New Technology for Environmental Assays – Improve Resolution, Run More Samples at Lower Cost

Pittcon 2008

Introduction – What are Some Issues with Typical Environmental HPLC Assays?

Lots of analytes need to be separated in one run.

This leads to long analysis times or less resolution.

In some cases multiple analyses – 2 different columns – may be recommended to complete the analysis.

Methods with only a few analytes may have compounds that are difficult to separate and more efficiency would improve resolution.

Analytes may have a wide range of polarity and gradient methods are often used. This can add complexity.

Sample preparation is typically required – can it be good enough to use sub 2-micron columns for these assays?

Can we use sub 2-micron column technology to resolve any of these issues?

➢Sub 2-micron technology can improve resolution and reduce analysis time.

➢Improved resolution can mean more analytes separated in one run and secondary columns may not be required.

➢Sufficient bonded phase choices need to be available to handle complex samples separated in gradient methods with varying polarity.

➢Adequate sample preparation can be done to use sub 2-micron columns – sample filtration may be required through a finer filter than is currently used.

➢In some cases a change from 5um to 3.5um particles may be preferred

Rapid Resolution HT Columns - What are they?

A group of ZORBAX HPLC columns that provide *fast separations with more resolving power*

- ZORBAX Rapid Resolution HT columns are a *New Technology*:
  - Sub 2µm (1.8µm), totally porous packing for ultra-fast separations.
  - The product line consists of the 400 bar column options, and 600 bar columns for use with the 1200 Series Rapid Resolution LC
- The small particles provide the high resolving power while very short column lengths (15-50mm) enable high-speed analyses.
- Ideal for ***High Throughput*** requirements while still maintaining resolution
- Longer columns (> 50mm) are ideal for separations requiring ***High Resolution*** or peak capacity

Environmental Methods Under Considerations

1. **PAH's** – typical methods are at least 16 PAH's, so sample complexity is high

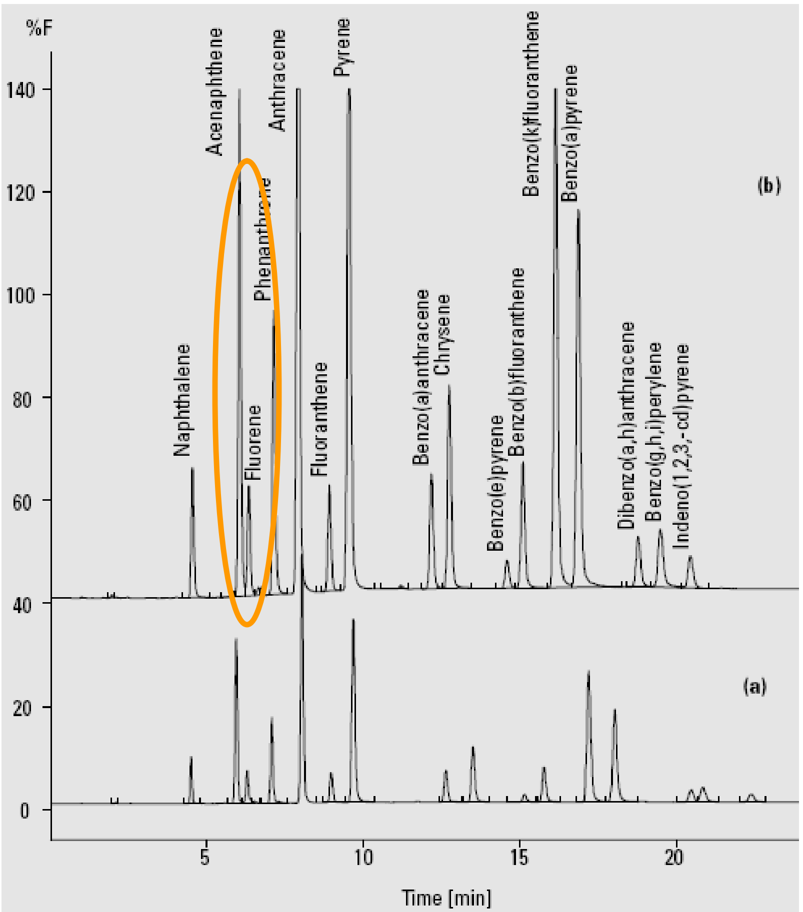
- Reduce method costs by speeding up separation
- Reduce method costs by reducing solvent use

