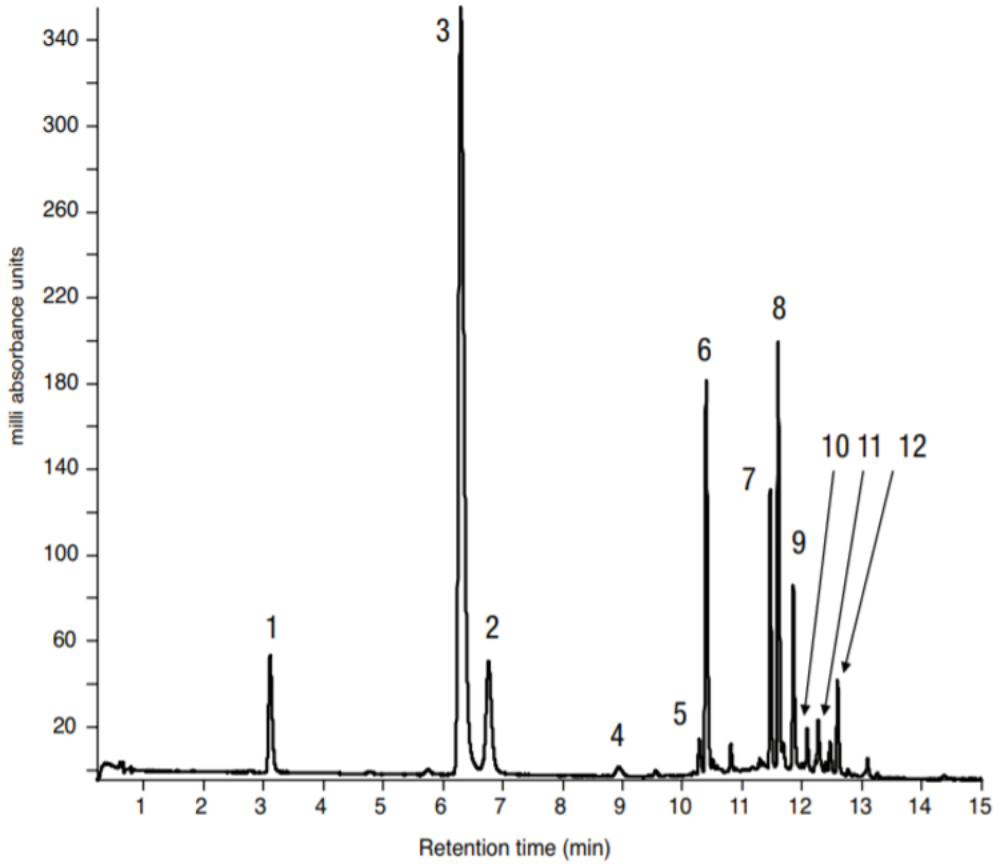


Figure 2: Analysis of a coffee bean extract using a 15 minute gradient on a solid core Accucore RP-MS HPLC column (2.6 µm particle size, 150 x 3.0 mm)

Artificial Sweeteners- Accucore RP-MS

Artificial Sweeteners- Accucore RP-MS

Artificial Sweeteners
Application 20675

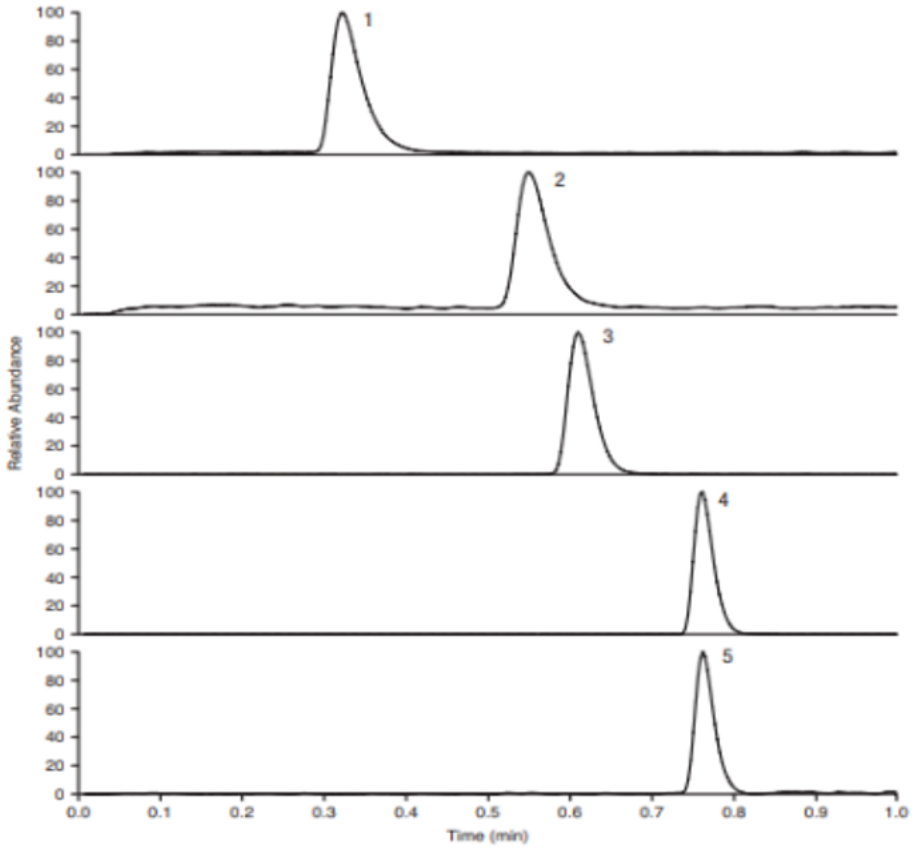
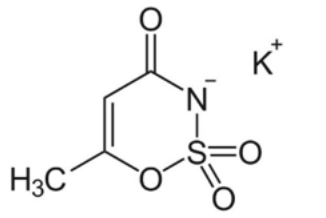
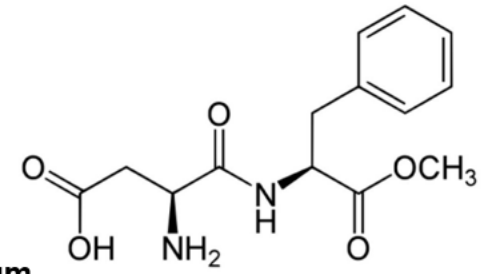


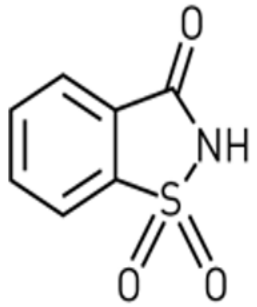
Figure 6: Selected ion chromatograms of acesulfame potassium (1), saccharin (2), sodium cyclamate (3), aspartame (4), and sucralose (5) at 200 ng/mL



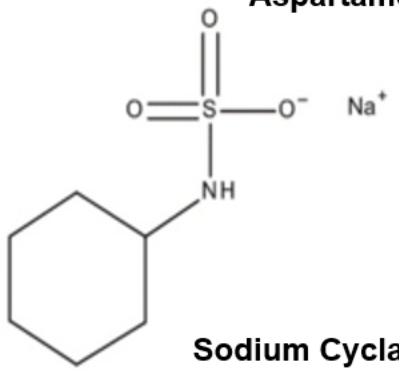
Acesulfame Potassium



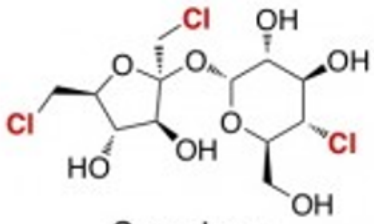
Aspartame



Saccharin



Sodium Cyclamate

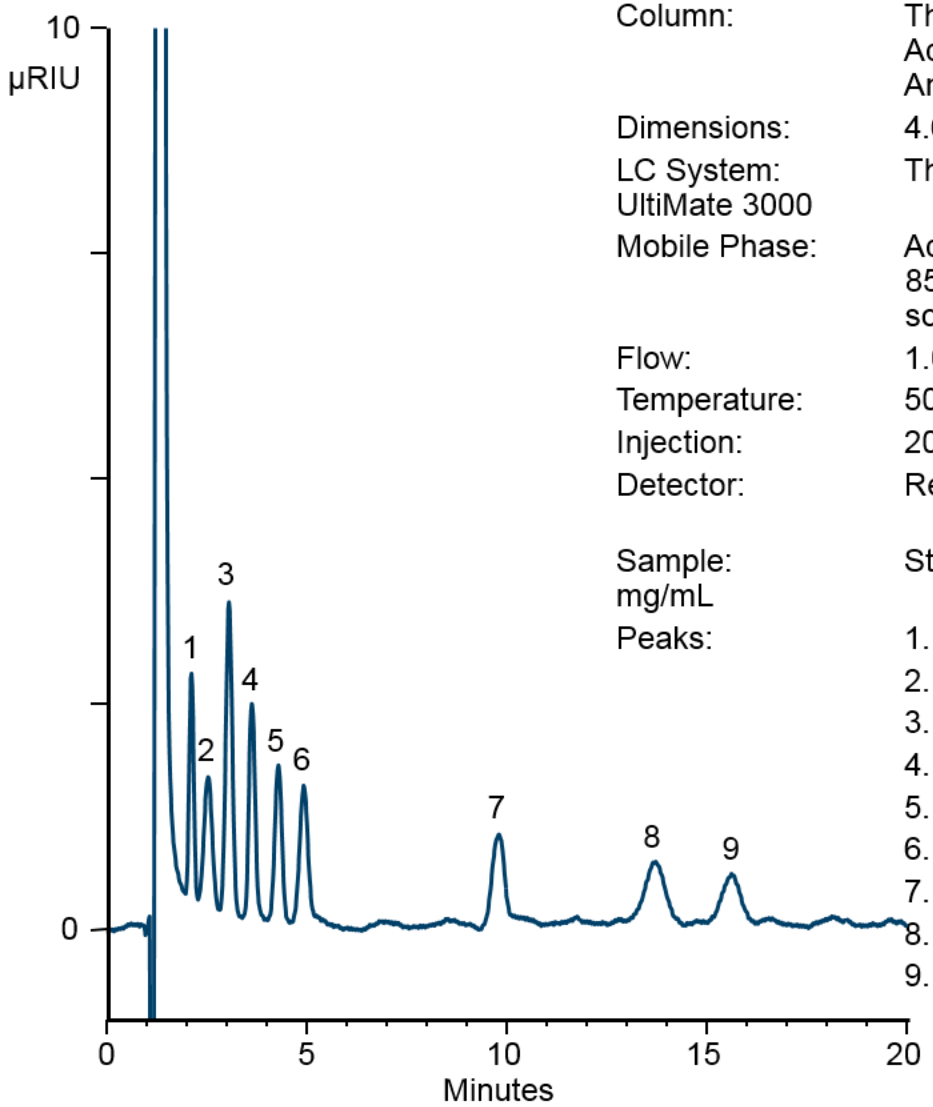


Sucralose
(Splenda)

