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Evaluation of the Current Capabilities of Ultra High Pressure LC and High Temperature LC

William Hedgepeth, Masatoshi Takahashi, Shimadzu Scientific Instruments

Introduction

UHPLC is becoming an increasingly popular technique for sample analysis due to the ability to reduce analysis times or improve resolution. However, the use of ultrahigh pressures can reduce column lifetime and increase the maintenance requirements of the LC system. Another less commonly used technique to reduce analysis times is the use of high-temperature LC. High-temperature LC has the advantage of reducing system pressure by lowering solvent viscosity and may provide the ability to reduce the organic content of the mobile phase. The use of high-temperature LC has been limited by the number of available columns for this technique. A number of applications will be presented up to 19,000 psi and 150 degrees C for the technique.

High Temperature Column Tested

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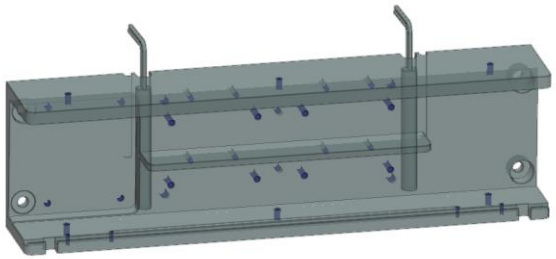
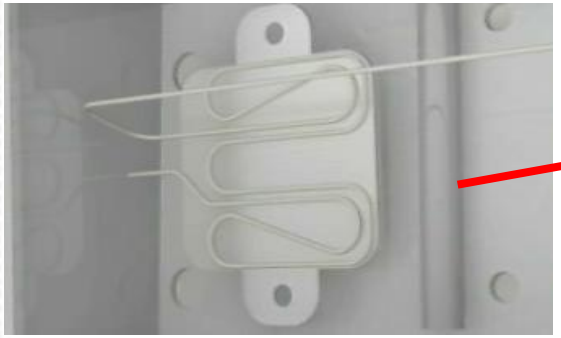
Column:	Shodex ET-RP1 4D
Particles:	Porous polymeric
Dimensions:	3 x 150mm, 4.6 x 150 mm
Functional Group:	Octadecyl
Temperature range:	20 ~ 150°C

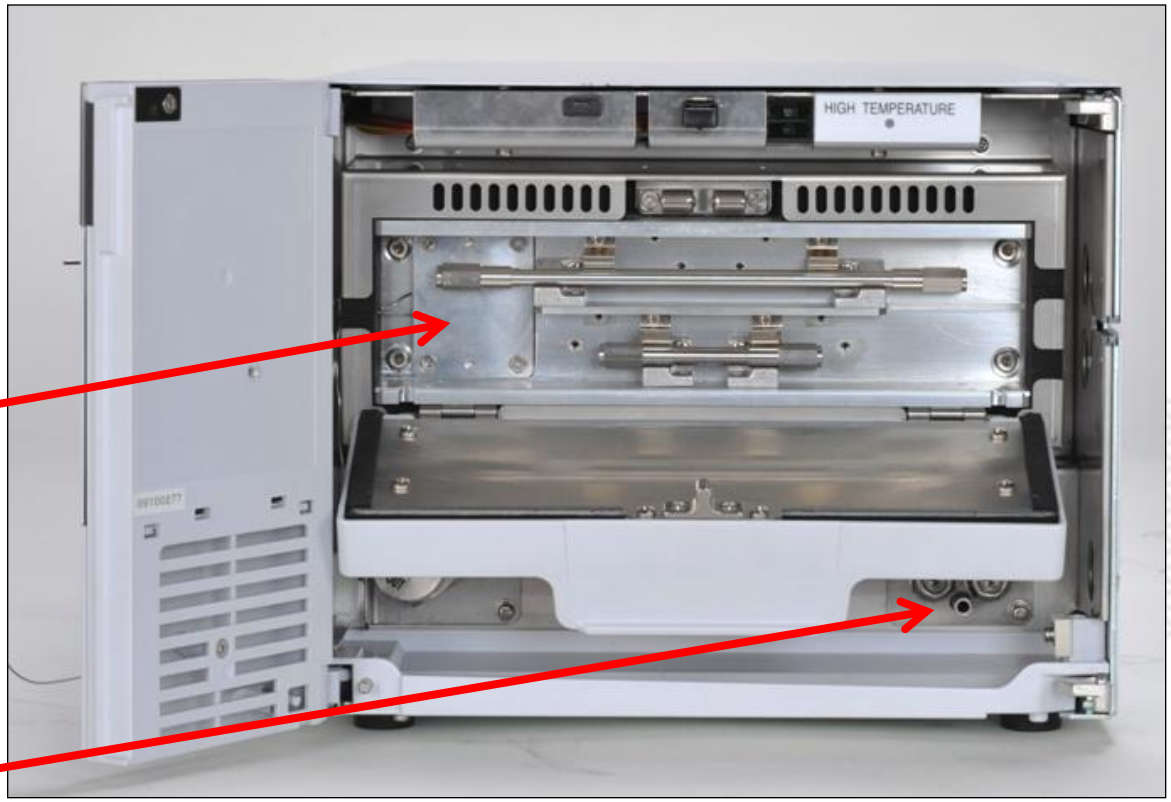
Column Oven Tested

Two independently controllable heaters minimize temperature distribution across column





Low volume preheater (< 3uL)



