Transfer and Speed-up of Methods to Fused-Core Particle Columns – EPA Method 1694

Ed Mauney, Hillel K. Brandes, William H. Campbell, Wayne K. Way, Richard A. Henry, David S. Bell, and Carmen T. Santasania

Supelco, Div. of Sigma-Aldrich, Bellefonte, PA 16823 USA

www.sigma-aldrich.com

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Abstract

EPA Method 1694 is used in the determination of pharmaceuticals and personal care products (PPCPs) in environmental samples. These PPCPs have received considerable press attention in recent years, with growing concerns over the presence of these compounds in the world's drinking waters. This method uses solid phase extraction (SPE) and high performance liquid chromatography combined with tandem mass spectrometry (LC-MS-MS) to quantitate more than 70 PPCPs in a variety of environmental matrices. In this work, the Group 1 compounds of EPA Method 1694 were run on a column containing Fused-Core™ particles.

HPLC columns packed with Fused-Core particles or porous particles in the sub-2 µm range can both enhance resolution and speed by producing either higher efficiency (N) for the same column length or equivalent efficiency with a shorter column length. The use of ultra-high performance liquid chromatography (UHPLC) comes at a price of higher pressure when sub-2 µm particles are employed.

Abstract (contd.)

As an alternative, Fused-Core silica particles have gained acceptance because of the equivalent performance to particles in the sub-2 µm range. These Fused-Core columns employ conventional 2 µm frits and operate ruggedly at much lower pressures that are within the operating limits of conventional HPLC instruments. In this work, Group 1 compounds are shown to exhibit good resolution with MS-MS detection indicating these Fused-Core columns may be used in this EPA Method.

Experimental

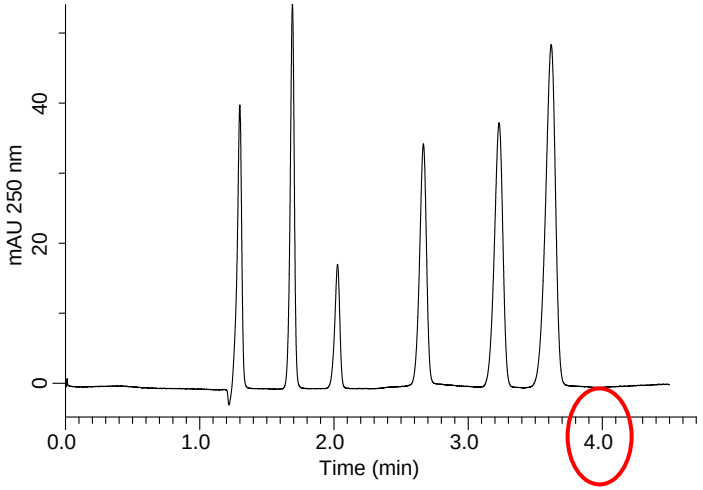
Example 1. Hydrophobic Test Mix

Initially, separation on a 5 µm C18 column is scaled to Ascentis® Express (FC column) to provide equivalent efficiency and retention for latest eluting peak, naphthalene. This involves changes in column length, mobile phase, and flow.

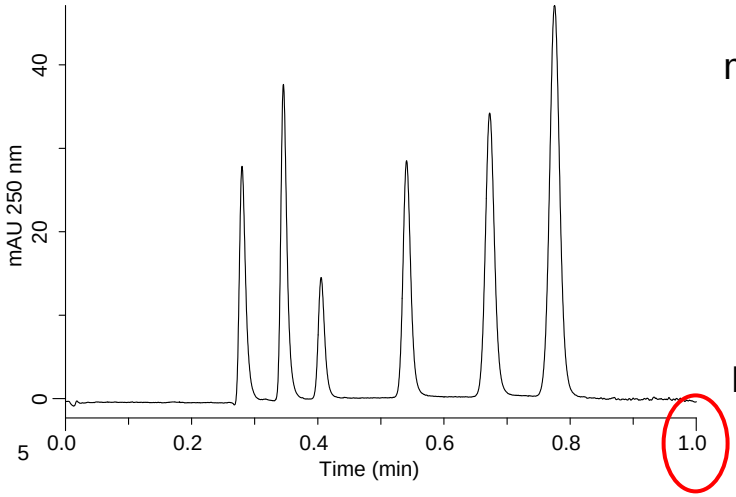
Runtime can be further shortened while still maintaining baseline resolution of critical pair.

Scaling Method to Fast LC, Moderate Pressure

Hydrophobic Test Mix



column:	C18, 15 cm x 3 mm I.D., 5 µm
mobile phase:	20:80, water:acetonitrile
flow rate:	0.4 mL/min.
temp.:	35 °C
injection:	1.5 µL
pressure:	885 psi (61 bar)
$N_{(naphthalene)}$:	~11000
$k_{(naphthalene)}$:	1.78
elution order:	uracil, phenol, acetophenone, benzene, toluene, naphthalene



column:	Ascentis Express C18, 5 cm x 3 mm I.D., 2.7 µm
mobile phase:	31:69, water:acetonitrile
flow rate:	0.6 mL/min.
temp.:	35 °C
injection:	0.5 µL
pressure:	1750 psi (121 bar)
$N_{(naphthalene)}$:	~11000
$k_{(naphthalene)}$:	1.75
$Rs_{(acetophenone)}$:	3.2

At least a 4-fold increase in throughput.