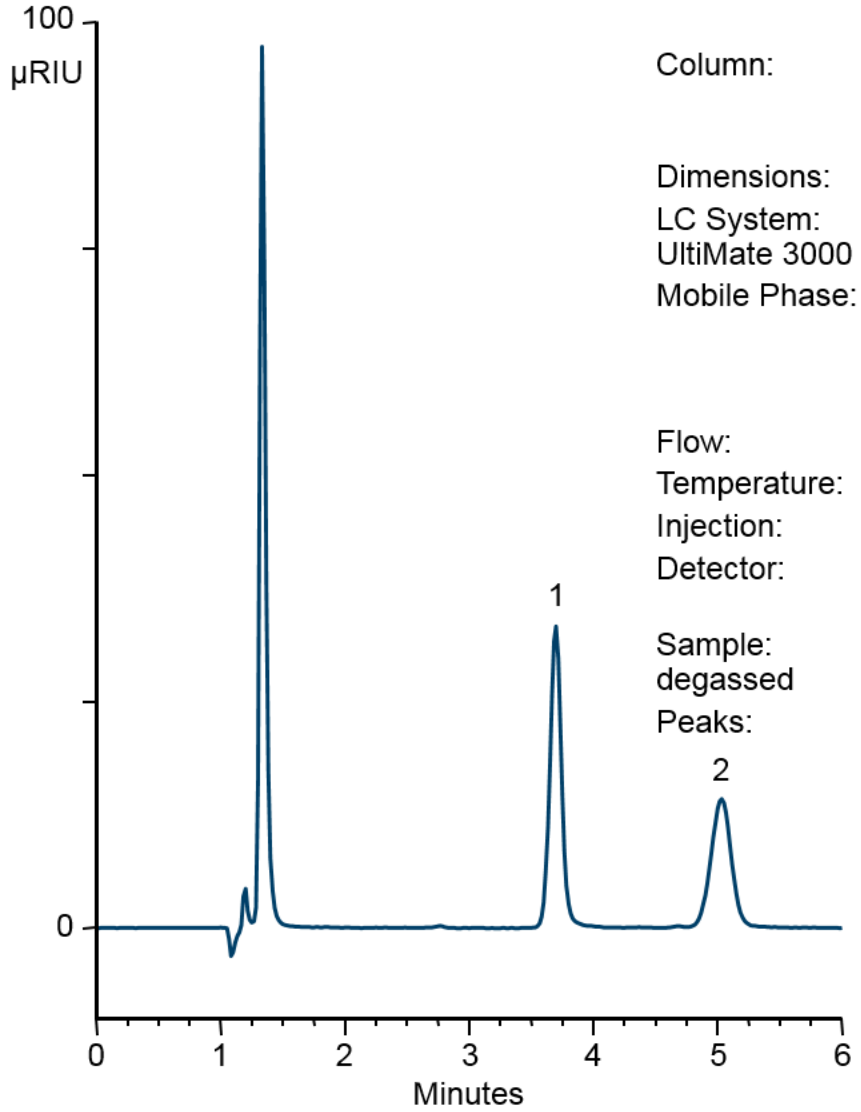
ThermoFisher SCIENTIFIC

Sugar Separation Using Accucore Amide HILIC

Sugar Separation Using Accucore Amide HILIC



Column:	Thermo Scientific Accucore HILIC-Amide, 2.6 µm
Dimensions:	4.6 x 100 mm
LC System: UltiMate 3000	Thermo Scientific
Mobile Phase:	Acetonitrile:Water 85:15 (v/v) + 10 mM sodium perchlorate
Flow:	1.00 mL/min
Temperature:	50°C
Injection:	20 µL
Detector:	Refractive Index
Sample: mg/mL	Standards, ~ 0.1
Peaks:	1. Rhamnose 2. Fucose 3. Arabinose 4. Fructose 5. Mannose 6. Glucose 7. Sucrose 8. Maltose 9. Lactose

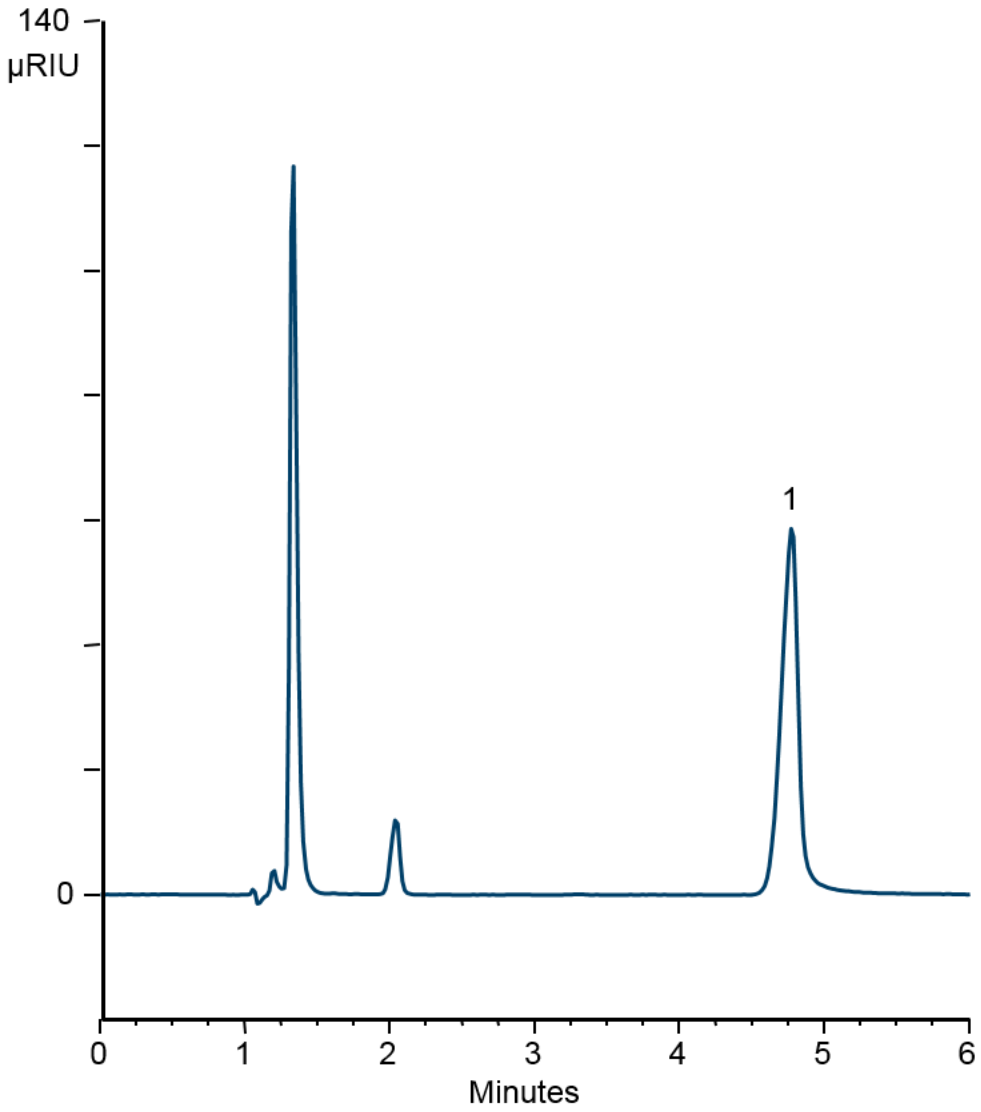


Column:	Thermo Scientific Accucore HILIC-Amide, 2.6 µm
Dimensions:	4.6 x 100 mm
LC System: UltiMate 3000	Thermo Scientific
Mobile Phase:	Acetonitrile:Water 85:15 (v/v) + 10 mM sodium perchlorate
Flow:	1.00 mL/min
Temperature:	50°C
Injection:	0.5 µL
Detector:	Refractive Index
Sample: degassed	Cola drink,
Peaks:	1. Fructose 2. Glucose