Post column cooler

Green HPLC

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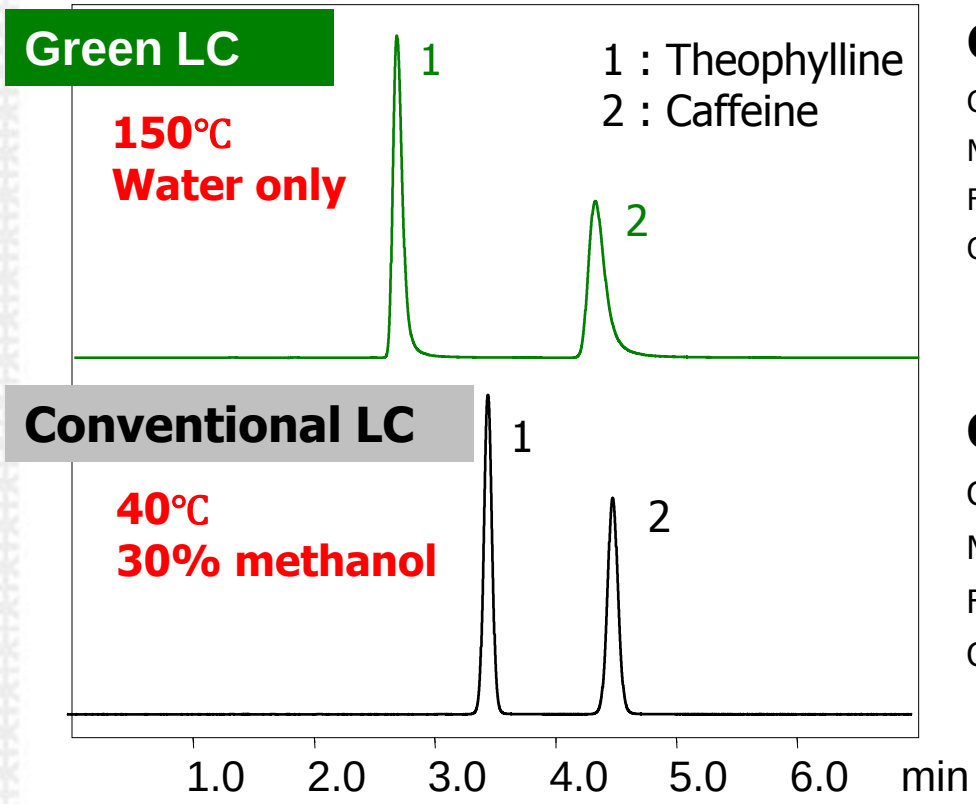
High-temperature analysis



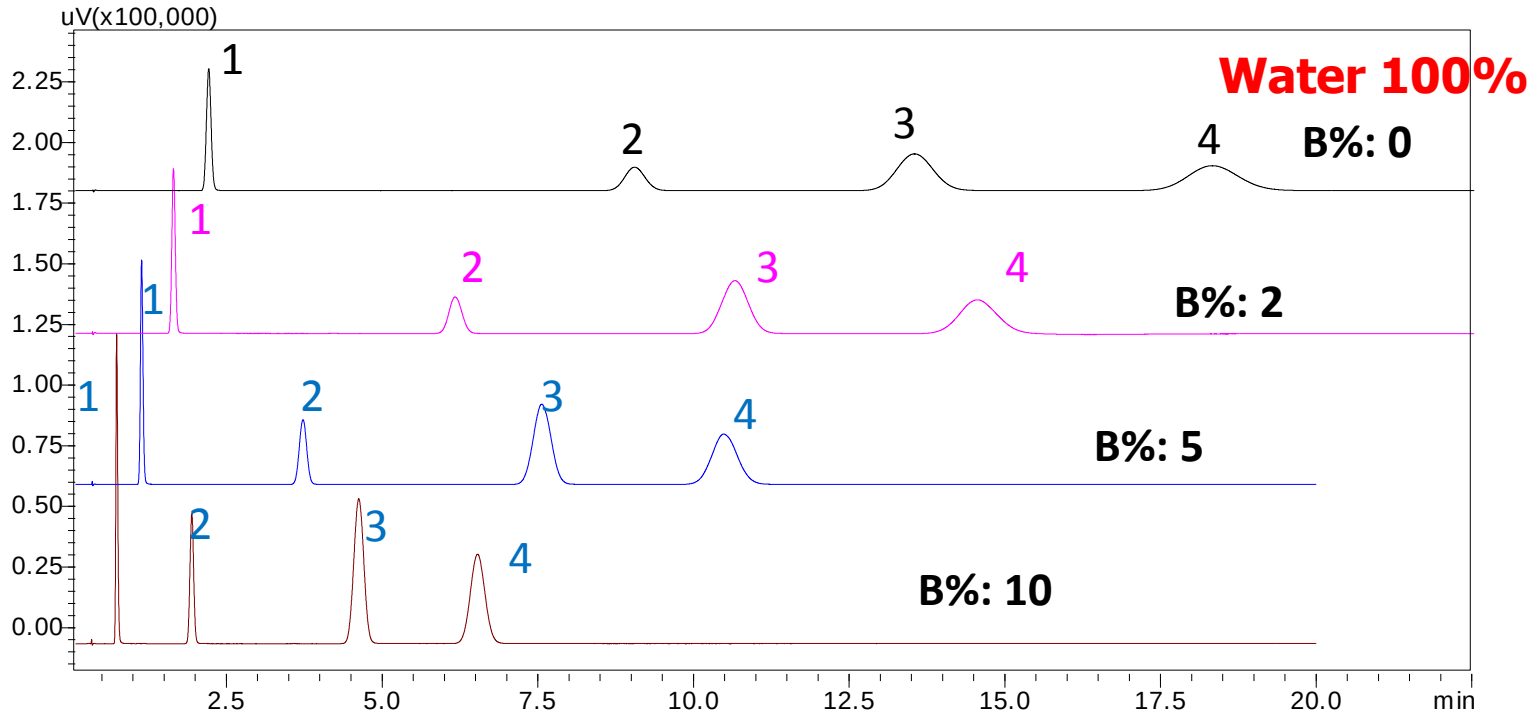
Green LC

Column : Shodex ET-RP1 (3.0mm x150mm)
Mobile phase : 100% H2O
Flow rate : 0.5 mL/min
Oven temp. : 150°C

Conventional LC

Column : ODS (4.6mm x 150mm, 5 um)
Mobile phase : methanol / water = 3/7
Flow rate : 1.0 mL/min
Oven temp. : 40°C