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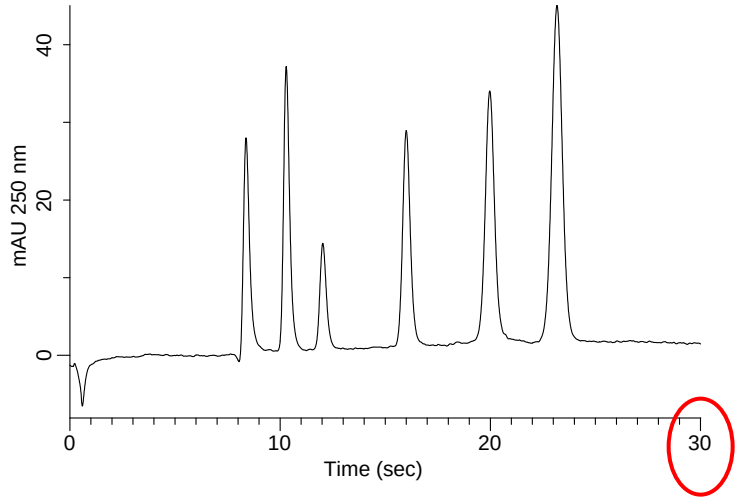
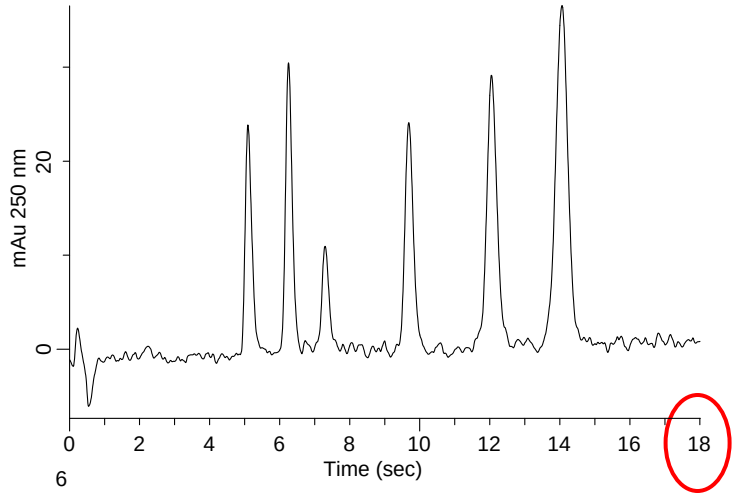
How fast do you want to go?
What system pressure limits exist?



column: Ascentis Express C18, 5 cm x 3 mm I.D., 2.7 µm
mobile phase: 31:69, water:acetonitrile
flow rate: 1.2 mL/min
temp.: 35° C
injection: 0.5 µL
pressure: 3700 psi (255 bar)
$Rs_{(acetophenone)}$: 3.1

9-fold increase in throughput compared to the original method on 5 µm particle.



column: Ascentis Express C18, 5 cm x 3 mm I.D., 2.7 µm
mobile phase: 31:69, water:acetonitrile
flow rate: 2.0 mL/min
temp.: 35° C
injection: 0.5 µL
pressure: 6400 psi (441 bar)
$Rs_{(acetophenone)}$: 2.8

15-fold increase in throughput compared to the original method on 5 µm particle.

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Experimental (contd.)

Example 2. EPA Method 1694

Pharmaceuticals in the water supply represent a growing public concern, as represented by stories in many newspapers nationwide.


CENTRE DAILY TIMES

MONDAY, SEPTEMBER 15, 2008 www.centredaily.com 50 CENTS

Blowout means more time for young Lions, B1

PHILLIES SWEEP BREWERS, B4

STEELERS HOLD OFF BROWNS, B1

Tons of drugs dumped into wastewater


CENTRE DAILY TIMES

Gypsy moths return just in time for spring

BACK IN THE GAME

Hometown crowd gives Flyers boost over Penguins, B1

WINGATE STUDENTS SPELL WAY TO SUCCESS, A8

COLUMBINE SHOOTING ANNIVERSARY

U.S. water tainted by drugs

Government has overlooked contamination

Current research at EPA includes development of testing methodologies for these compounds:

- Method 1694: Pharmaceutical and Personal Care Products in Water, Soil, Sediment, and Biosolids
- Method 1698: Steroids and Hormones in Water, Soil, Sediment, and Biosolids

Experimental (contd.)