2. **Explosives** – typical methods may call for 2 columns

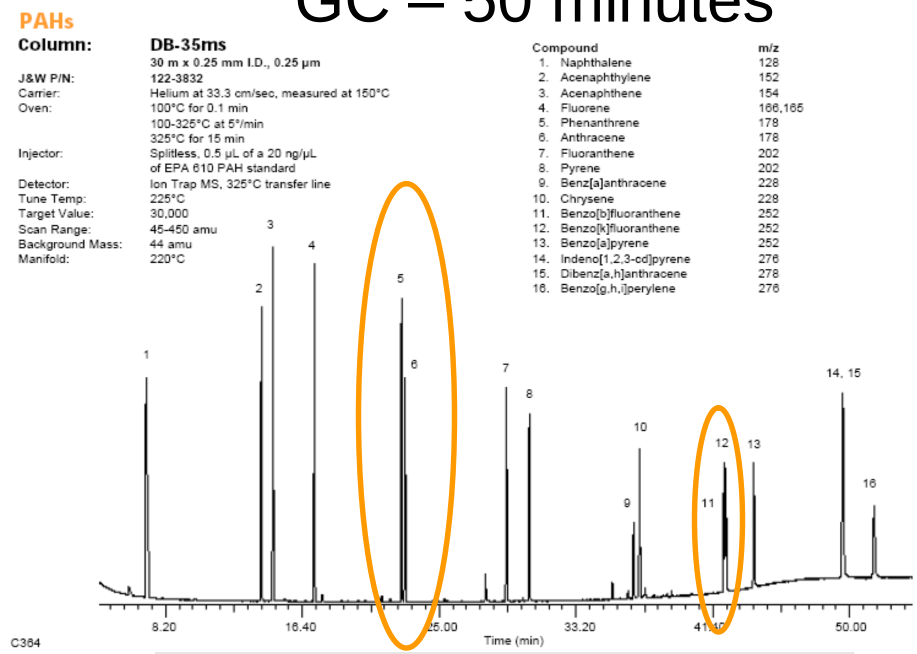
- Reduce method costs by separating analytes completely on one column

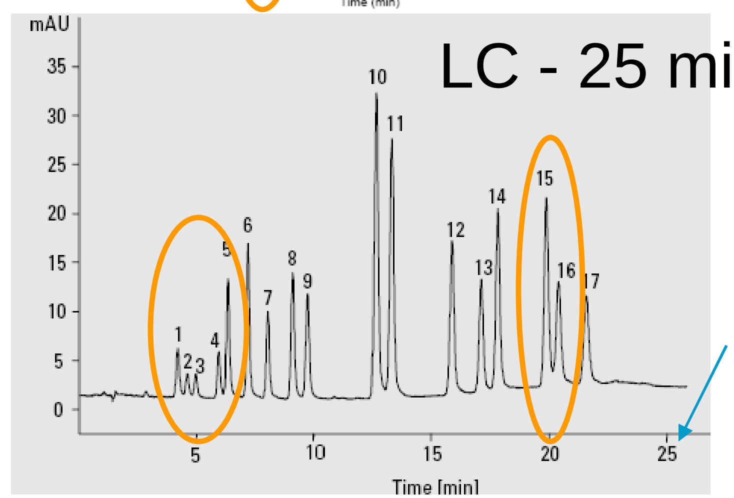
Typical LC and GC Analyses of EPA Priority Pollutants

LC - 25 min



GC – 50 minutes





LC - 25 min

PAH Column Choices – Balance Analysis Time, Solvent Use

1. A 250mm, 5um column is the most traditional column for the analysis of PAHs.

- The 4.6 mm ID will use the most solvent.
- The 3.0 mm ID will use 50% less solvent for the same analysis time.