ThermoFisher SCIENTIFIC

Mouthwash - Accucore Amide- HILIC

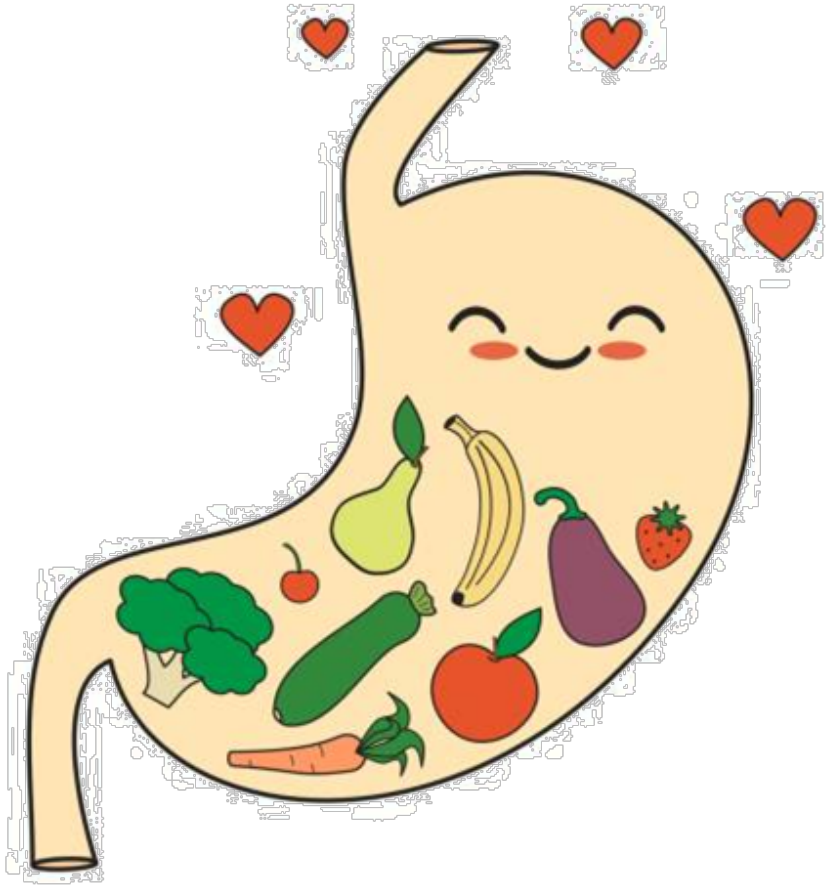
Mouthwash - Accucore Amide- HILIC



Sorbitol in Mouthwash

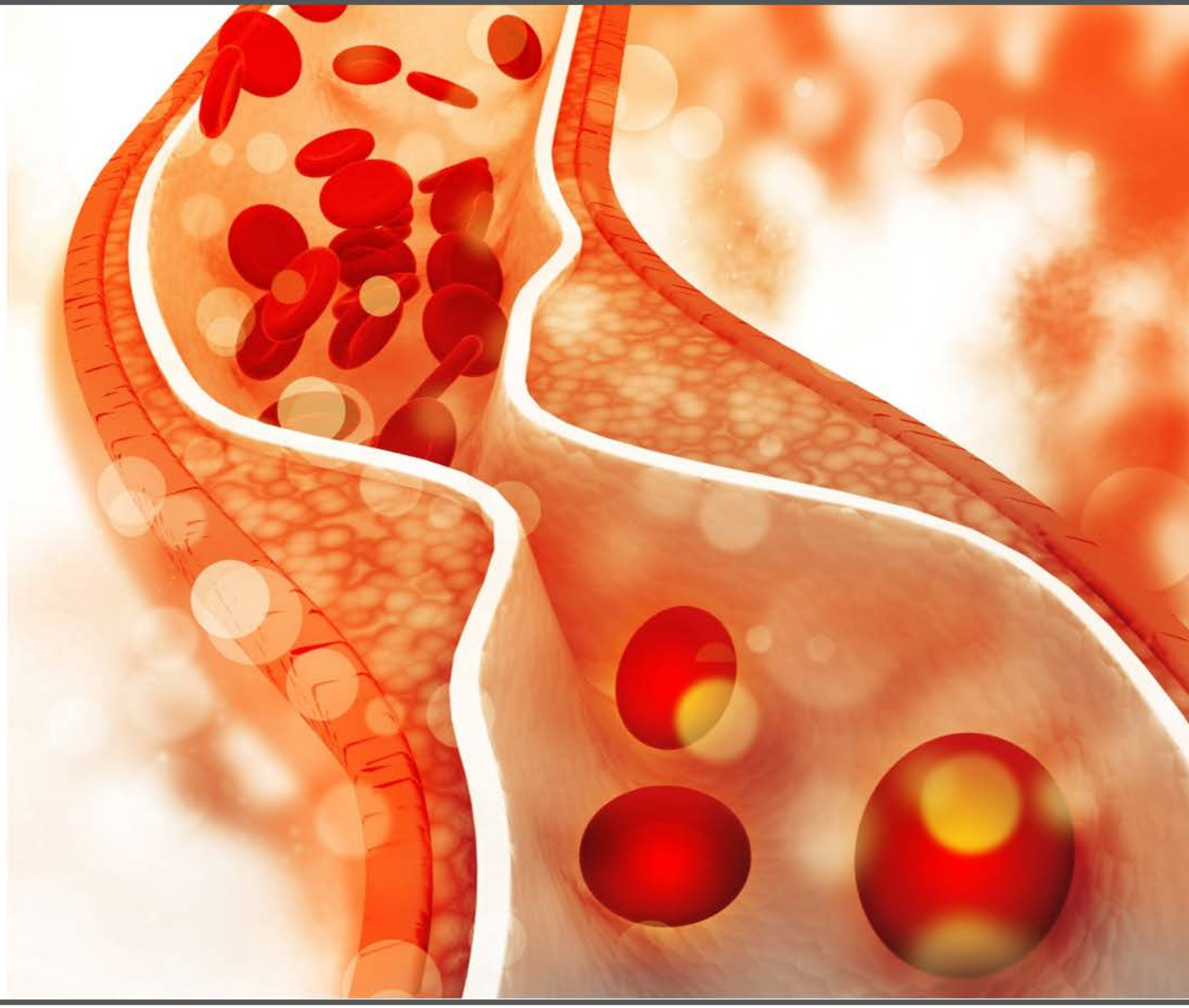
Column:	Thermo Scientific Accucore Amide-HILIC, 2.6 µm
Dimensions:	4.6 x 100 mm
LC System:	Thermo Scientific UltiMate 3000
Mobile Phase:	Acetonitrile:Water 85:15 (v/v) + 10 mM sodium perchlorate
Flow:	1.00 mL/min
Temperature:	50°C
Injection:	0.5 µL
Detector:	Refractive Index
Sample:	Mouthwash
Peaks:	1. Sorbitol

Digestion



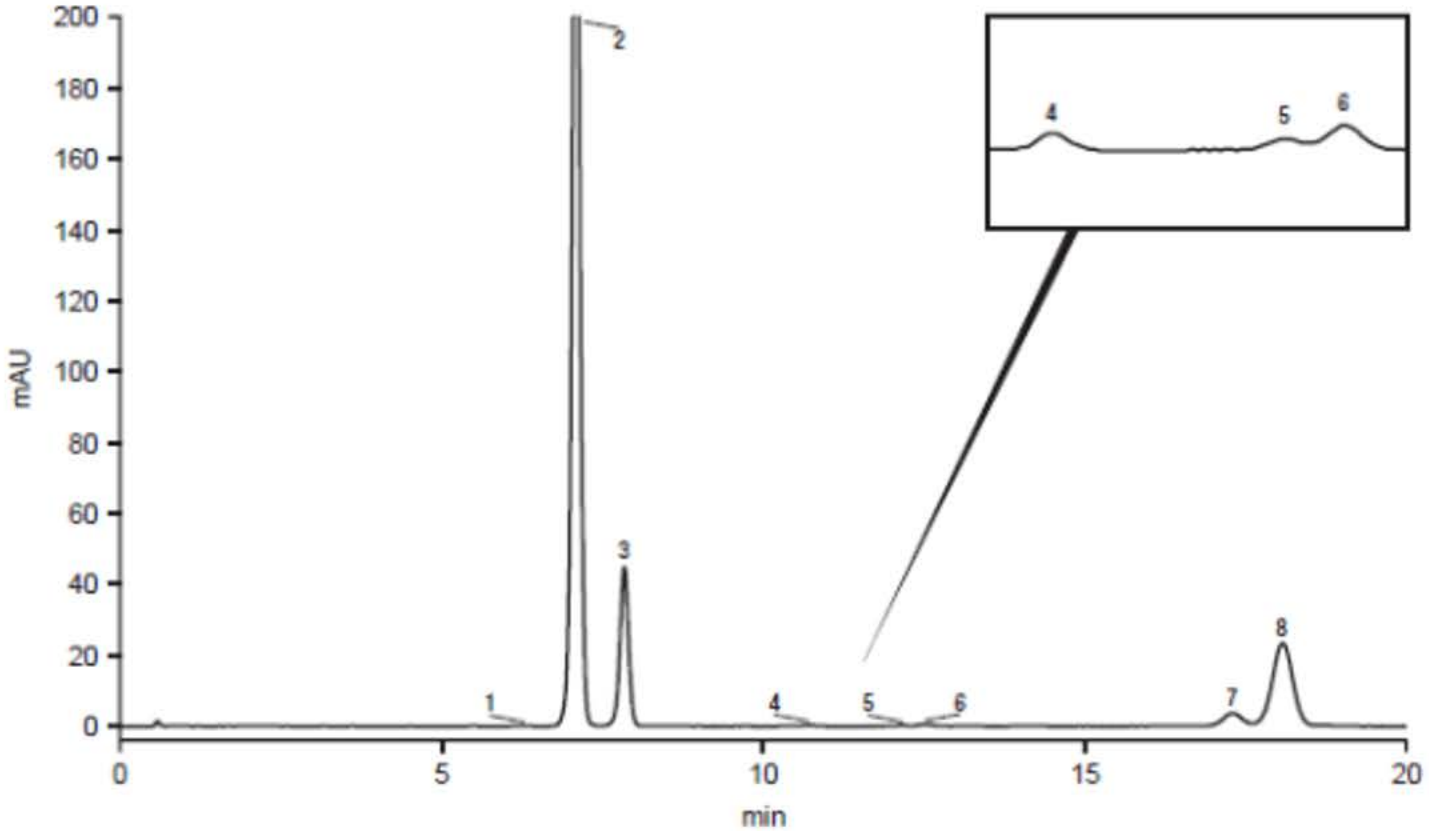
Accucore for EVERY Market

Cholesterol Check? Accucore PFP



Cholesterol Check? Accucore PFP

Rosuvastatin and its Degradants: Application Note 20534



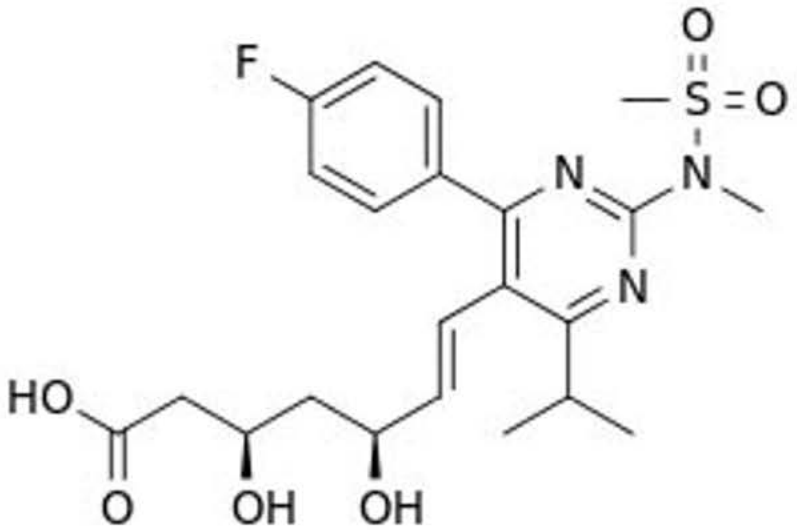


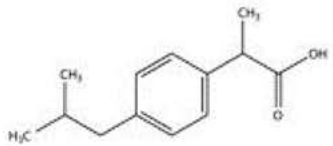
Figure 1: Chromatogram representing resolution of acid degradation for rosuvastatin