LC-MS/MS Conditions

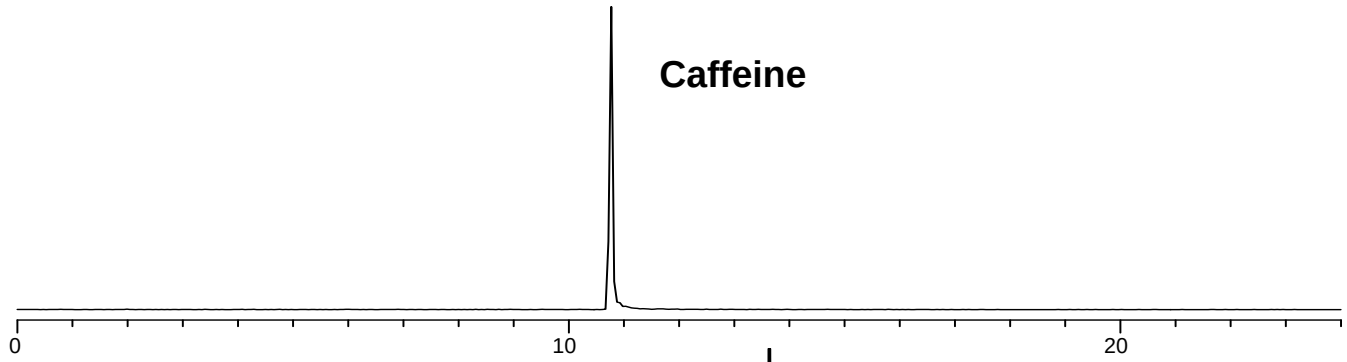
Instrument	Applied Biosystems 3200QT
Column	Ascentis Express C18, 10 cm x 2.1 mm, I.D., 2.7 µm
Mobile Phase	(A): 0.1% formic acid and 0.1% ammonium formate in water; (B): 50:50 methanol:acetonitrile
Gradient and Flow	**Time** **Flow µL/min** **%A** **%B** 0.00 150 95.0 5.0 4.00 250 95.0 5.0 22.50 300 12.0 88.0 23.00 300 0.0 100.0 26.00 300 0.0 100.0 26.50 150 95.0 5.0 33.00 150 95.0 5.0 43.00 150 95.0 5.0
Temperature	40 ° C
Injection Volume	5.0 µL
Source Conditions	Turbo ion spray ESI +, MRM
MS-MS Transitions	See Table 1
Dwell time	50 msec

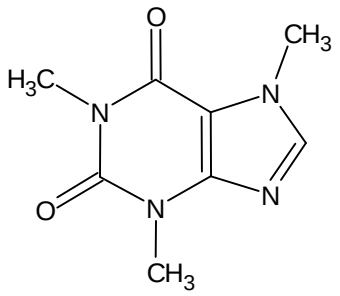
Experimental (contd.)

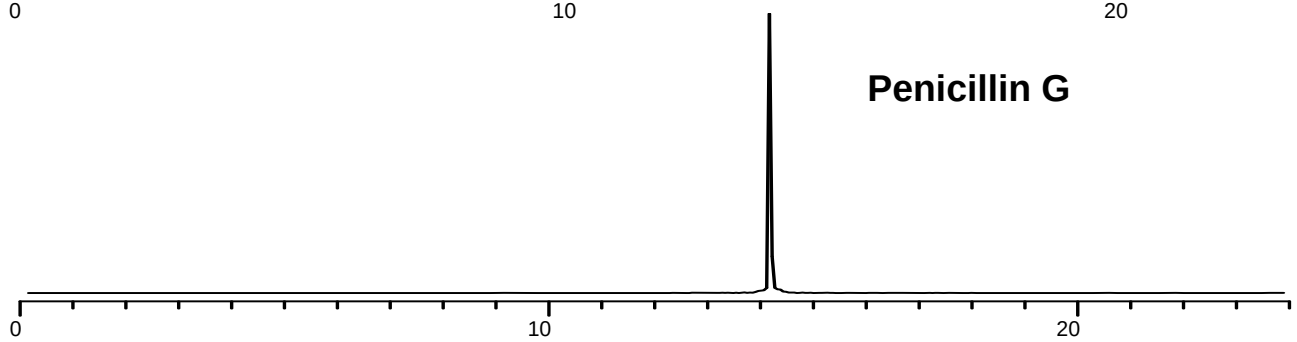
SPE Extraction Method

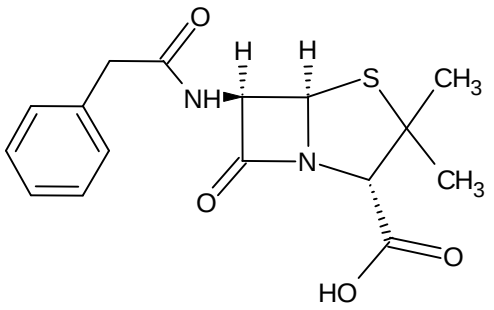
Extraction Cartridge	Supelco Select HLB SPE Tube, 500 mg/6 mL
Sample	500 mL of drinking water spiked with Group 1 compounds, adjusted to pH=4 with 6M HCl
Tube Conditioning	20 mL of methanol, 6 mL of water, 6 mL of water at pH 2 (with 6 M HCl)
Sample extraction	10 mL/min through tube
Dry time	5 min
Elution	12 mL, 50:50 methanol:acetonitrile
Dry down	40 ° C, under nitrogen stream
Reconstitution	To final volume of 2 mL using mobile phase