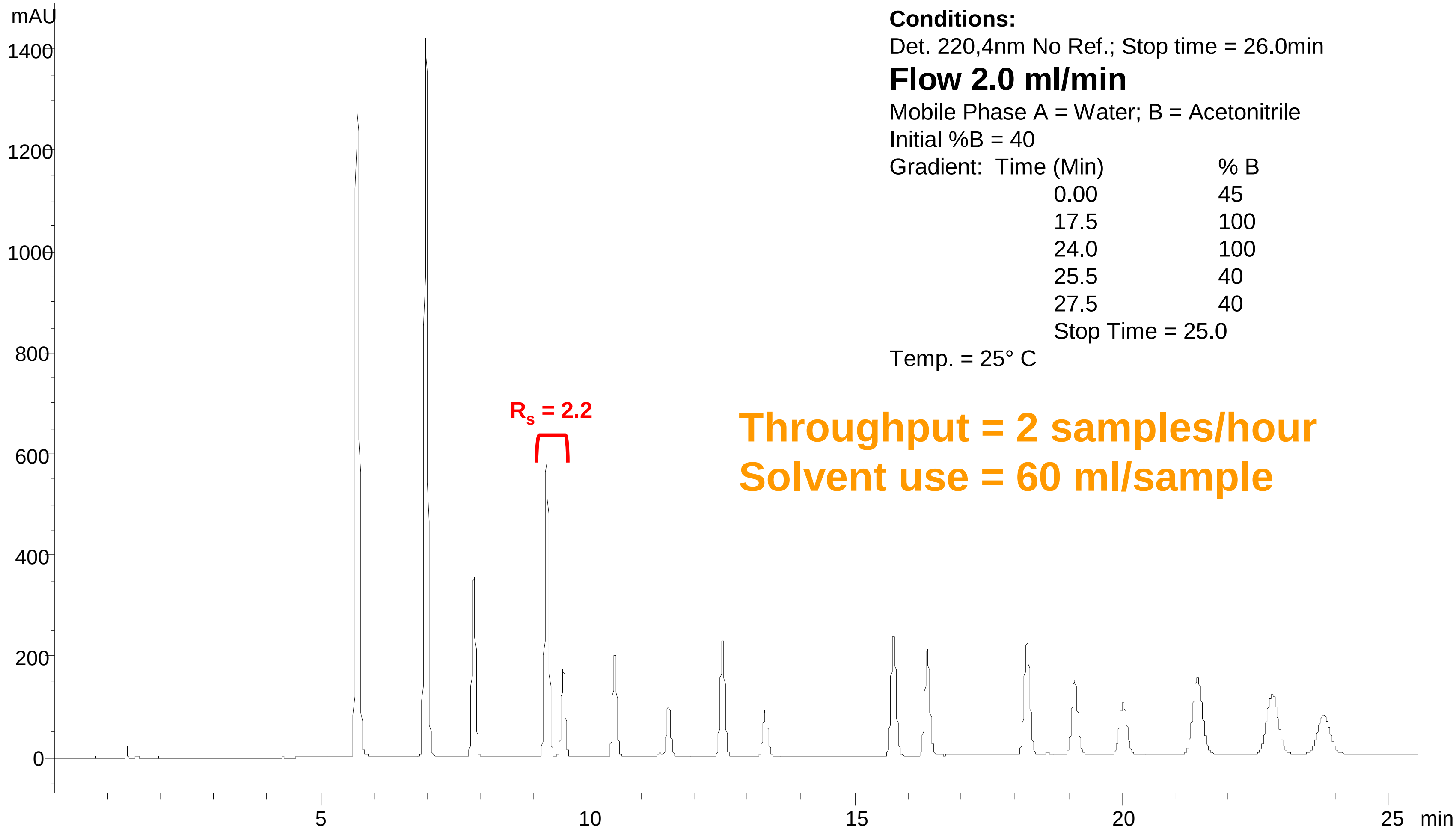
2. Shorter columns with smaller particle sizes – 3.5um or 1.8um – allow for faster analyses for higher throughput and increased productivity.

Choose Column Dimension for Time, Resolution

#	Column Description	Size	Analysis Time	Rs (5,4)
1	Analytical	4.6 x 250mm, 5um	25 min	2.2
2	Analytical	4.6 x 150mm, 5um	20 min	2.0
3	Analytical	4.6 x 100mm, 5um	19 min	2.2
4	Rapid Resolution	4.6 x 150mm, 3.5um	19 min	2.6
5	Rapid Resolution	4.6 x 100mm, 3.5um	14 min	2.4
6	Rapid Resolution	4.6 x 50mm, 3.5um	11 min	2.0
7	Rapid Resolution HT	4.6 x 100mm, 1.8um	16 min	3.6
8	Rapid Resolution HT	4.6 x 50mm, 1.8um	5 min	2.0
9	Rapid Resolution HT	4.6 x 30mm, 1.8um	2 min	1.7
10	Solvent Saver	3.0 x 250mm, 5um	27 min	2.1
11	Narrow Bore	2.1 x 250mm, 5um	27 min	2.0
12	Narrow Bore	2.1 x 150mm, 5um	25 min	2.5
13	Narrow Bore RR	2.1 x 100mm, 3.5um	16 min	2.5
14	Narrow Bore RRHT	2.1 x 100mm, 1.8um	16 min	3.6
15	Narrow Bore RRHT	2.1 x 50mm, 1.8um	7 min	2.1

- **Max resolution with 100mm, 1.8um – but would have max pressure as well – above 400 bar.**
- **For max resolution below 400 bar – choose 4.6 x 150mm, 3.5um**
- **For shortest time choose 4.6 x 50mm, 1.8um – pressure below 400 bar**
- **For screening choose 4.6 x 30mm, 1.8um – pressure only 150 bar and 2 minutes!!**
- **Choose 3.0 mm ID columns for 50% solvent savings and reduced costs.**

Traditional Separation of EPA 610 PAH Mix on 4.6x250mm, 5.0µm Eclipse PAH Column



Conditions:

Det. 220,4nm No Ref.; Stop time = 26.0min

Flow 2.0 ml/min

Mobile Phase A = Water; B = Acetonitrile

Initial %B = 40

Gradient: Time (Min)	% B
0.00	45
17.5	100
24.0	100
25.5	40
27.5	40

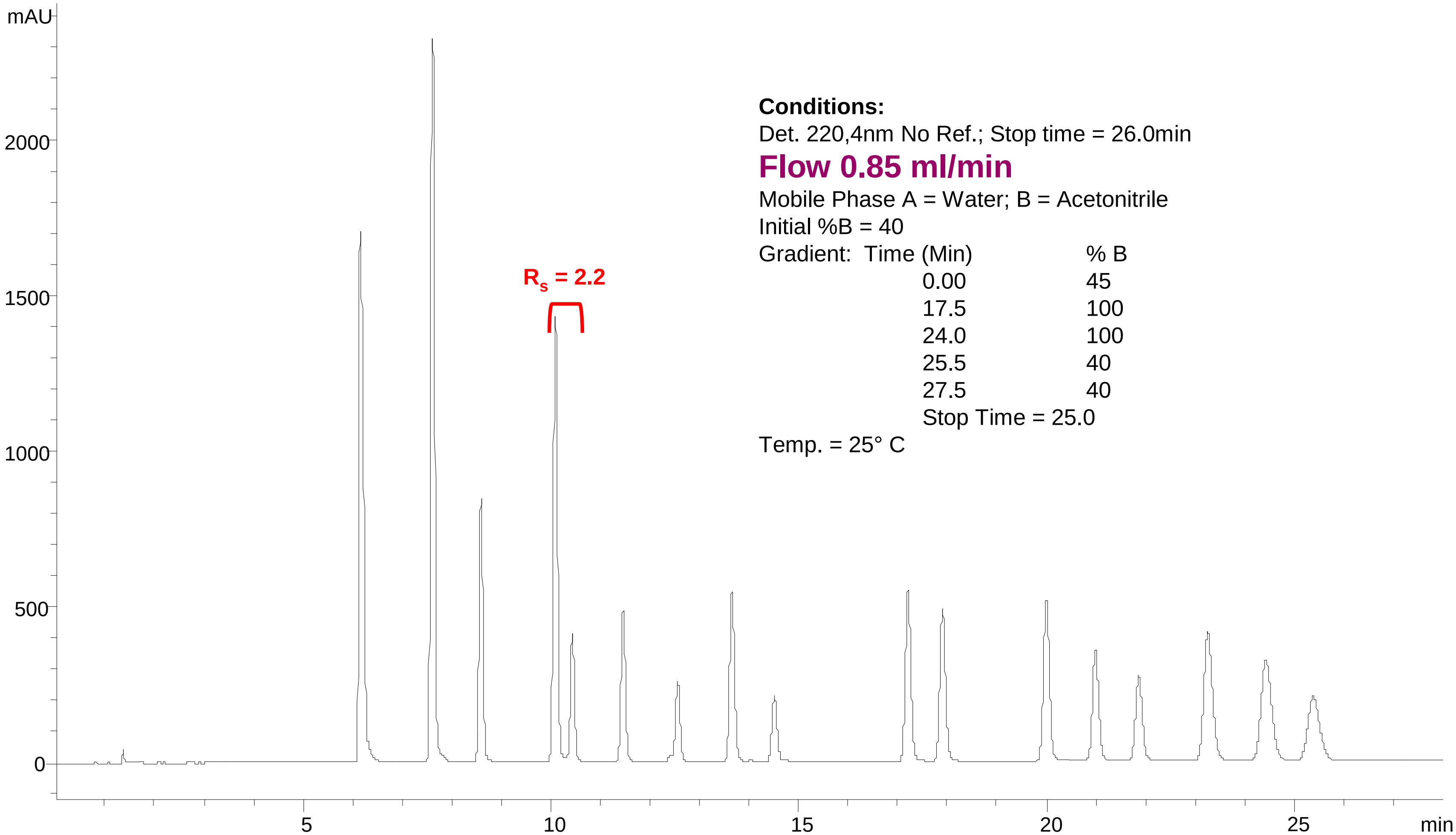
Stop Time = 25.0

Temp. = 25° C

Throughput = 2 samples/hour
Solvent use = 60 ml/sample

A traditional PAH separation, with a typical 25 min analysis time on a long 5um column.

Separation of EPA 610 PAH Mix on 3.0x250mm, 5.0µm Eclipse PAH Column – 55% Less Solvent Used with 3.0mm ID



Conditions:

Det. 220,4nm No Ref.; Stop time = 26.0min

Flow 0.85 ml/min

Mobile Phase A = Water; B = Acetonitrile

Initial %B = 40

Gradient:

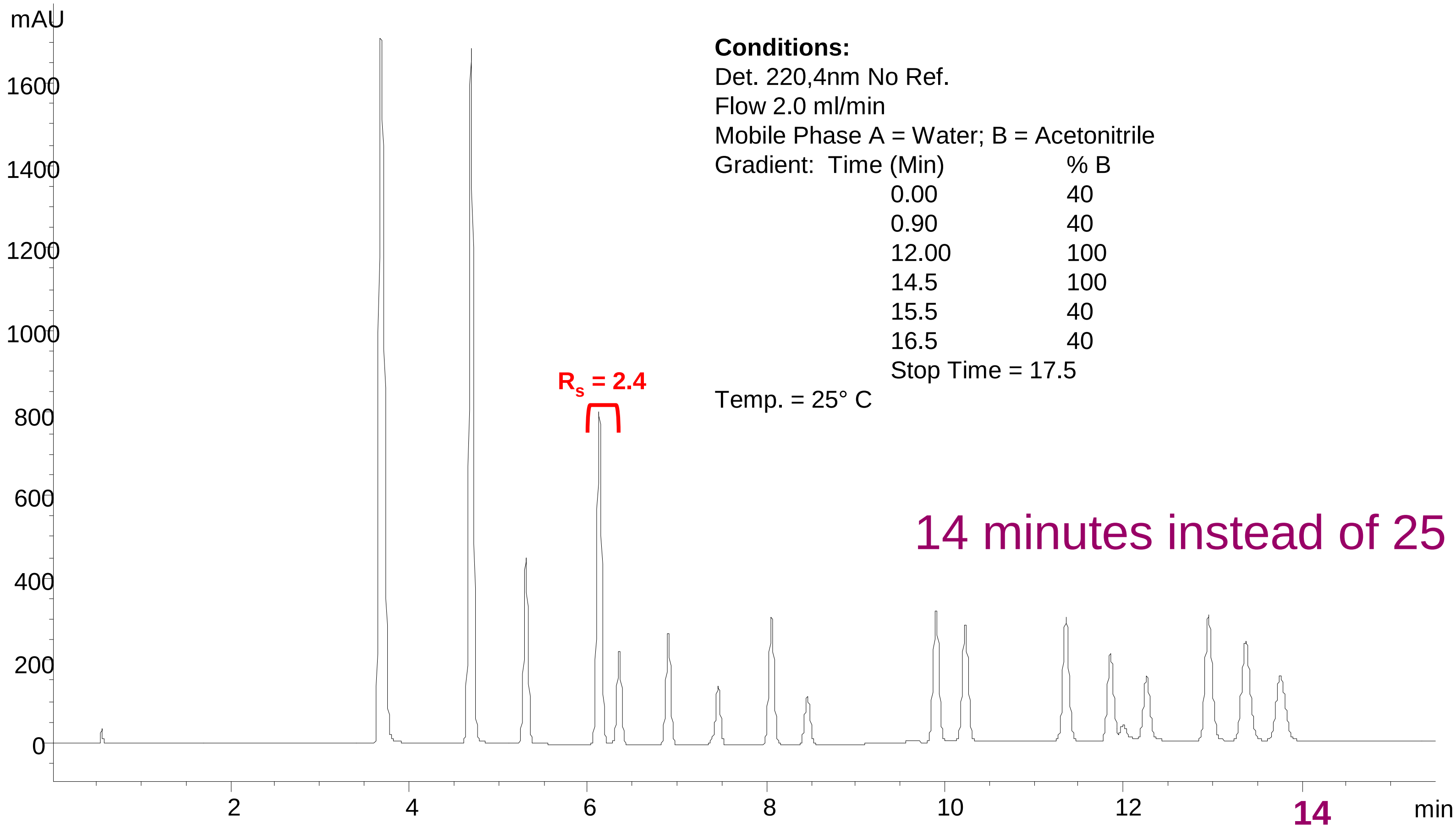
Time (Min)	% B
0.00	45
17.5	100
24.0	100
25.5	40
27.5	40

Stop Time = 25.0

Temp. = 25° C

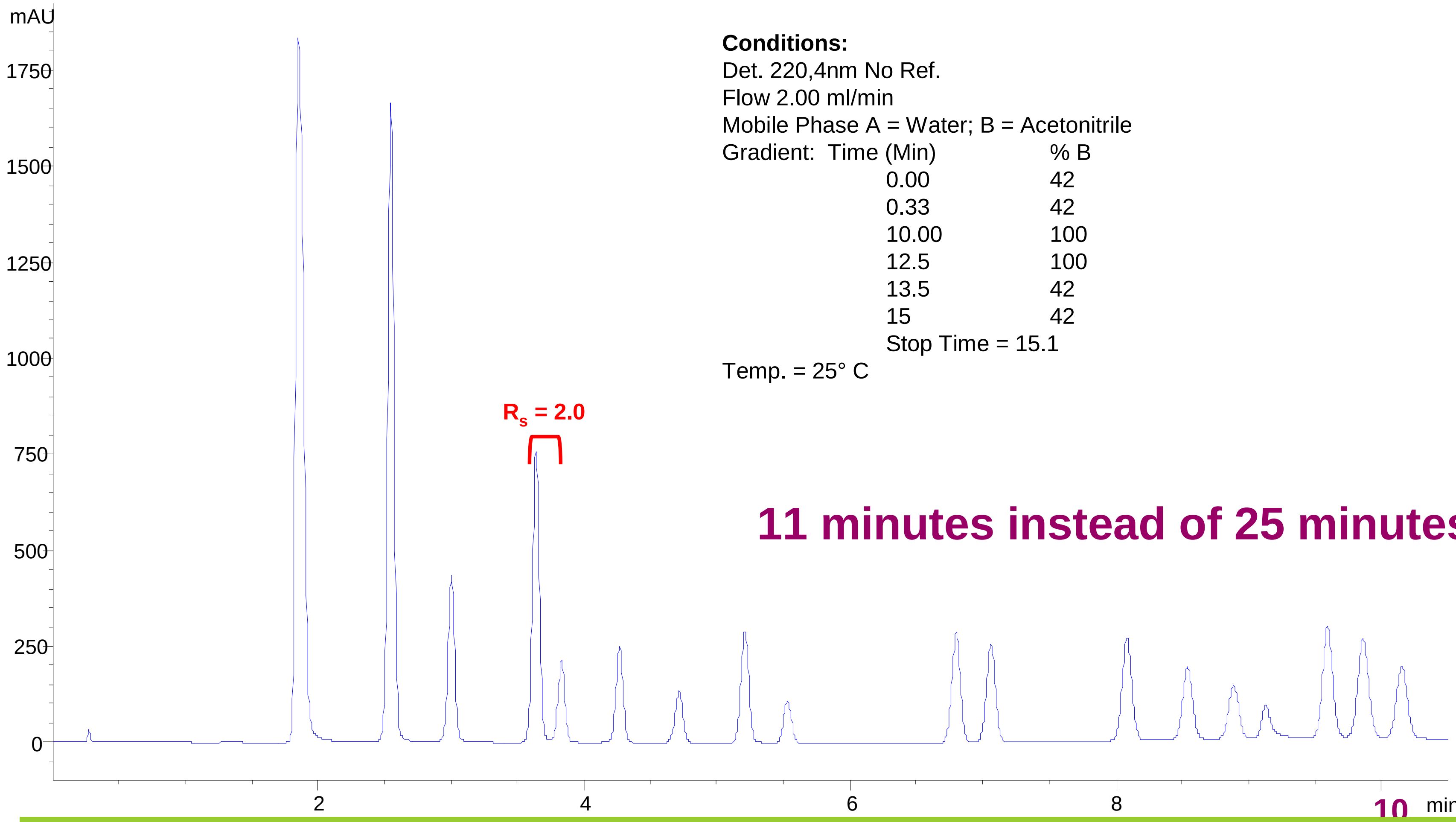
Many contract labs are cost sensitive and changing to a Solvent Saver column and reducing solvent use by 55% is an easy way to reduce costs!