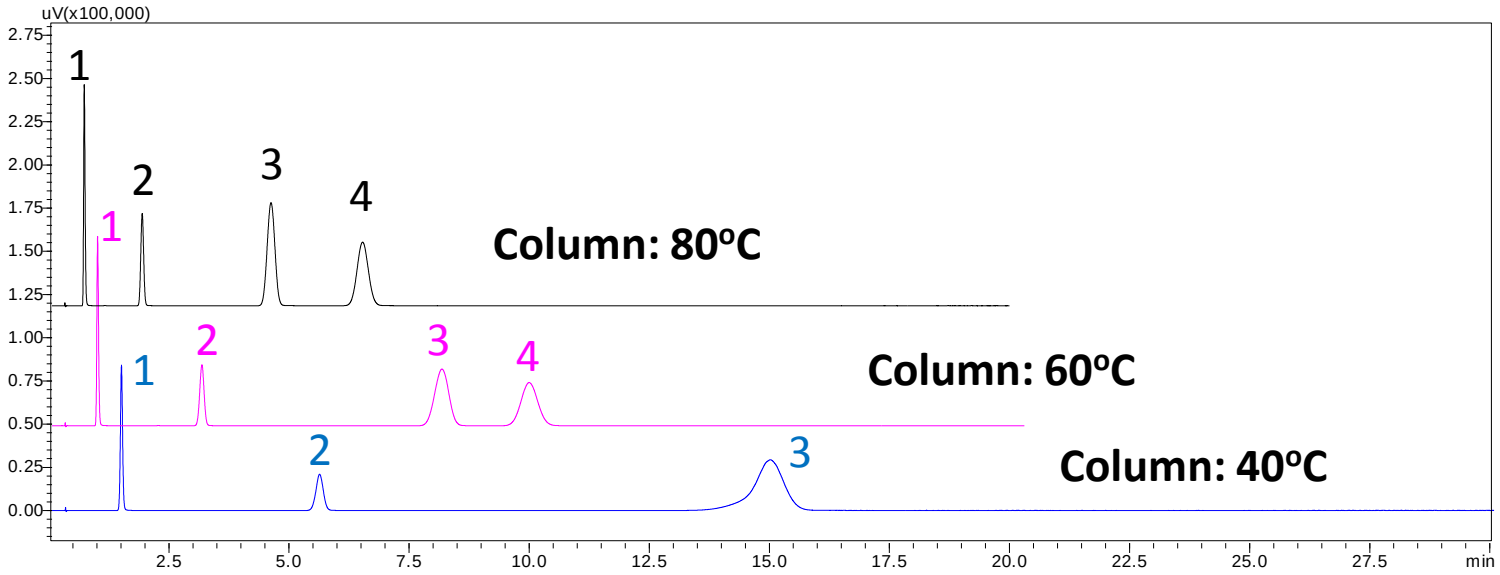
Effect of Organic Modifier on Retention



1. Theobromine
2. Caffeine
3. Methyl paraben
4. Acetophenone

- Mobile phase: Water/MeOH, 0.8 mL/min
- Column: 2.2 um, 3 x 50 mm
- Temp: 80°C
- Detector: 260 nm (100Hz)

Effect of Temperature on Retention

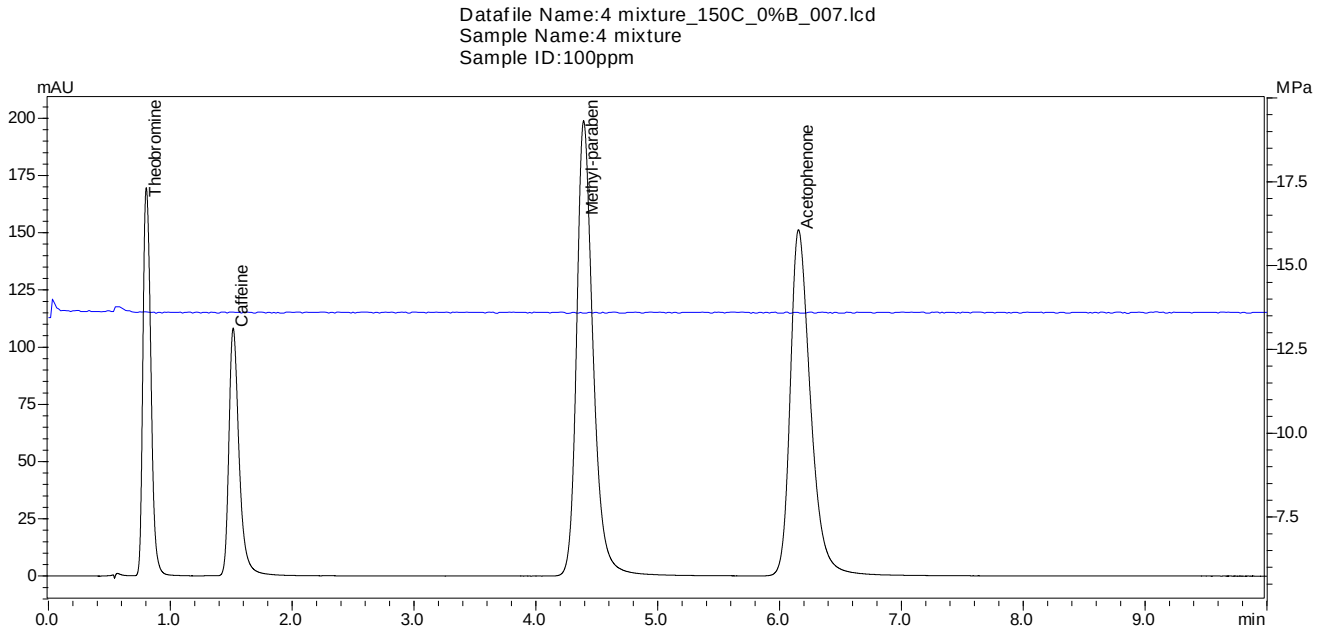


1. Theobromine
2. Caffeine
3. Methyl paraben
4. Acetophenone

Method:

- Mobile phase: Water/MeOH = 90/10 (v/v)
- Column: XR-ODS 2.2 um, 3 x 50 mm
- Detector: 260 nm, 100Hz