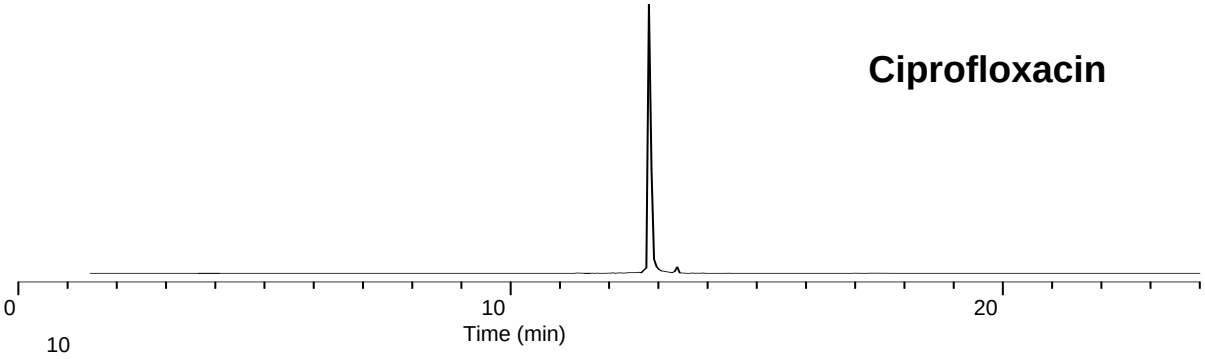
Figure 2. EPA Method 1694
Select MRM Transitions from Figure 1











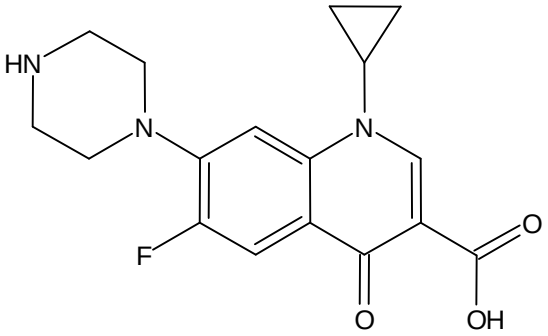


Figure 1. LC-MS-MS Chromatogram of Group 1 Compounds

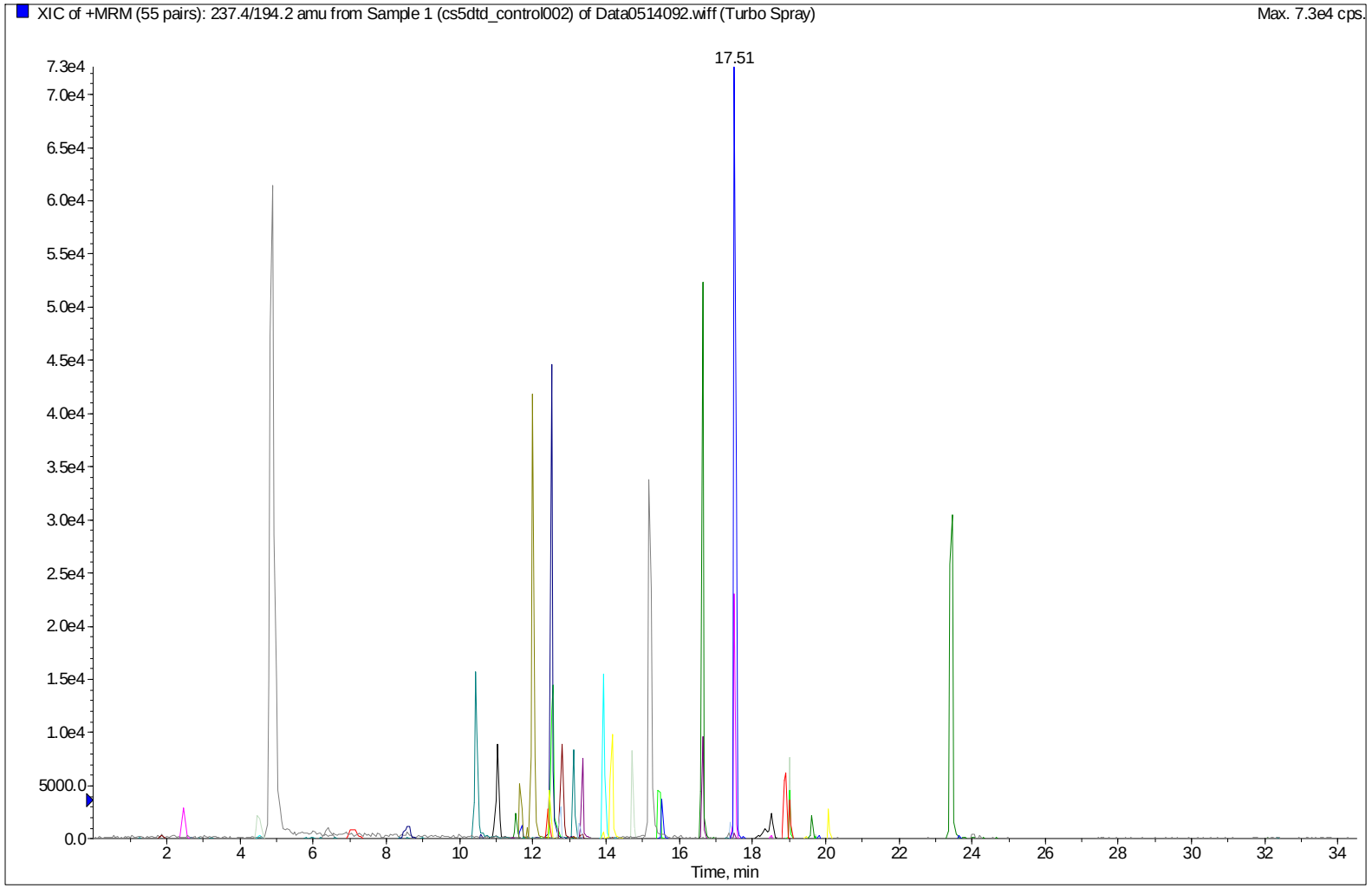


Figure 2. EPA Method 1694
Select MRM Transitions from Figure 1 (contd.)