Faster Separation of 16 PAH's 4.6x**100** mm, 3.5µm Eclipse PAH Column



A faster separation is another way to improve productivity and reduce solvent costs. The 100mm, 3.5um column would reduce solvent use by 45% and improves resolution.

PAH 610 Mix on 4.6x50mm, 3.5µm PAH Column – Fast with Excellent Resolution



Conditions:
Det. 220,4nm No Ref.
Flow 2.00 ml/min
Mobile Phase A = Water; B = Acetonitrile

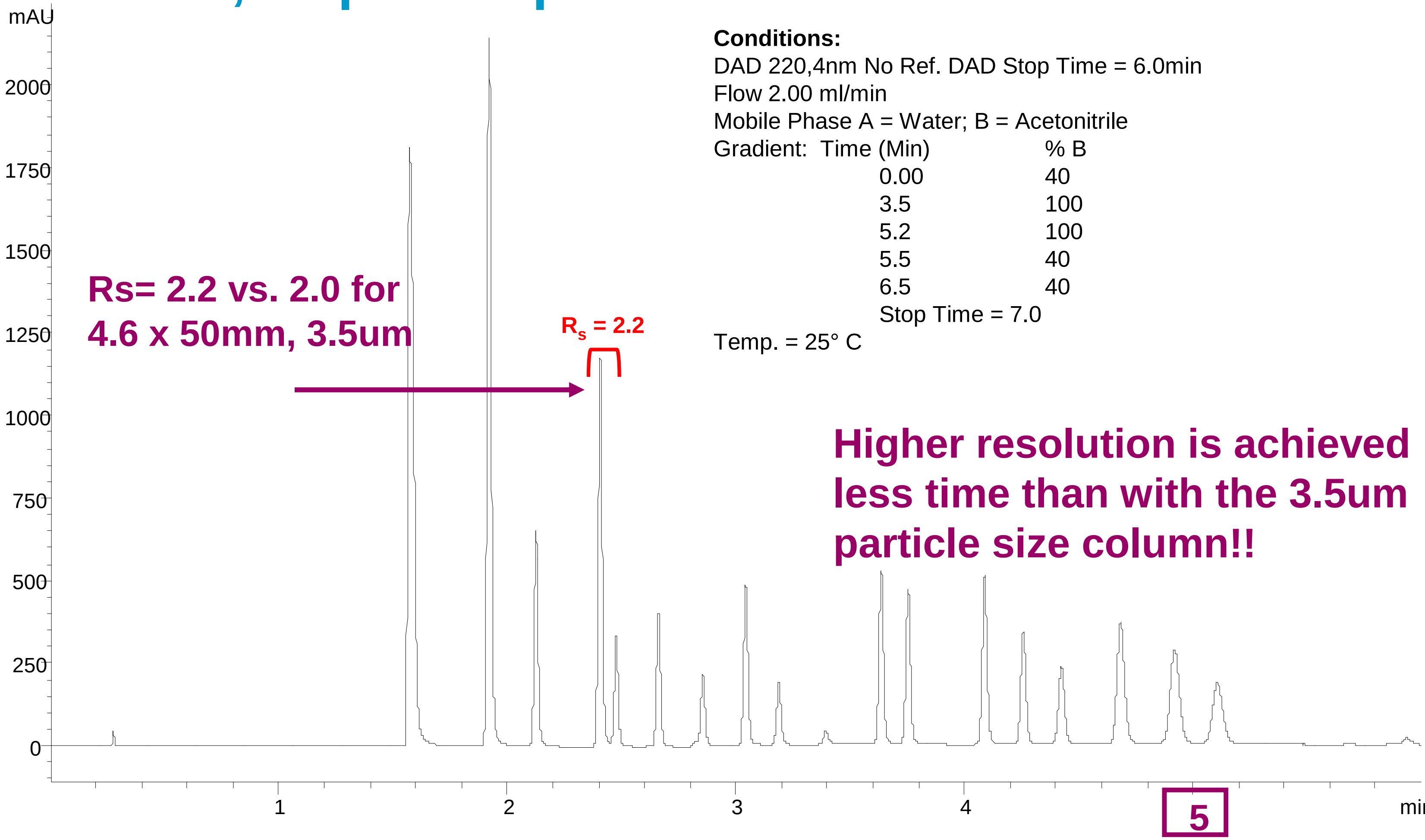
Gradient: Time (Min)	% B
0.00	42
0.33	42
10.00	100
12.5	100
13.5	42
15	42