Green LC at High Temperature



Sample: mixture of 4 compounds

1. Theobromine
2. Caffeine
3. Methyl paraben
4. Acetophenone

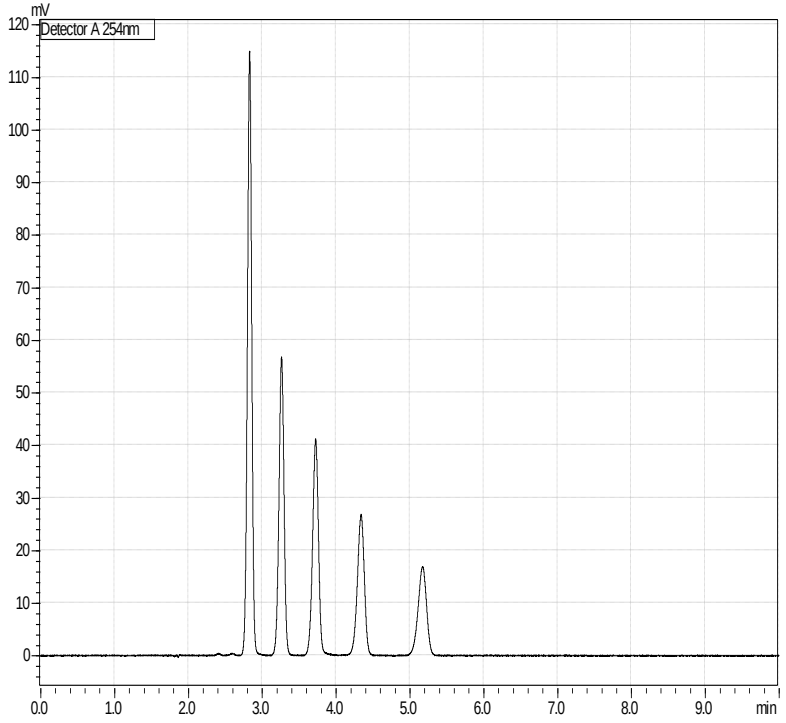
Method:

- Mobile phase: Water (100%)
- Column: ET-RP1, 3 x 150 mm
- Temperature: 150°C
- Detector: UV-VIS (100 Hz), 260 nm

Effect of Preheating at 150°C

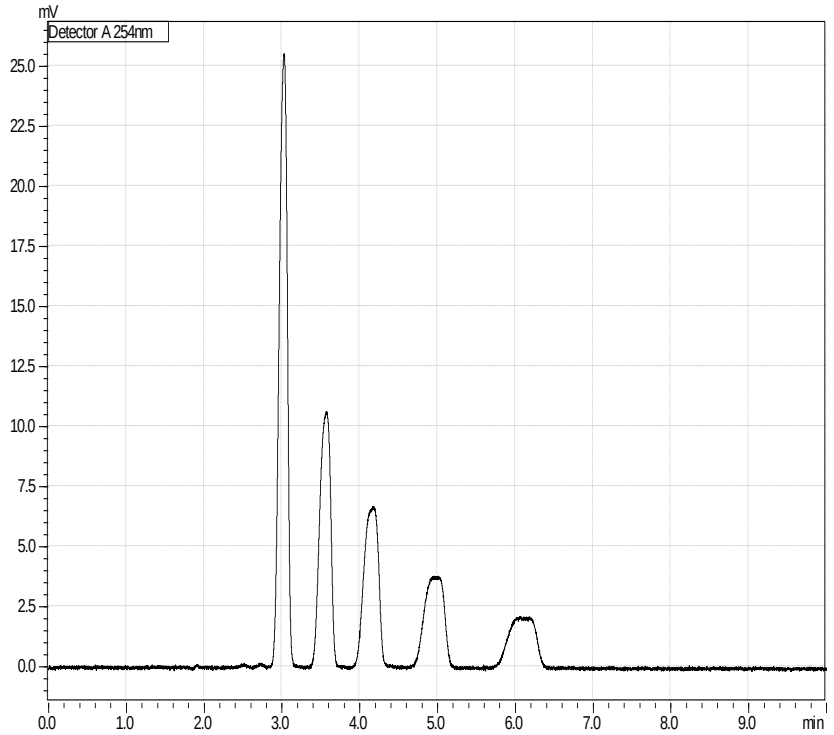
With preheating

Datafile Name:Phenons5mix-50ppm-150C-06.lcd
Sample Name:Phenone mix
Sample ID:Phenone mix006



Without preheating

Datafile Name:Phenones5-50ppm-150Cnopre-2.lcd
Sample Name:alkyl 5
Sample ID:alkyl 5