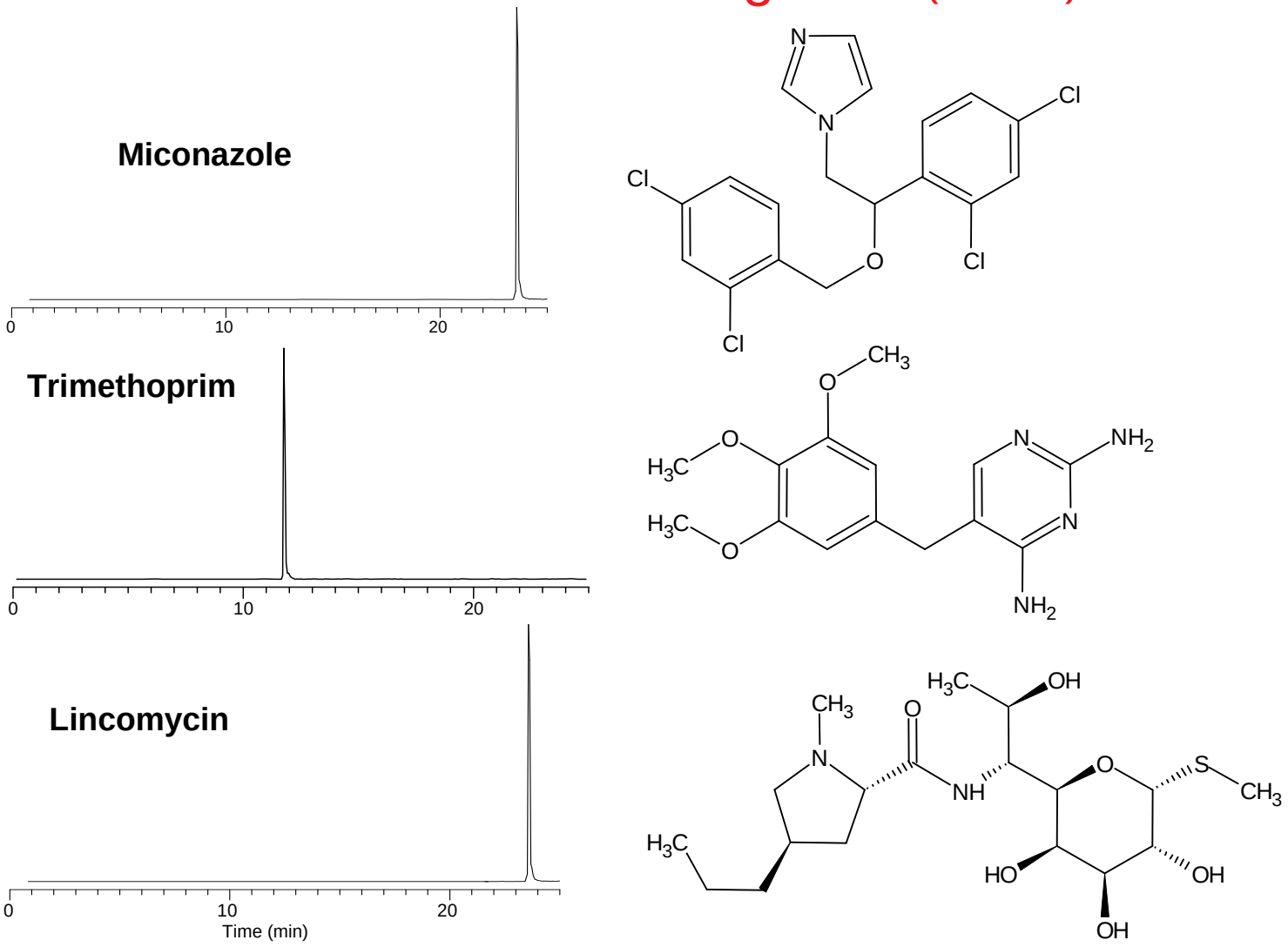


Table 1. Group 1 Compounds from EPA Method 1694

Compound	MRM Transition
Acetaminophen	152.2-126.2
Ampicillin	350.3-106.1
Azithromycin	749.9-591.6
Caffeine	195.0-138.0
Carbadox	263.2-231.2
Carbamazepine	237.4-194.2
Cefotaxime	456.4-396.1
Ciprofloxacin	332.2-314.2
Clinafloxacin	366.3-348.1
Cloxcillin	469.1-160.1
Codeine	300.0-152.0
Cotinine	177.0-98.0
Dehydronifedipine	345.5-284.1
Digoxigenin	391.2-355.2
Digoxin	781.5-113.1
Diltiazem	415.5-178.0
1,7-Dimethylxanthine	181.2-124.0
Diphenhydramine	256.8-168.1
Enrofloxacin	360.0-316.0
Erythromycin	734.4-158.0
Erythromycin anhydrate	716.4-158.0
Flumeqine	262.0-173.7
Fluoxetine	310.3-148.0
Lincomycin	407.5-126.0

Table 1. Group 1 Compounds from EPA Method 1694 (contd.)