1. unknown impurity-1 2. rosuvastatin 3. anti isomer 4. unknown impurity-2 5. unknown impurity-3
6. unknown impurity-4 7. unknown impurity-5 8. lactone impurity

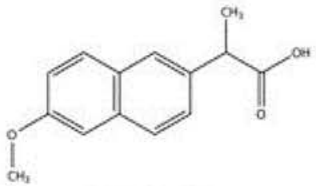
Stop the Madness: Accucore Polar Premium

Stop the Madness: Accucore Polar Premium

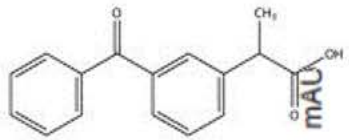
Anti-Inflammatory Drugs: Application Note 20596



Ibuprofen



Naproxen



Ketoprofen

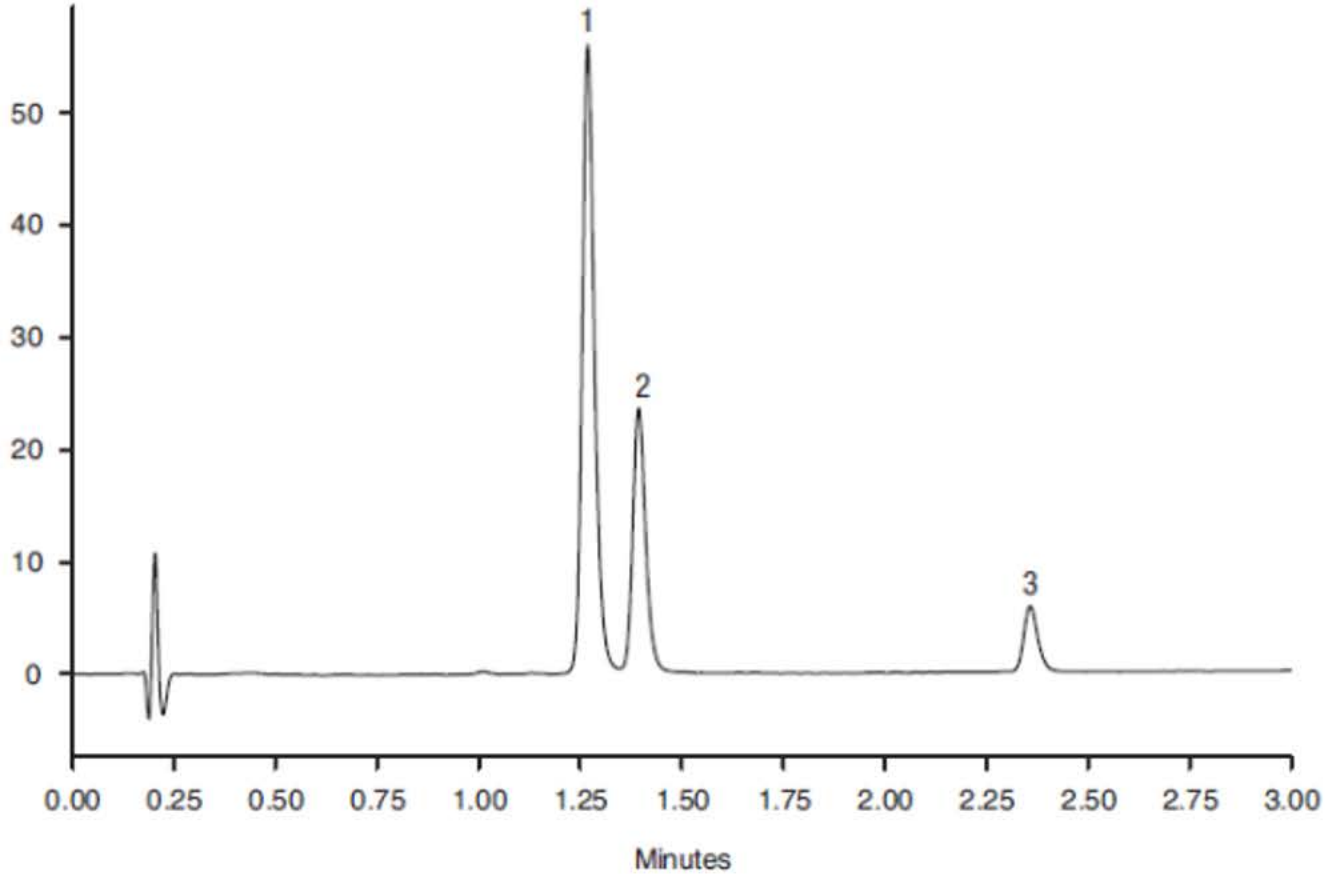
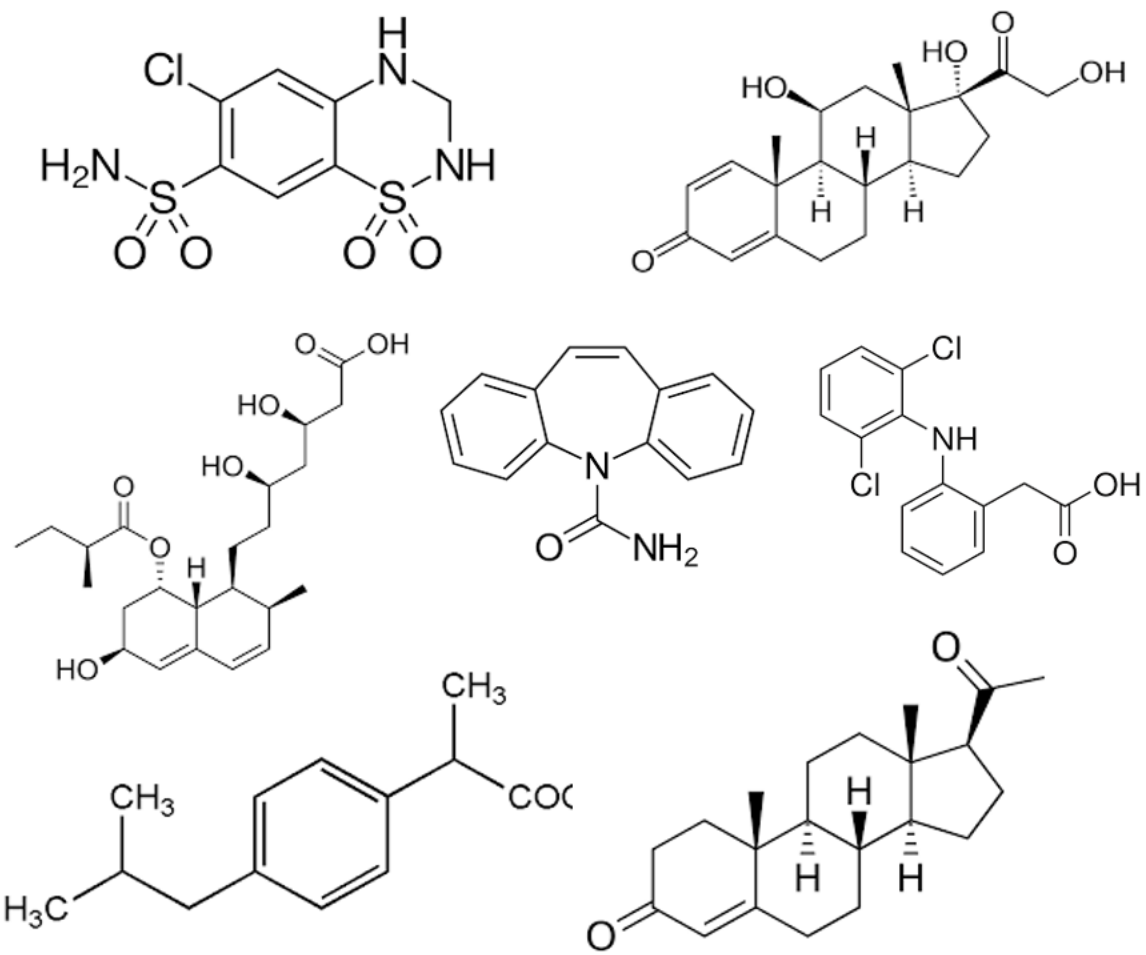


Figure 1: Chromatogram for ketoprofen 1. naproxen 2. and ibuprofen 3. separated on an Accucore Polar Premium 2.6 µm, 50 x 2.1 mm column.