Alkylphenone 50 ppm mix, preheater < 3 uL

Temperature Effect on Retention

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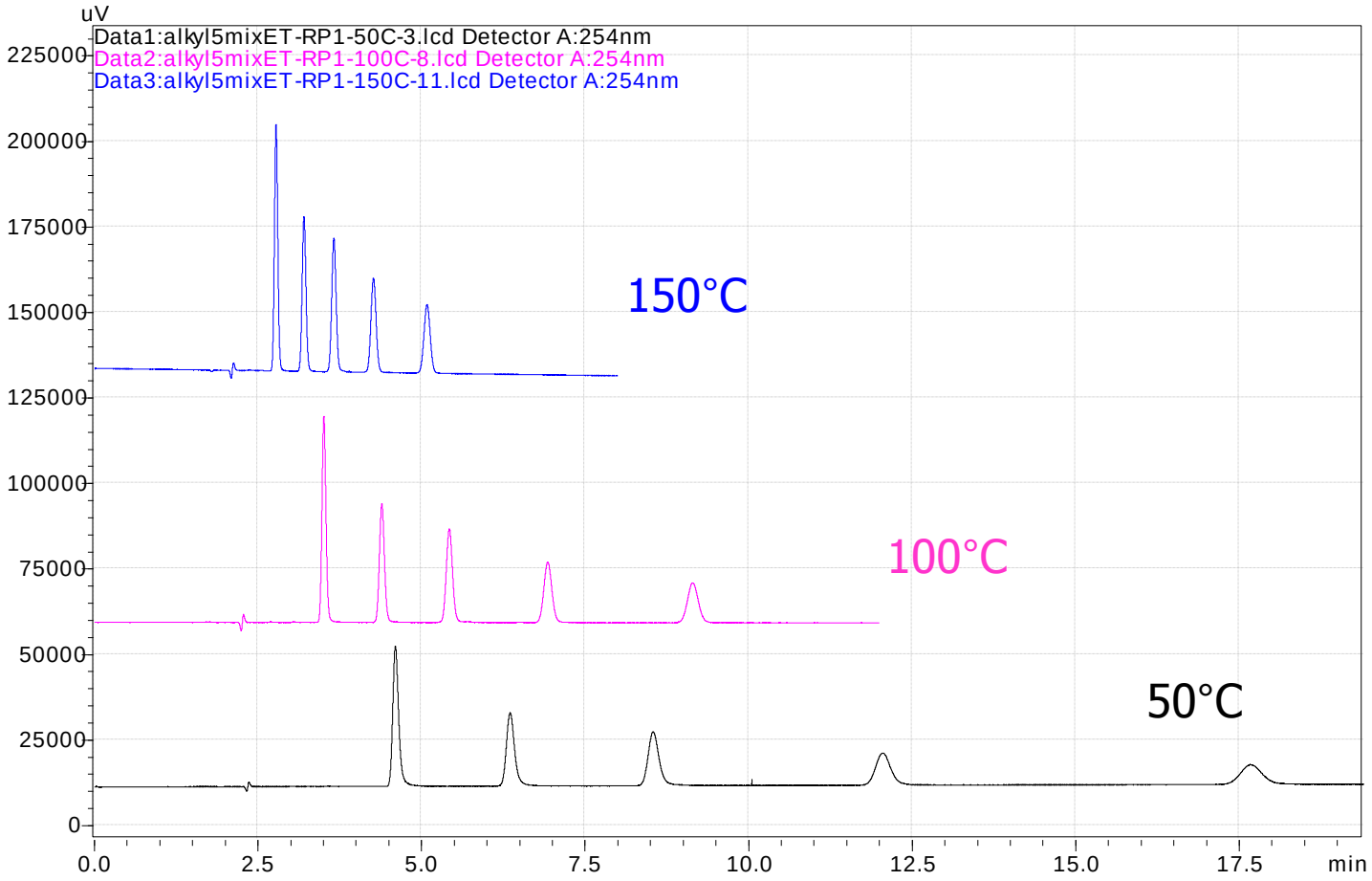
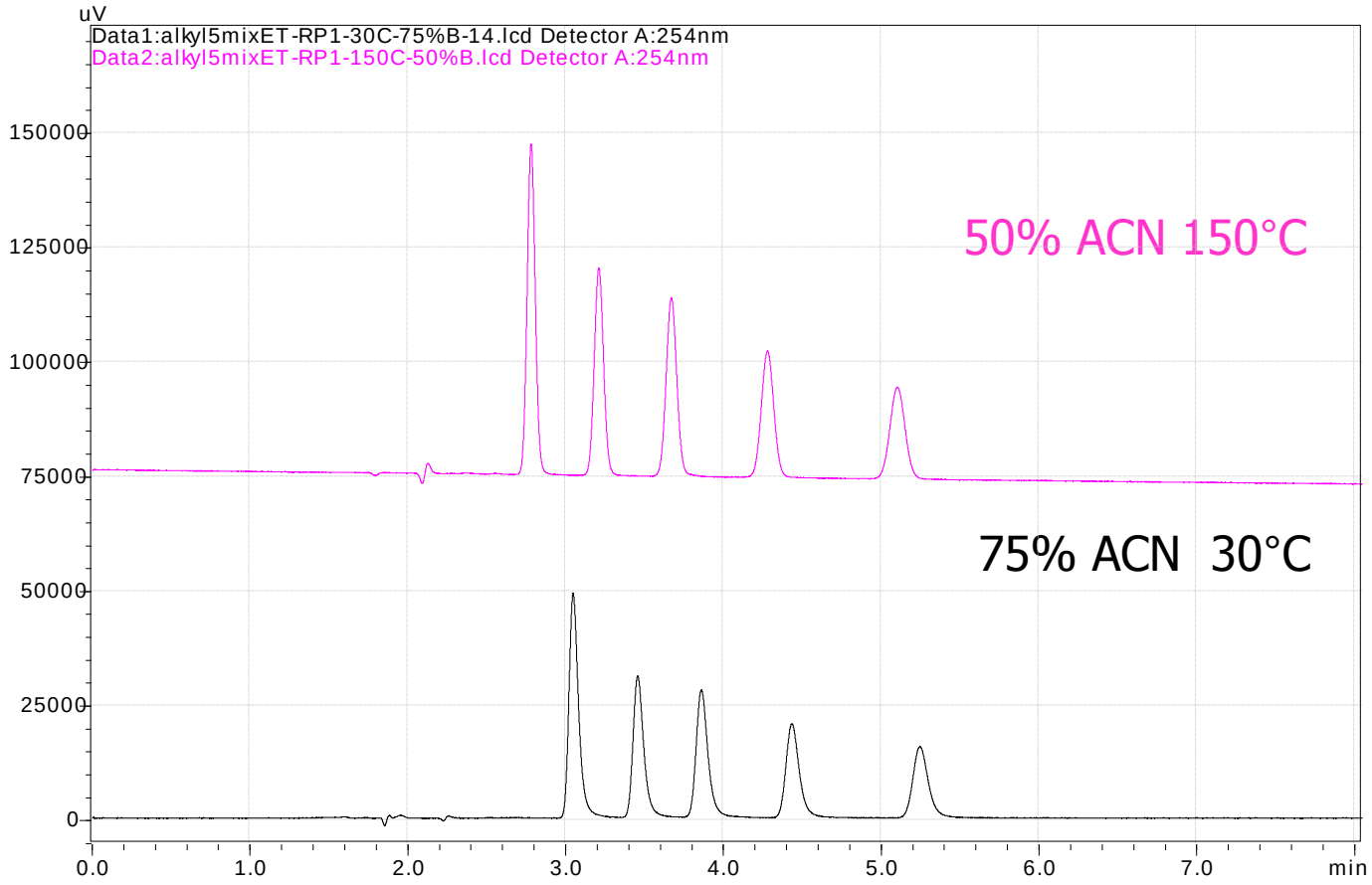
ET-RP1 column, Alkylphenone mix