Compound	MRM Transition
Lomefloxacin	352.2-308.1
Miconazole	417.0-161.0
Norfloxacin	320.0-302.0
Norgestimate	370.5-124.0
Ofloxacin	362.2-318.0
Ormetoprim	275.3-259.1
Oxacillin	402.2-160.2
Oxolinic acid	244.1-216.1
Penicillin G	335.1-160.1
Penicillin V	373.2-182.2
Roxithromycin	837.0-158.8
Sarafloxacin	386.0-299.0
Sulfachloropyridazine	285.0-156.0
Sulfadiazine	251.2-156.1
Sulfadimethoxine	311.0-156.0
Sulfamerazine	265.0-156.0
Sulfamethazine	279.0-156.0
Sulfamethizole	271.0-1560.
Sulfamethoxazole	254.0-156.0
Sulfanilamide	173.2-108.2
Sulfathiazole	256.3-156.0
Thiabendazole	202.1-175.1
Trimethoprim	291.0-230.0
Tylosin	916.6-174.2
Virginiamycin	526.3-508.3

Results

Figure 1 shows the LC-MS-MS chromatogram of a standard mix of the Group 1 compounds at the CS5 level in Method 1694. Good chromatographic resolution was obtained using the Fused-Core LC column.

A few compounds were not detected in the spiked sample. Most of these were –cillin type compounds, and further experiments with the SPE phase are needed to check the retention characteristics on the SPE phase for these compounds.

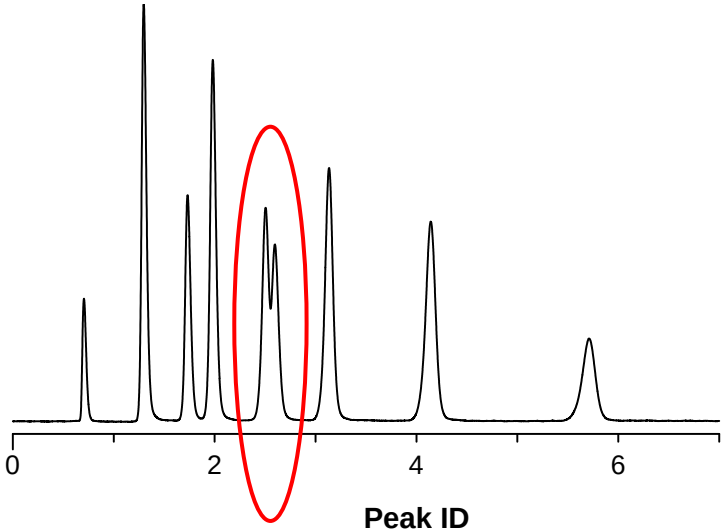
Finally, a number of differences between the published method MRM transitions and those used here were seen. All transitions used are listed in Table 1.

Example 3. Tricyclic Antidepressants

Initially, drop-in substitution is made with Ascentis Express for 3 µm C18 column, with adjustment in mobile phase composition to yield isoelutropic elution of latest eluting peak.

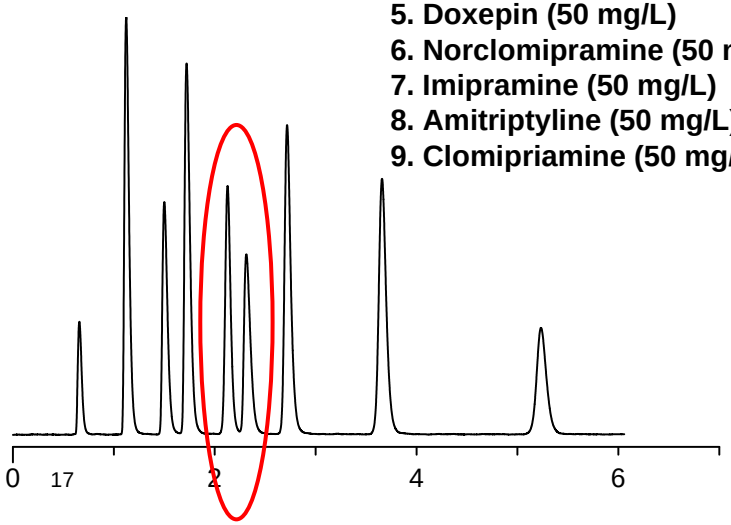
While backpressure is higher, though within operating conditions of a conventional system, resolution of critical pair is achieved.