Stop Time = 15.1
Temp. = 25° C

11 minutes instead of 25 minutes!

Speed, productivity can be increased more with a short column.
Resolution is still excellent and this column size can be used successfully on any LC.

High Resolution and Fast Analysis on Rapid Resolution HT 4.6x50mm, 1.8µm Eclipse PAH Column



Conditions:
DAD 220,4nm No Ref. DAD Stop Time = 6.0min
Flow 2.00 ml/min
Mobile Phase A = Water; B = Acetonitrile

Gradient:

Time (Min)	% B
0.00	40
3.5	100
5.2	100
5.5	40
6.5	40

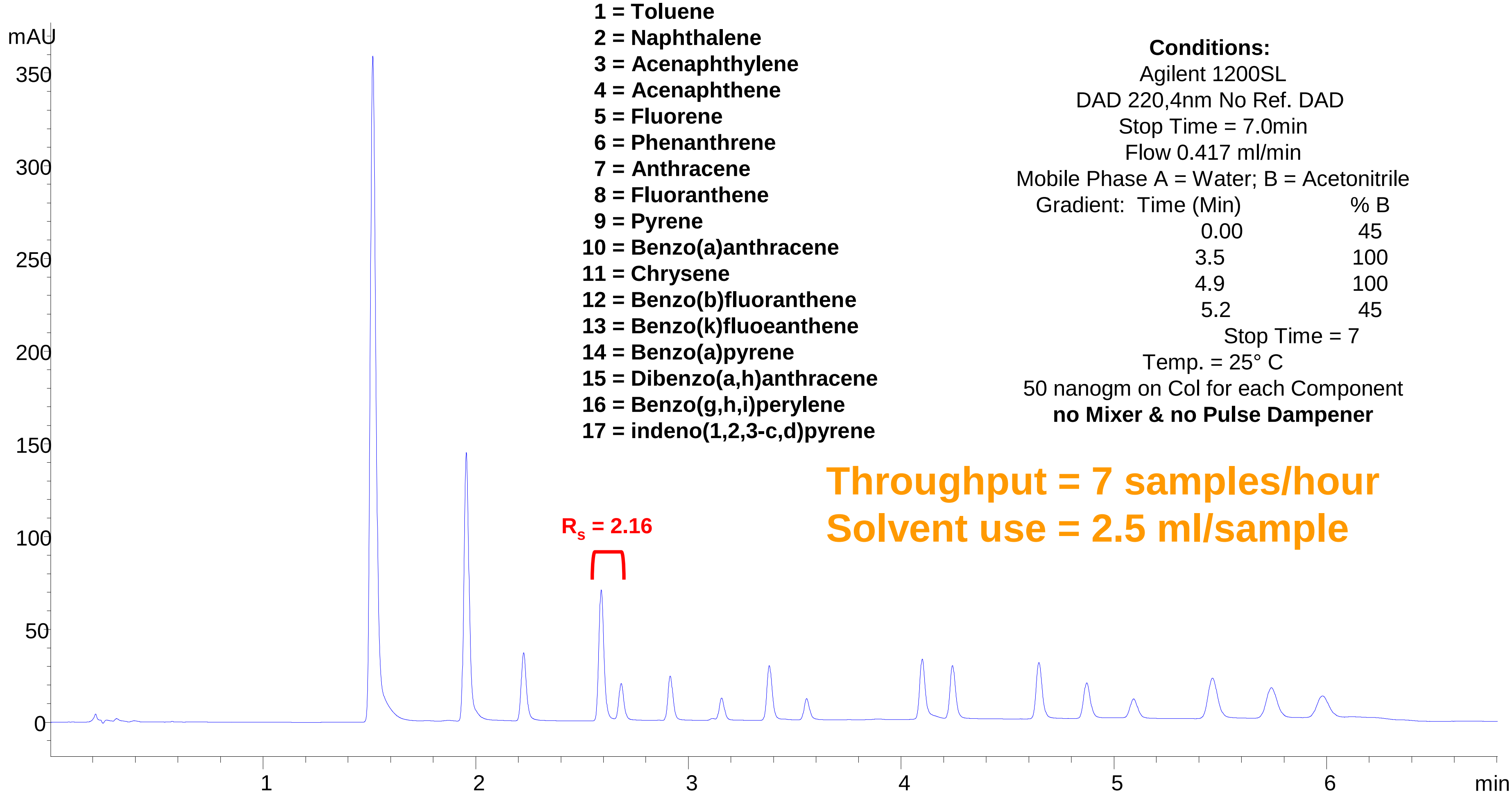
Stop Time = 7.0

Temp. = 25° C

Higher resolution is achieved in less time than with the 3.5um particle size column!!

The RRHT (1.8um) column is the ideal choice for the fastest analysis with resolution equivalent to the 250mm, 5um column.