High Temperature vs. High Organic

Flow: 0.75 mL/min each run, Column: ET-RP1



Reducing Organic Modifier Results

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High Temperature 50% ACN at 150°C

50%B 150°C							
Peak#	Ret. Time	Area	Height	Area%	NTP	Resolution	Width(10%)
1	2.781	260600	71683	28.35	13547	--	0.104
2	3.21	187561	44917	20.40	13624	4.19	0.119
3	3.67	185443	38841	20.17	13725	3.92	0.136
4	4.279	152092	27457	16.54	13594	4.49	0.158
5	5.102	133618	19971	14.54	13161	5.09	0.191
75%B 30°C							
Peak#	Ret. Time	Area	Height	Area%	NTP	Resolution	Width(10%)
1	3.047	212006	48784	28.71	12895	--	0.127
2	3.457	149905	30832	20.30	13174	3.60	0.142
3	3.86	149582	27788	20.26	13459	3.19	0.157
4	4.434	120811	20346	16.36	13988	4.06	0.173
5	5.245	106079	15570	14.37	14515	5.02	0.2

High Organic 75% ACN at 30°C

Peak efficiency and resolution can be maintained with reduced organic modifier.

Efficiency at Elevated Temperature

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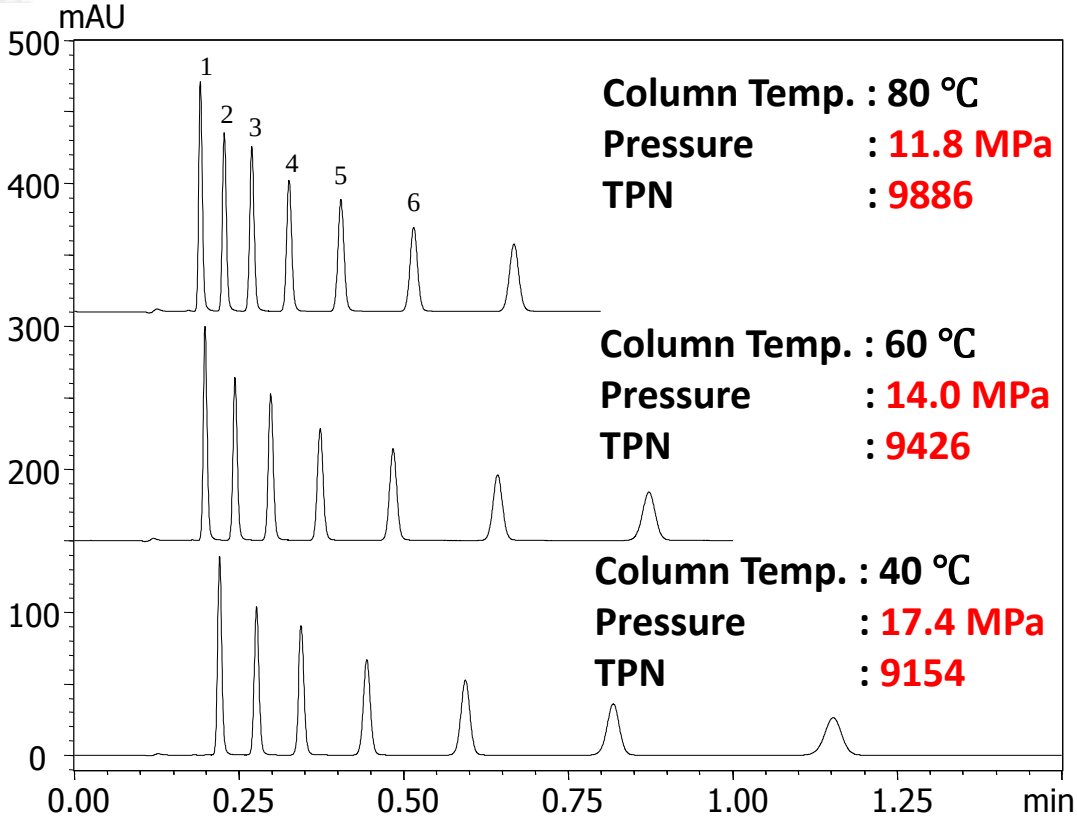
Improved separation efficiency at elevated temperature

Increase of theoretical plate

Decrease of column pressure



Column : Shim-pack XR-ODS (3.0mmi.d., 50mm, 2.2um)
Mobile phase : water/acetonitrile (3/7, v/v)
Flow rate : 1.5 mL/min
Detection : UV 245 nm.

Peaks;
1: acetophenone
2: propiophenone
3: butyrophenone
4: valerophenone
5: hexanophenone
6: heptanophenone
7: octanophenone