Method Transfer with Gains in Resolution



Peak ID

1. **Uracil**
2. **Nordoxepin (50 mg/L)**
3. **Desipramine (50 mg/L)**
4. **Nortiptyline (50 mg/L)**
5. **Doxepin (50 mg/L)**
6. **Norclomipramine (50 mg/L)**
7. **Imipramine (50 mg/L)**
8. **Amitriptyline (50 mg/L)**
9. **Clomipriamine (50 mg/L)**



column:	3 µm C18, 10 cm x 2.1 mm I.D.
mobile phase A:	100 mM ammonium acetate, pH 7.0 (titrated with ammonium hydroxide)
mobile phase B:	water
mobile phase C:	methanol
online mixing:	A:B:C = 10:25:65
flow rate:	0.4 mL/min.
temp.:	55 °C
det.:	250 nm
injection:	1 µL
pressure:	1750 psi (121 bar)
$k_{(clomipramine)}$:	7
$Rs_{(norclomipramine)}$:	0.61

column:	Ascentis Express C18, 10 cm x 2.1 mm I.D.
mobile phase A:	100 mM ammonium acetate, pH 7.0 (titrated with ammonium hydroxide)
mobile phase B:	water
mobile phase C:	methanol
online mixing:	A:B:C = 10:28:62 (isoelutropic for clomipramine)
flow rate:	0.3 mL/min.
temp.:	55 °C
det.:	250 nm
injection:	1 µL
pressure:	2990 psi (206 bar)
$k_{(clomipramine)}$:	7
$Rs_{(norclomipramine)}$:	1.71

Example 4. Comparisons to sub-2 µm columns

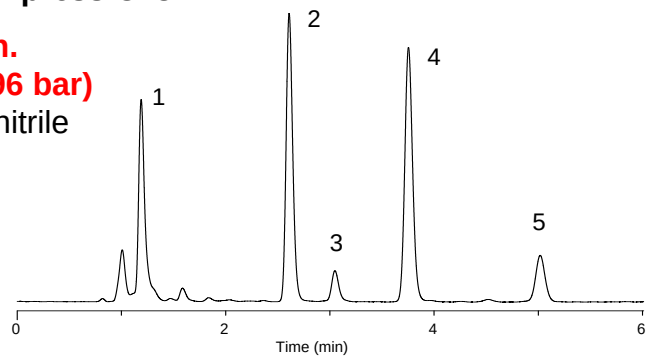
At a given column length, Ascentis Express provides similar performance at much lower backpressures. This affords the opportunity for reducing runtime all within the constraints of whatever instrument is available.

At equivalent flow and backpressure, Ascentis Express provides superior performance by virtue of longer column length.

Fast LC, Moderate Pressure vs sub-2 µm[1]

Ascentis Express C18

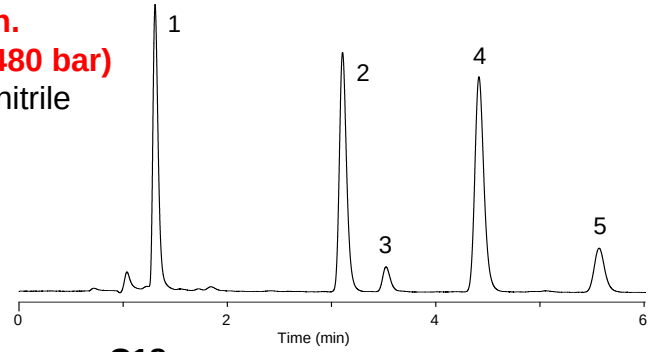
0.2 mL/min.
1390 psi (96 bar)
45% acetonitrile



column: 10 cm x 2.1 mm I.D.
mobile phase: water:acetonitrile; isoelutropic for β-Estradiol
flow rate: variable
det.: 200 nm
injection: 1 µL

Sub-2 µm competitor 2

0.2 mL/min.
7000 psi (480 bar)
51% acetonitrile

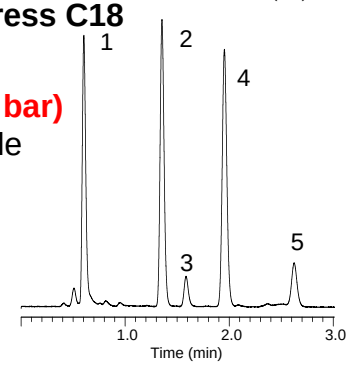


Peak ID:

1. **Estriol**
2. **β-Estradiol**
3. **Contaminant**
4. **Estrone**
5. **Estrone Degradant**

Ascentis Express C18

0.4 mL/min.
2880 psi (199 bar)
45% acetonitrile



Column	Flow mL/ min.	Peak 1 N	Peak 2 N	Peak 3 N	Peak 3 Rs	Peak 4 N	Peak 5 N
Ascentis Express C18	0.2	2180	7970	9040	3.58	11760	14160
Ascentis Express C18	0.4	1540	5820	6700	3.12	9020	11200
Sub-2 µm competitor	0.2	3150	9590	10430	3.15	12200	14070