Pharmaceuticals: Accucore C18

Pharmaceuticals: Accucore C18

Acidic and Neutral Drug Separation: Application Note ANCCSCETACNEUT



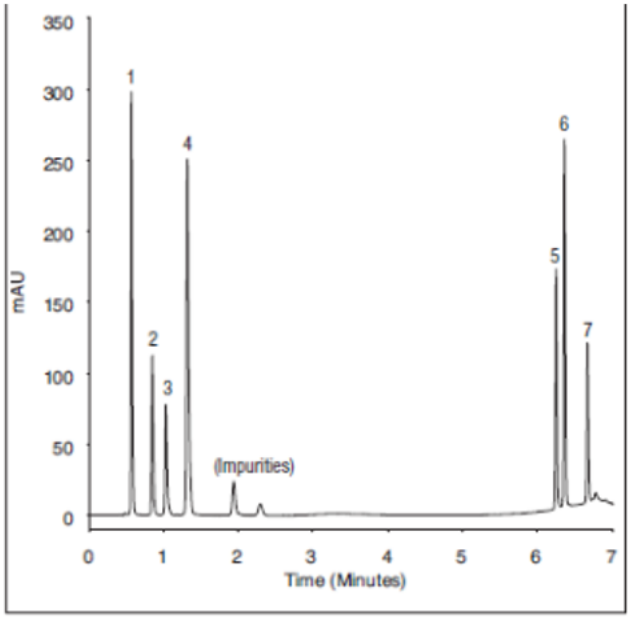


Figure 1: Chromatogram of acidic and neutral drugs separated on an Accucore C18 2.6 µm 100 x 2.1 mm column

	Compounds	Sample Concentration (µg/mL)	t_R/min	%RSD (t_R/min) n=6
1	Hydrochlorothiazide	20	0.57	0.13
2	Prednisolone	50	0.85	0.19
3	Pravastatin	50	1.03	0.27
4	Carbamazepine	20	1.32	0.20
5	Diclofenac	20	6.29	0.59
6	Ibuprofen	50	6.40	0.57
7	Progesterone	50	6.71	0.54

Drugs of Abuse: Accucore C18

Drugs of Abuse: Accucore C18

Separation of 40 Psychoactive Stimulants in Urine*: Journal of Chromatography A 2015 Jun 5; 1397:32-42

J Chromatogr A. Author manuscript; available in PMC 2016 Jun 5.
Published in final edited form as:
J Chromatogr A. 2015 Jun 5; 1397: 32–42.
Published online 2015 Apr 8. doi: 10.1016/j.chroma.2015.04.002

PMCID: PMC4433760
NIHMSID: NIHMS679366
PMID: 25931378

Simultaneous determination of 40 novel psychoactive stimulants in urine by liquid chromatography-high resolution mass spectrometry and library matching

Marta Concheiro,[1,2] Marisol Castaneto,[1,3] Robert Kronstrand,[4] and Marilyn A. Huestis[1,*]

[1]Chemistry and Drug Metabolism, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, USA
[3]Program in Toxicology, University of Maryland Baltimore, Baltimore, MD, USA
[4]Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden Division of Drug Research, Department of Medical and Health Sciences, Linköping University, Linköping, Sweden
Corresponding author Professor Dr. Dr. (h.c.) Marilyn A. Huestis, Chief, Chemistry and Drug Metabolism, Biomedical Research Center (BRC), National Institute on Drug Abuse, NIH, 251 Bayview Blvd, Suite 200 Room 05A721, Baltimore, MD 21224, mhuestis@intra.nida.nih.gov, Phone: 443-740-2524, Fax: 443-740-2823
[2]Currently Department of Sciences, John Jay College of Criminal Justice, City University of New York, New York, NY, USA

Abstract Go to:

The emergence of novel psychoactive substances is an ongoing challenge for analytical toxicologists. Different analogs are continuously introduced in the market to circumvent legislation and to enhance their pharmacological activity. Although detection of drugs in blood indicates recent exposure and link intoxication to the causative agent, urine is still the most preferred testing matrix in clinical and forensic settings. We developed a method for the simultaneous quantification of 8 piperazines, 4 designer amphetamines and 28 synthetic cathinones and 4 metabolites, in urine by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). Data were acquired in full scan and data dependent MS^2 mode. Compounds were quantified by precursor ion exact mass, and confirmed by product ion spectra library matching, taking into account product ions' exact mass and intensities. One-hundred µL urine was subjected to solid phase cation exchange extraction (SOLA SCX). The chromatographic reverse-phase separation was achieved with gradient mobile phase of 0.1% formic acid in water and in acetonitrile in 20 min. The assay was linear from 2.5 or 5 to 500µg/L. Imprecision (n=15) was <15.4%, and accuracy (n=15) 84.2-118.5%. Extraction efficiency was 51.2-111.2%, process efficiency 57.7-104.9% and matrix effect ranged from -41.9 to 238.5% (CV<23.3%, except MDBZP CV<34%). Authentic urine specimens (n=62)

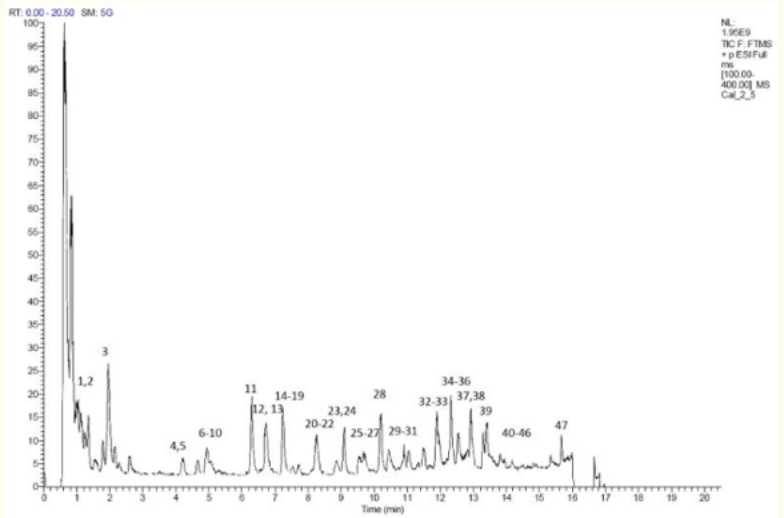


Fig. 1

Full scan total ion chromatogram (TIC) of urine sample fortified at 2.5 µg/L. 1, BZP; 2, MDBZP; 3, cathinone; 4, methcathinone; 5, methiopropamine; 6, 4-fluoromethcathinone; 7, methylone; 8, ethylcathinone; 9, MeOPP; 10, pFPP; 11, α-PPP; 12, buphedrone ephedrine; 13, ethylone, 14, methedrone; 15, buphedrone; 16, normephedrone; 17, diethylcathinone; 18, 5-APDB; 19, MDPPP; 20, 4-methylephedrine; 21, butylone; 22, mephedrone; 23, 2C-B-BZP; 24, 6-APB; 25, 4-MEC; 26; 4-MEC-metabolite; 27, α-PVT; 28, MDPBP; 29, αPBP; 30, pentedrone; 31, mCPP; 32, α-ethylaminopentiophenone; 33, pentylone; 34, 3,4-DMMC; 35, α-PVP; 36, DBZP; 37, 4-MPBP; 38, MDPV; 39, 4-Cl-2,5-DMA; 40, 4-methoxy-α-PVP; 41, TFMPP; 42, pyrovalerone; 43, trazodone; 44, benzedrone; 45, MPHP; 46, PV8; 47, naphyrone.

*For research use only

ThermoFisher SCIENTIFIC

Drug Testing: Accucore PFP

Drug Testing: Accucore PFP

43 Drugs in Urine* Dilute and Shoot LC-MS/MS: Application Note 576

Application Note 576

Simultaneous Quantitation of 43 Drugs in Human Urine with a "Dilute-and-Shoot" LC-MS/MS Method

Xiang He and Marta Kozak, Thermo Fisher Scientific, San Jose, CA

Key Words
TSQ Quantum Access MAX, forensic toxicology, drugs of abuse, pain management drugs, urine, quantitation

Goal
The goal of this work was to develop a simple "dilute-and-shoot" liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the simultaneous quantitation of 43 drugs of abuse, including pain management drugs, in human urine for forensic toxicology purposes. The drugs to be analyzed included opioids, amphetamines, benzodiazepines, cocaine, buprenorphine, methadone, and some of their metabolites. An additional objective was to use ultra-high-pressure liquid chromatography (UHPLC) to improve throughput and sensitivity of the method.

Introduction
LC-MS/MS has become more accepted as the tool for quantitative analysis of drugs in forensic toxicology laboratories. This technique enables simultaneous detection of multiple analytes of interests and is compatible with a simple "dilute-and-shoot" sample preparation method for urine samples.

Methods
Sample Preparation
Nine individual human urine and pure water samples were spiked with 20 and 200 ng/mL of the 43 drugs of

LC-MS/MS Conditions
LC-MS/MS analysis was performed on a Thermo Scientific™ Accela™ 1250 pump and Accela Open autosampler coupled to a Thermo Scientific TSQ Quantum Access MAX™ triple stage quadrupole mass spectrometer. The analytical column was a Thermo Scientific Accucore™ PFP column (50 × 2.1 mm, 2.6 µm particle size) maintained at room temperature. Details of the LC gradient and mobile phases (MP) are as follows:

Time (min)	Flow rate (mL/min)	Gradient	MPA (%)	MPB (%)	MPC (%)
0.00	0.75	Step	95	5	0
0.50	0.75	Ramp	60	40	0
2.60	0.75	Ramp	5	95	0
4.50	1.00	Step	0	100	0
5.50	1.00	Step	0	0	100
5.75	1.00	Step	95	5	0

MPA: 10 mM NH_4Ac and 0.1% formic acid in water
MPB: 10 mM NH_4Ac and 0.1% formic acid in methanol
MPC: acetonitrile/isopropanol/acetone 9:9:2 (v/v/v)

The mass spectrometer was operated with a heated electrospray ionization (HESI-II) source in positive ionization mode. The MS conditions were as follows:

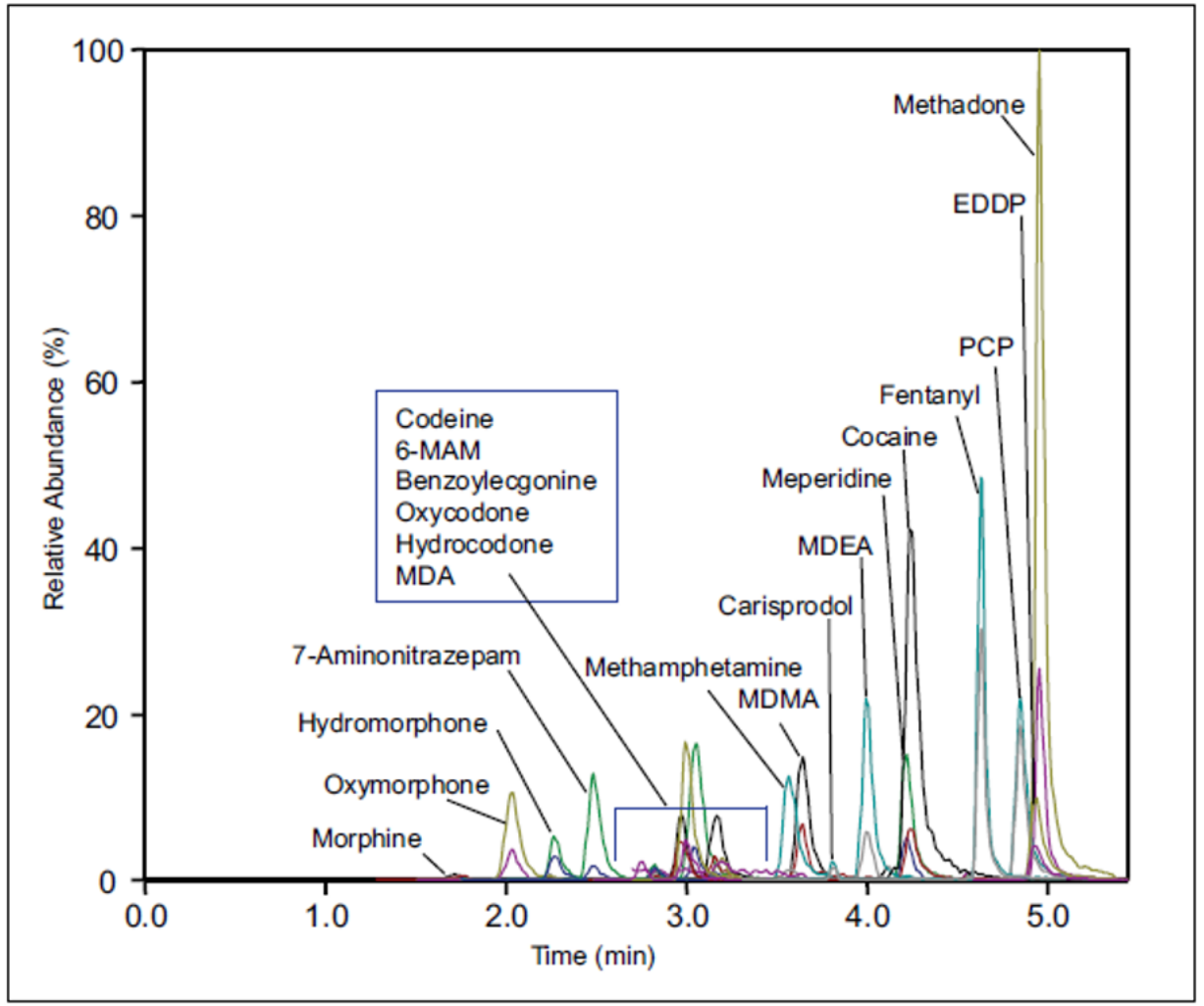


Figure 2. SRM chromatograms of 20 selected drugs at 20 ng/mL in spiked human urine

*For research use only

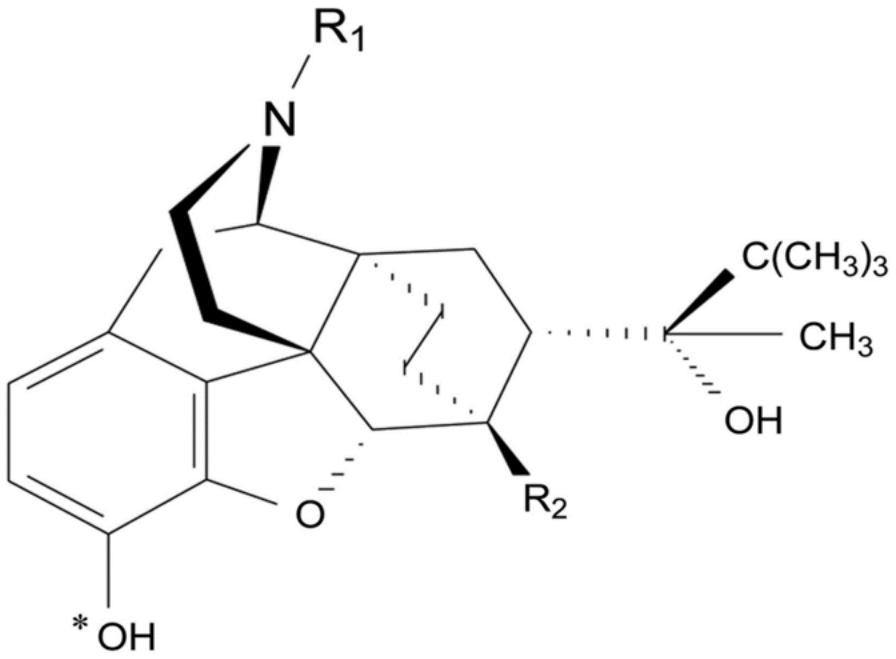
ThermoFisher SCIENTIFIC

Rehabilitation: Accucore Phenyl-Hexyl

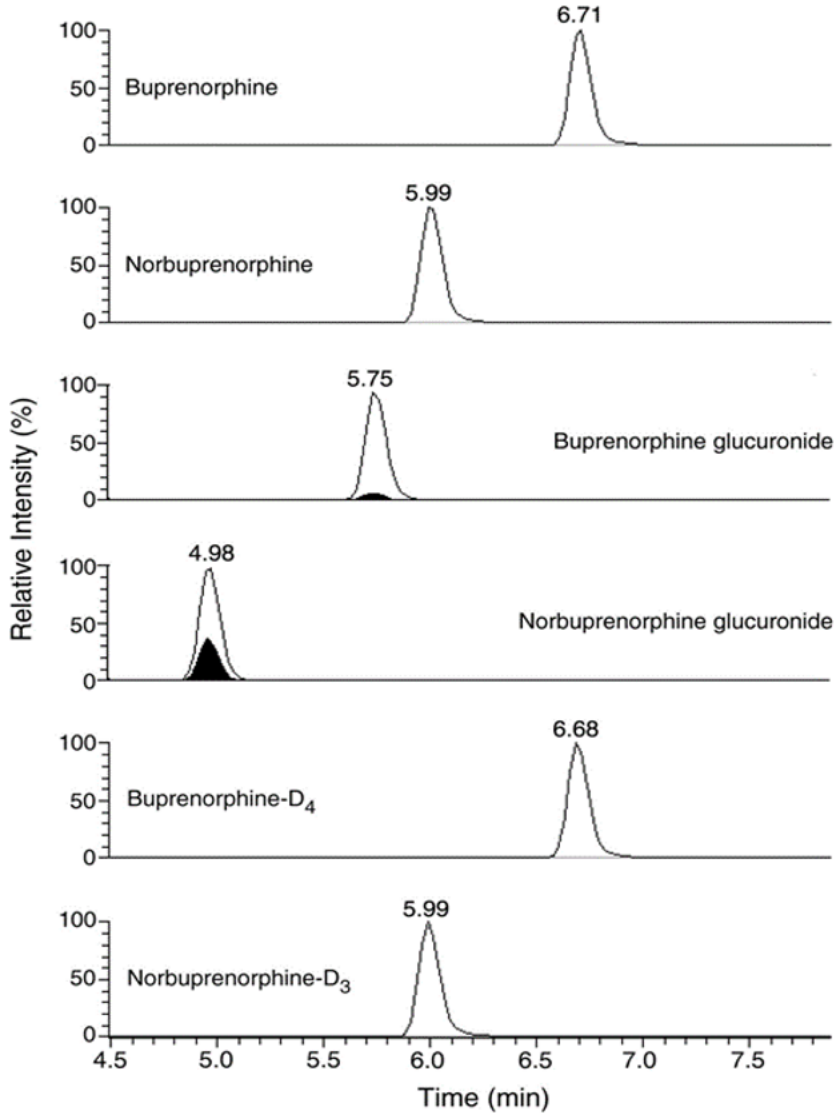
ThermoFisher SCIENTIFIC

Rehabilitation: Accucore Phenyl-Hexyl

Separation of Buprenorphine in Urine*:
Journal of Analytical Toxicology, Volume 38, Issue 7, Sept. 2014, Pages 438-443



	R_1	R_2
Buprenorphine	$CH_2CH(CH_2)_2$	OCH_3
Norbuprenorphine	H	OCH_3
Buprenorphine-D_4	$CD_2CH(CH_2CD_2)$	OCH_3
Norbuprenorphine-D_3	H	OCD_3



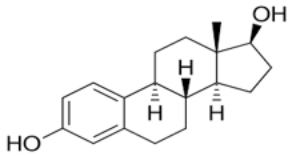
*For research use only

VICTORY! Steroid Hormones in Serum*- Accucore aQ or Phenyl Hexyl

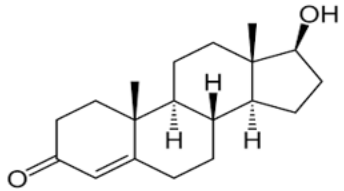
VICTORY! Steroid Hormones in Serum*- Accucore aQ or Phenyl Hexyl

Steroids in plasma:
Technical Note 64973

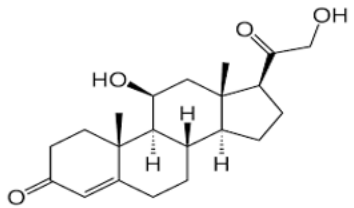
Steroids in serum: *Anal Bioanal Chem. 2017 Oct; 409(25): 5943–5954.*



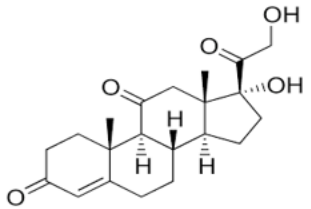
estradiol



testosterone



corticosterone



cortisone

Anal Bioanal Chem. Author manuscript; available in PMC 2017 Nov 17.
Published in final edited form as:
Anal Bioanal Chem. 2017 Oct; 409(25): 5943–5954.
Published online 2017 Aug 11.
doi: 10.1007/s00216-017-0529-x
PMCID: PMC5693763
NIHMSID: NIHMS909567
PMID: 28801832

Simultaneous measurement of total Estradiol and Testosterone in human serum by isotope dilution liquid chromatography tandem mass spectrometry

Hui Zhou,[1] Yuesong Wang,[1] Matthew Gatcombe,[1] Jacob Farris,[1] Julianne C. Botelho,[1] Samuel P. Caudill,[1] and Hubert W. Vesper[1,*]

[1]Centers For Disease Control And Prevention, National Center For Environmental Health, Division of Laboratory Sciences, Clinical Chemistry Branch, 4770 Buford Hwy NE, Atlanta, Georgia, USA

*Send correspondence to this author: Hubert W Vesper, Centers for Disease Control and Prevention, 4770 Buford HWY NE, MS-F25, Atlanta, Georgia 30341, Fax (770) 488-7030, HVesper@cdc.gov

*For research use only

Quantitative analysis of estradiol and testosterone in plasma for clinical research using the TSQ Altis triple quadrupole mass spectrometer

Authors
Xiaolei Xie, Kristine Van Natta, Neloni Wijeratne, Claudia Martins

Thermo Fisher Scientific, San Jose, CA

Keywords
Estradiol, LC-MS/MS, LLE, testosterone, TSQ Altis MS

Goal
To develop a sensitive LC-MS/MS method for quantitative analysis of estradiol and testosterone in plasma for clinical research using liquid chromatographic separation coupled to a triple quadrupole mass spectrometer.