Use of Alternate Solvents for Separation

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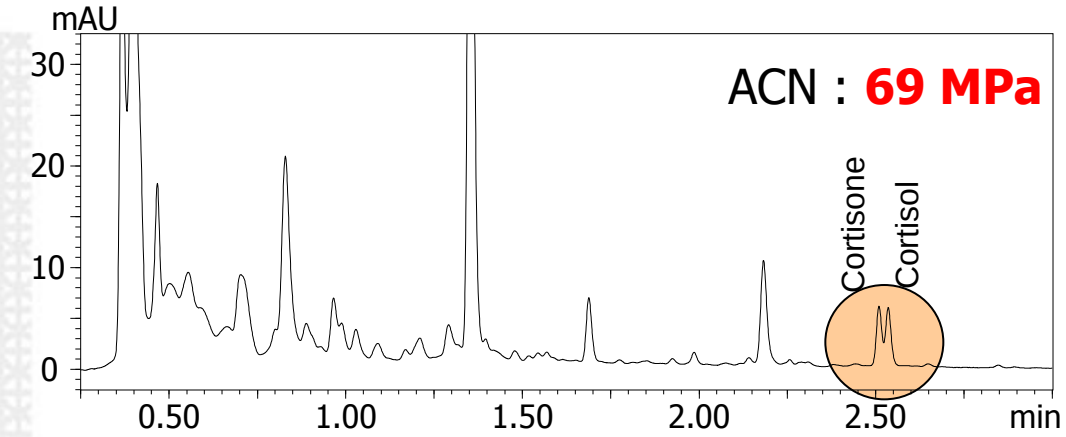
Higher pressure allows alternate solvent usage for selectivity purposes

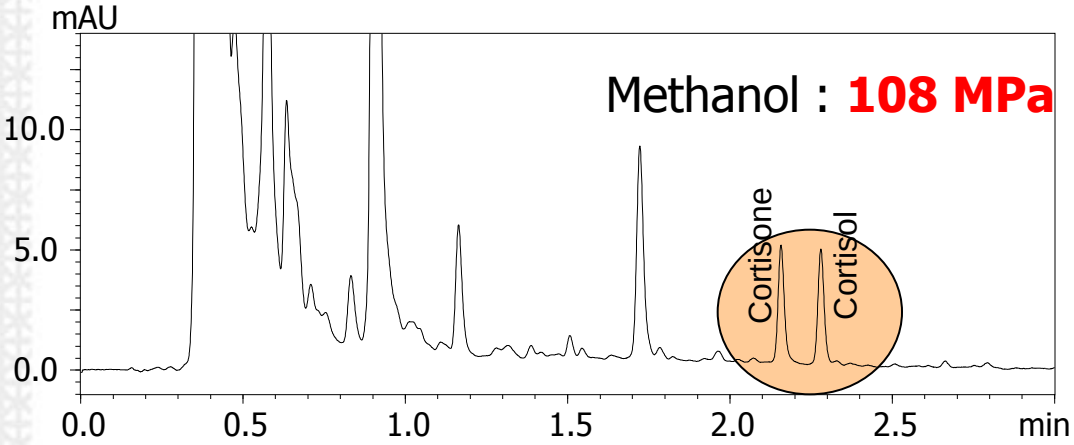


Mobile phase

: A :0.1 % formic acid in water

: B : **acetonitrile**

Methanol



Mobile phase

: A : 0.1 % formic acid in water

: B : **methanol**

Analysis of cortisone and cortisol in urine (each 5 ppm, spiked: UV @ 254nm)

Ultra-high Pressure Evaluation

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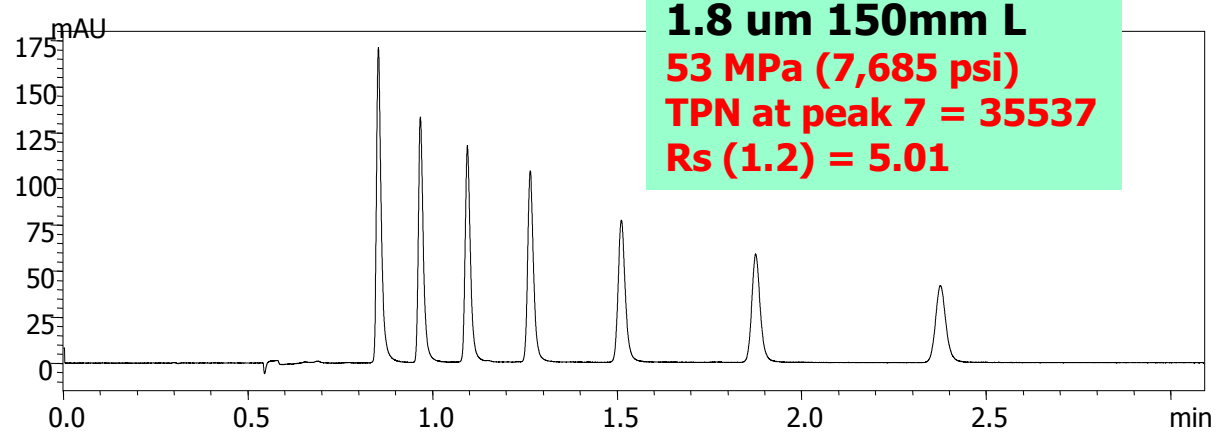
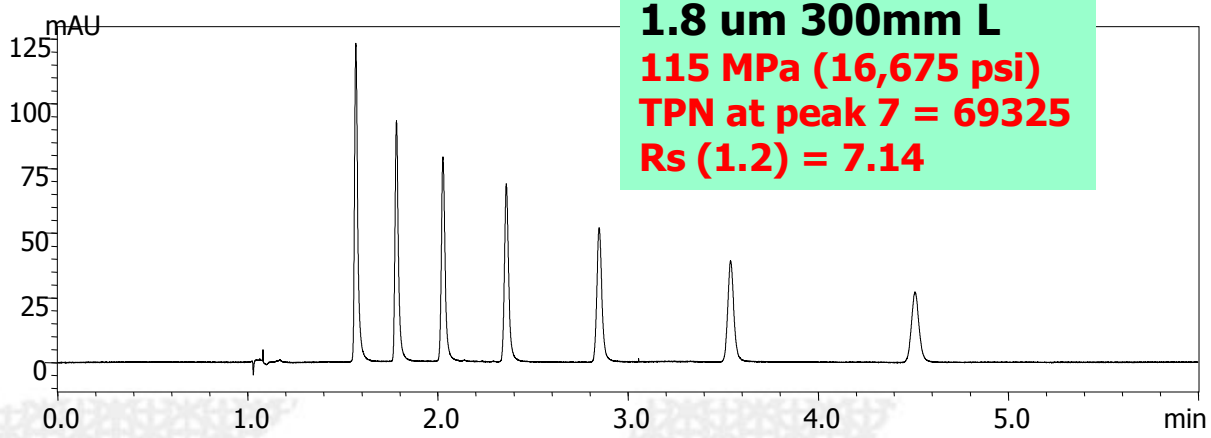
Theoretical plate number is not compromised at ultra-high pressures.