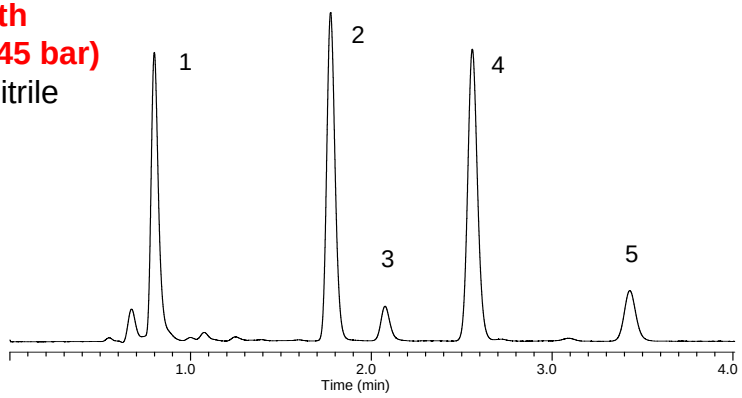
1. **This estrogen application was performed on a nonoptimized system.**

Fast LC, Moderate Pressure vs sub-2 µm

column: 2.1 mm I.D.
mobile phase: water:acetonitrile; isoelutropic for β-Estradiol
flow rate: 0.3 mL/min
det.: 200 nm
injection: 1 µL

Ascentis Express C18

10 cm length
2100 psi (145 bar)
45% acetonitrile

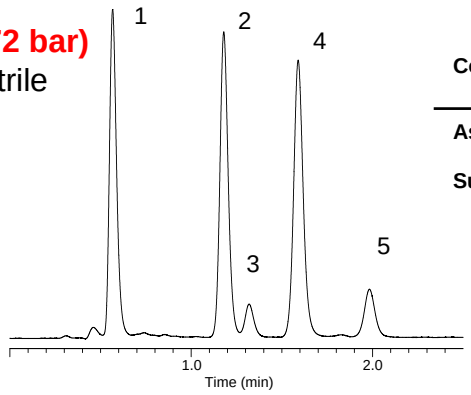


Peak ID:

1. **Estriol**
2. **β-Estradiol**
3. **Contaminant**
4. **Estrone**
5. **Estrone degradant**

Sub-2 µm competitor 1

5 cm length
2500 psi (172 bar)
46% acetonitrile



Column	Peak 1 N	Peak 2 N	Peak 3 N	Peak 3 Rs	Peak 4 N	Peak 5 N
Ascentis Express C18	1780	6890	7180	3.28	10360	12500
Sub-2 µm competitor	980	3160	3260	1.58	4330	5210

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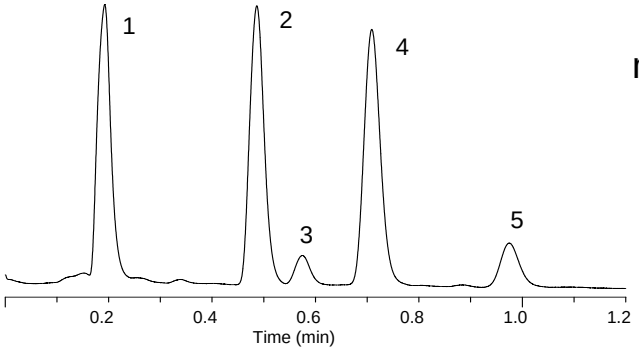
Fast LC, High Pressure vs sub-2 µm

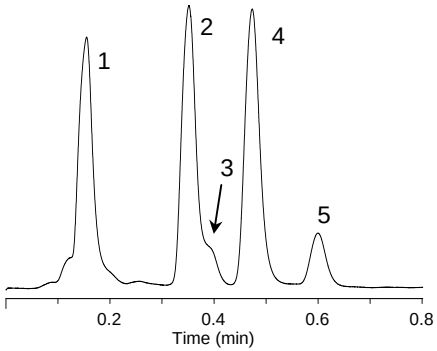
Ascentis Express C18

10 cm length
45% acetonitrile
1.5 mL/min.



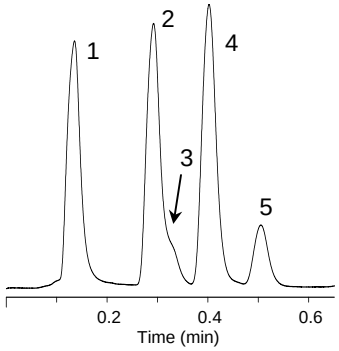
Sub-2 µm competitor 1

5 cm length
46% acetonitrile
1.38 mL/min.



Sub-2 µm competitor 2

5 cm length
51% acetonitrile
1.35 mL/min.



column: 2.1 mm I.D.
mobile phase: water:acetonitrile; isoelutropic for β-Estradiol
flow rate: variable
det.: 200 nm
injection: 1 µL
pressure: 11,700 psi (807 bar)

Peak ID:

1. **Estriol**
2. **β-Estradiol**
3. **Contaminant**
4. **Estrone**
5. **Estrone degradant**

Conclusions

The relatively low backpressure of Fused-Core columns permits transfer of methods from traditional porous particle columns while realizing shorter run times and/or improved performance, all with conventional HPLC instrumentation.

The extent of improvements in throughput are determined by system limitations and/or objectives of the method.

For a given column length, Fused-Core columns provide similar performance to sub-2 µm columns, but at much lower backpressure.

At constant pressure, improved performance of Fused-Core columns over sub-2 µm columns is realized by use of approximately twice the column length.

Trademarks

Ascentis, Supel – Sigma-Aldrich Biotechnologies LP

Fused-Core – Advanced Materials Technology, Inc.