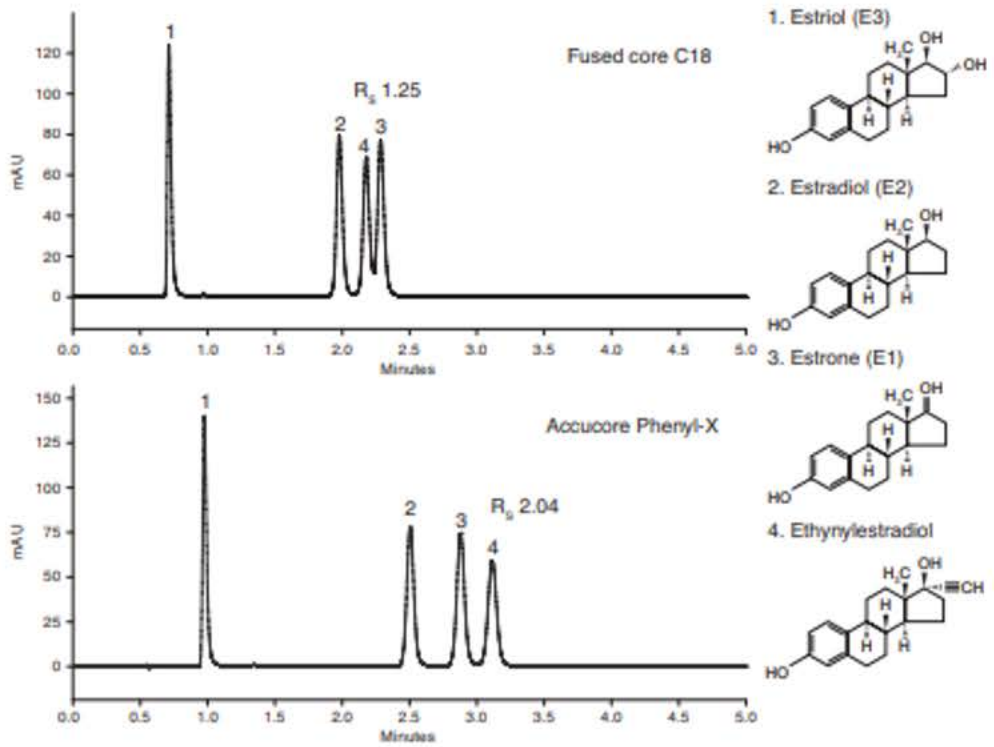
Introduction
Analysis of estradiol and testosterone in plasma samples for clinical research requires a sensitive analytical method. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has been widely adopted as an analytically sensitive and selective technique for estradiol and testosterone analysis in complex matrices such as human serum or plasma.

Experimental
Sample preparation
To prepare the samples, 10 µL of spiking solution (final concentration range: 0.5–10 pg/mL) and 20 µL of internal standard (2 pg/mL testosterone-^{13}C3

ThermoFisher SCIENTIFIC

VICTORY! Steroid Hormones in Serum*- Accucore aQ or Phenyl Hexyl

Estrogens on Phenyl X: Technical Note 20594



Application Note 20594

Analysis of Estrogens Using a Solid Core HPLC Column

Jamil Ali, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words
Accucore Phenyl-X, fused core, superficially porous, estrogens, estrone (E1), estradiol (E2), estriol, ethynylestradiol

Abstract
This application note demonstrates the use of the Thermo Scientific™ Accucore™ Phenyl-X HPLC column for the analysis of aromatic steroids. When compared with a C18 column the Accucore™ Phenyl-X HPLC column provides high aromatic selectivity, good hydrophobic retention and unique, complementary selectivity.

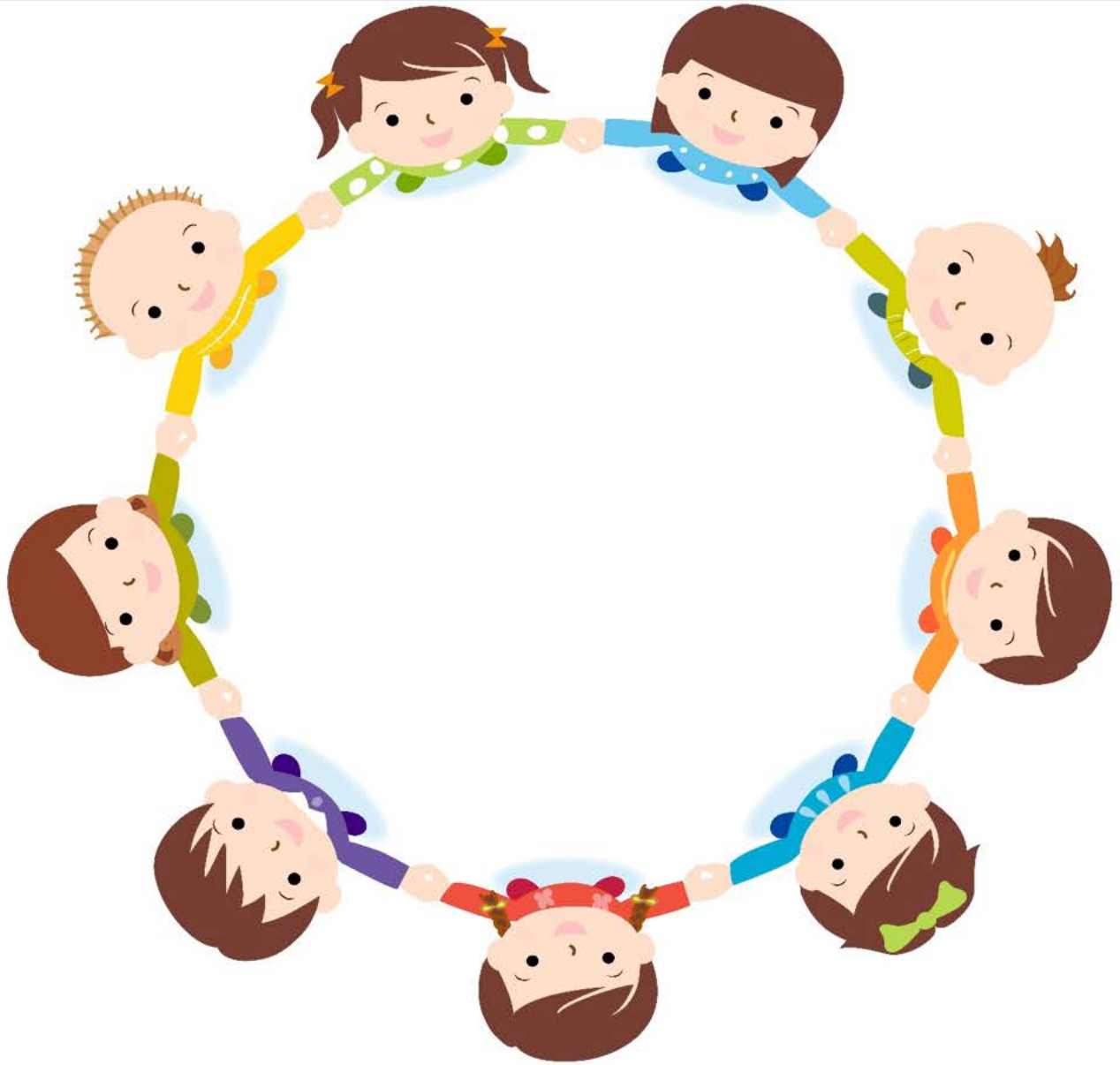
Accucore Phenyl-X HPLC column can baseline resolve them isocratically, providing good retention and unique selectivity.

Introduction
Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials. The proprietary Accucore Phenyl-X alkyl aromatic bonded phase provides a unique selectivity when compared to other reversed phase materials such as C18 or Phenyl. The advanced design of the bonded phase makes it robust and compatible with highly aqueous mobile phases.

Aromatic steroids can present a challenge in liquid chromatography as in reversed phase it is difficult to get good separation. The use of a highly selective phase is the key to overcoming this challenge. In this application the Accucore Phenyl-X phase was employed to achieve the separation of four structurally related aromatic steroids classed as estrogens. Estrogens are a group of steroids thus named for their importance in the estrous cycle. They function as the primary female sex hormone.