1.8 um 150mm L
53 MPa (7,685 psi)
TPN at peak 7 = 35537
Rs (1.2) = 5.01



1.8 um 300mm L
115 MPa (16,675 psi)
TPN at peak 7 = 69325
Rs (1.2) = 7.14

Zorbax eclipse plus C18 ODS
(2.1mmi.d. 150mm, 1.8 um)
0.5 mL/min
Water/ACN = 20/80
50°C
245nm

1. Acetophenone
2. Propiophenone
3. Butyrophenone
4. Valerophenone
5. Hexanophenone
6. Heptanophenone
7. Octanophenone

Zorbax eclipse plus C18 ODS
(2.1mmi.d. 300mm, 1.8 um)
0.5 mL/min
Water/ACN = 20/80
50°C
245nm

UHPLC vs. Conventional LC

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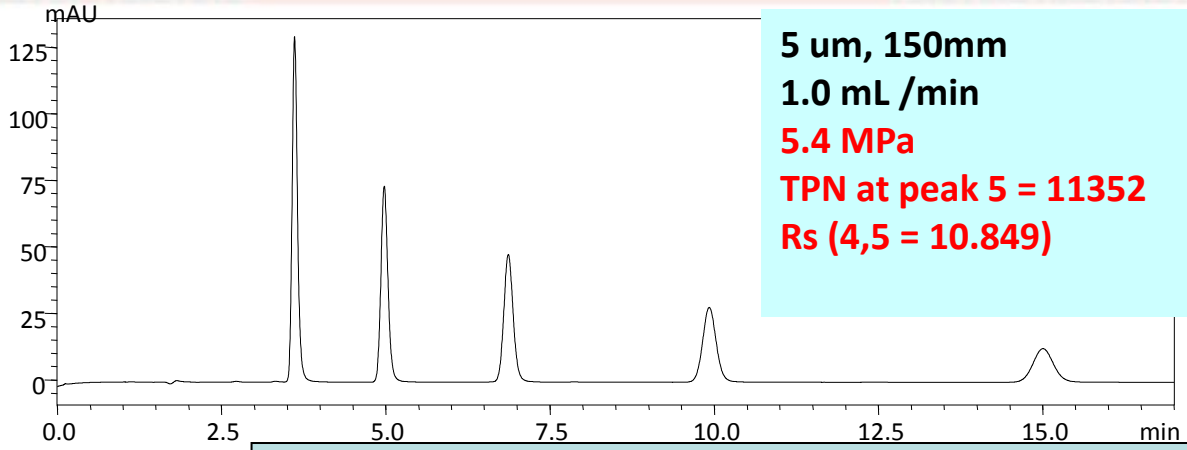
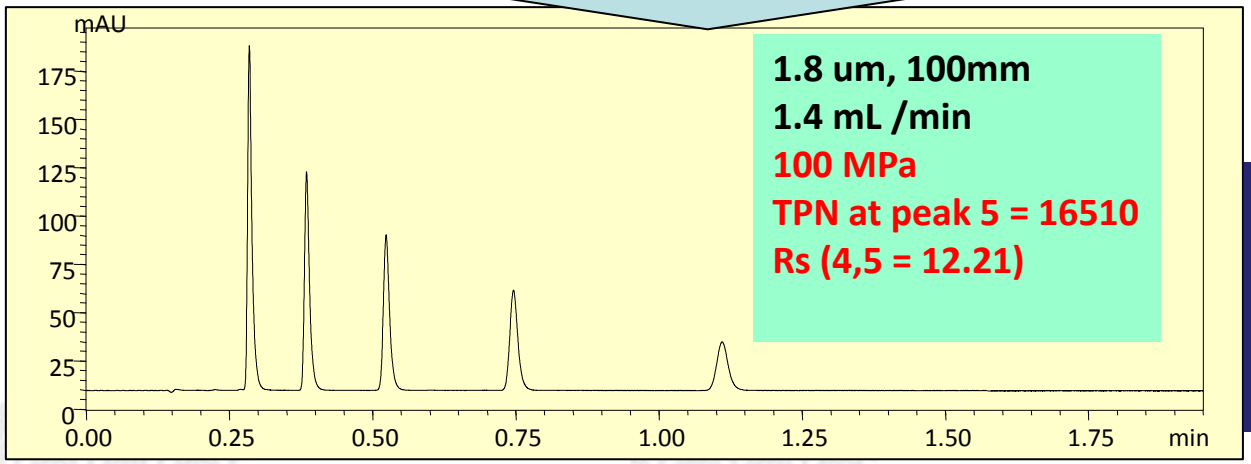


5 um, 150mm
1.0 mL /min
5.4 MPa
TPN at peak 5 = 11352
Rs (4,5 = 10.849)

Shim-pack VP-ODS
(4.6mmi.d.150mm, 5um)
1.0 mL/min
Water/acetonitrile = 45/55
40°C
245nm

14 times faster
1.5 times higher separation efficiency

1. Acetophenone
2. Propiophenone
3. Butyrophenone
4. Valerophenone
5. Hexanophenone



1.8 um, 100mm
1.4 mL /min
100 MPa
TPN at peak 5 = 16510
Rs (4,5 = 12.21)

Zorbax eclipse plus C18 RRHD
(2.1mmi.d. 100mm, 1.8 um)
1.4mL/min
Water/acetonitrile = 45/55
60 °C
245nm

Conclusions

- Similar results were obtained while decreasing organic mobile phase content and increasing column temperature.
- Reduction of the organic content of mobile phases can reduce analysis cost and waste toxicity.
- Use of a UHPLC system allowed doubling of column length to above 16,000 psi without loss of expected efficiency.
- Column heat balancing maintains column efficiency at temperatures up to 150°C.