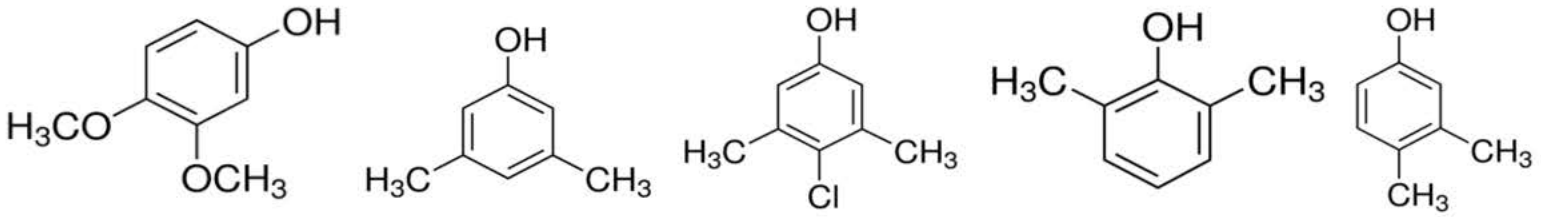
ThermoFisher SCIENTIFIC

PFP vs C18



PFP vs C18

Separation of 14 Positional Isomers: Application Note ANCCSCETISOMER



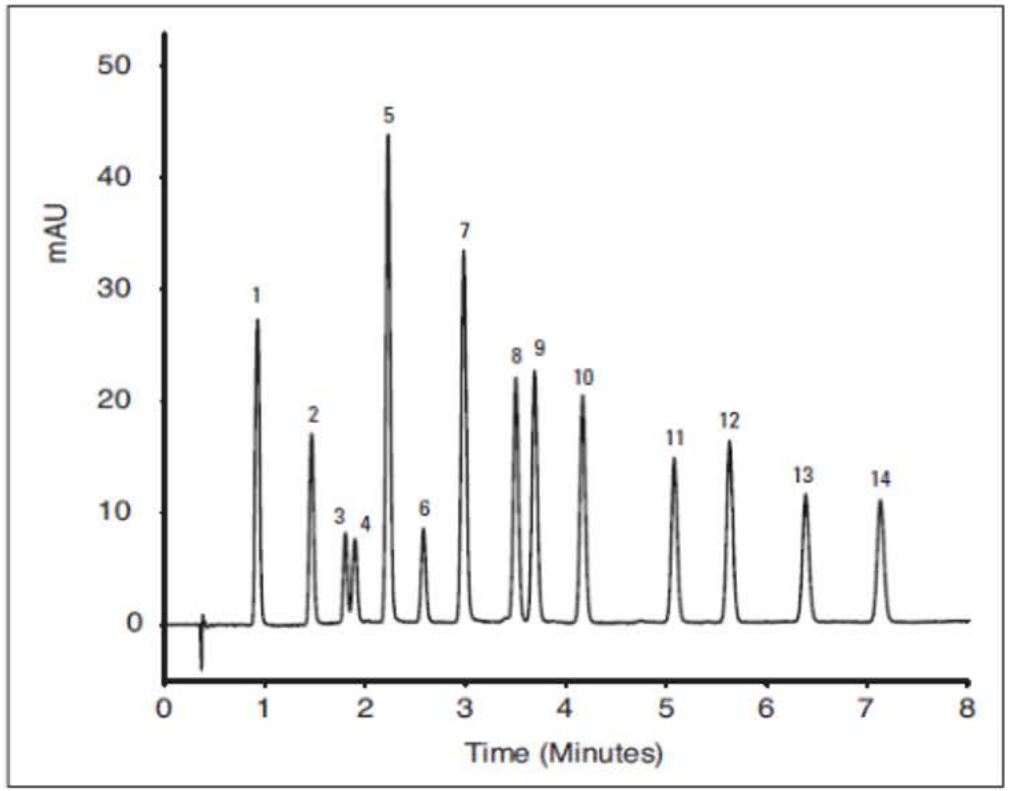


Figure 1: Separation of 14 positional isomers on Accucore PFP 2.6 µm, 100 x 2.1 mm

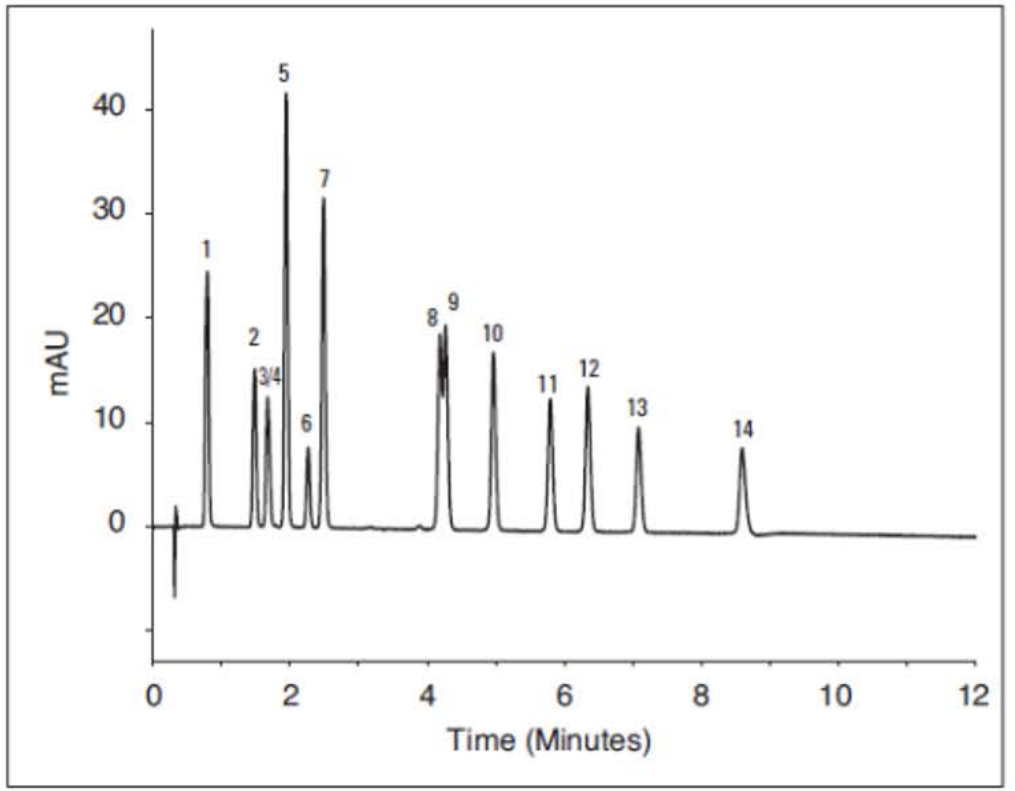


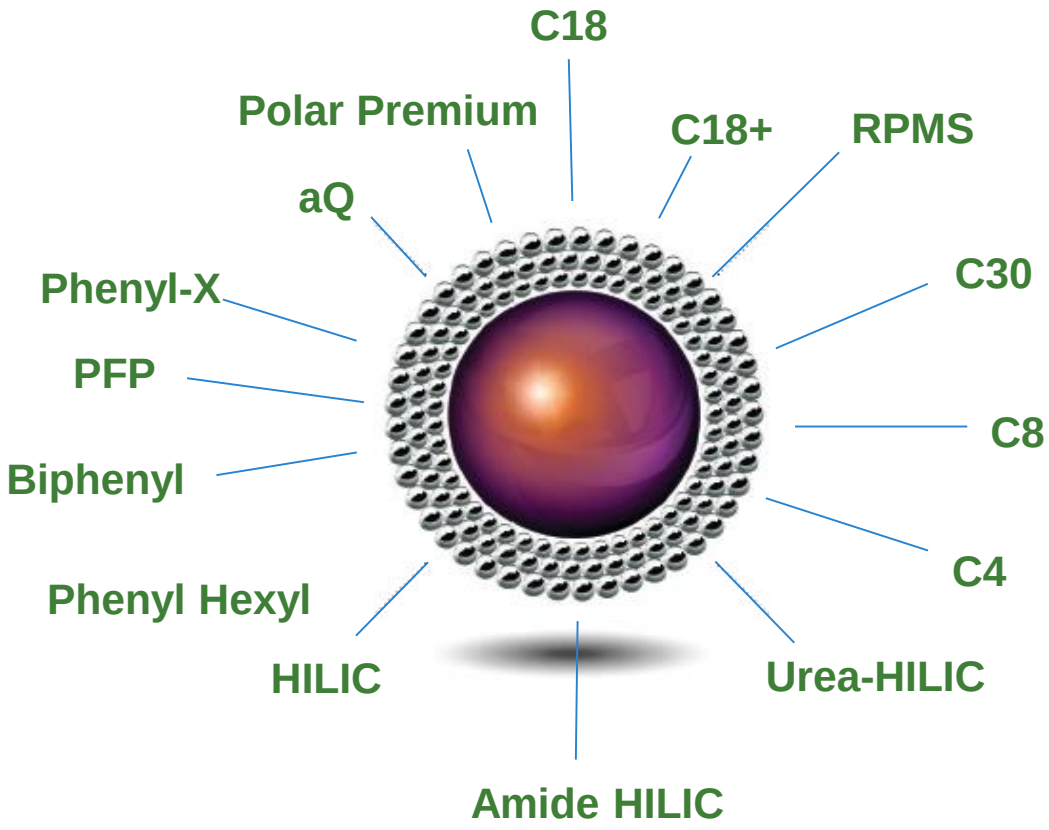
Figure 2: Separation of 14 positional isomers on Accucore C18 2.6 µm, 100 x 2.1 mm

Position	Analyte name
1	3,4 - Dimethoxyphenol
2	2,6 - Dimethoxyphenol
3	2,6 - Difluorophenol
4	3,5 - Dimethoxyphenol
5	2,4 - Difluorophenol
6	2,3 - Difluorophenol
7	3,4 - Difluorophenol
8	3,5 - Dimethylphenol
9	2,6 - Dimethylphenol
10	2,6 - Dichlorophenol
11	4 - Chloro-3-Methylphenol
12	4 - Chloro-2-Methylphenol
13	3,4 - Dichlorophenol
14	3,5 - Dichlorophenol

Summary

Accucore columns are rugged columns that can be used in a wide variety of applications and market spaces. We have a large portfolio of solid core columns and unique chemistries help solve challenging separations.

- 4µm columns are used to improve legacy methods on 5µm columns, for lower pressure HPLC systems
- 2.6µm columns have the biggest range of phase chemistries, for bridging the gap of HPLC to UHPLC
- 1.5µm columns allow for high resolution, high throughput separations on a UHPLC system



Thank you for listening!

Questions?

Thank You

Please return our survey to receive a drink ticket for our daily networking event where you can continue discussions with our experts!