Narrow Bore RRHT Eclipse PAH 2.1x50mm, 1.8µm PAH Column – Maximum Solvent Savings, 2.4 mL/run



1 = Toluene
2 = Naphthalene
3 = Acenaphthylene
4 = Acenaphthene
5 = Fluorene
6 = Phenanthrene
7 = Anthracene
8 = Fluoranthene
9 = Pyrene
10 = Benzo(a)anthracene
11 = Chrysene
12 = Benzo(b)fluoranthene
13 = Benzo(k)fluoeanthene
14 = Benzo(a)pyrene
15 = Dibenzo(a,h)anthracene
16 = Benzo(g,h,i)perylene
17 = indeno(1,2,3-c,d)pyrene

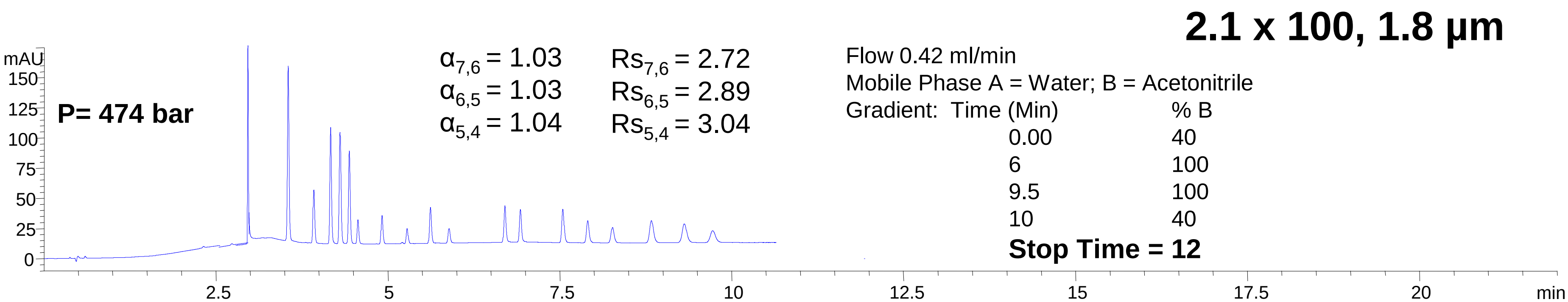
Conditions:
Agilent 1200SL
DAD 220,4nm No Ref. DAD
Stop Time = 7.0min
Flow 0.417 ml/min
Mobile Phase A = Water; B = Acetonitrile

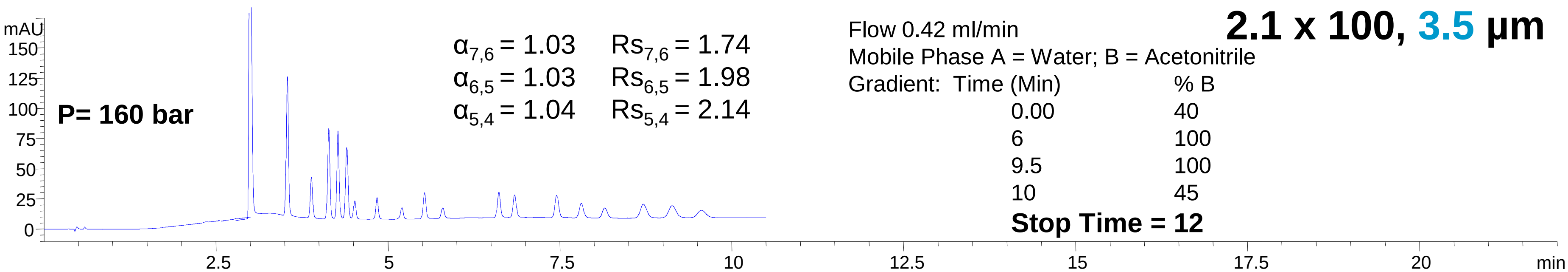
Gradient: Time (Min)	% B
0.00	45
3.5	100
4.9	100
5.2	45

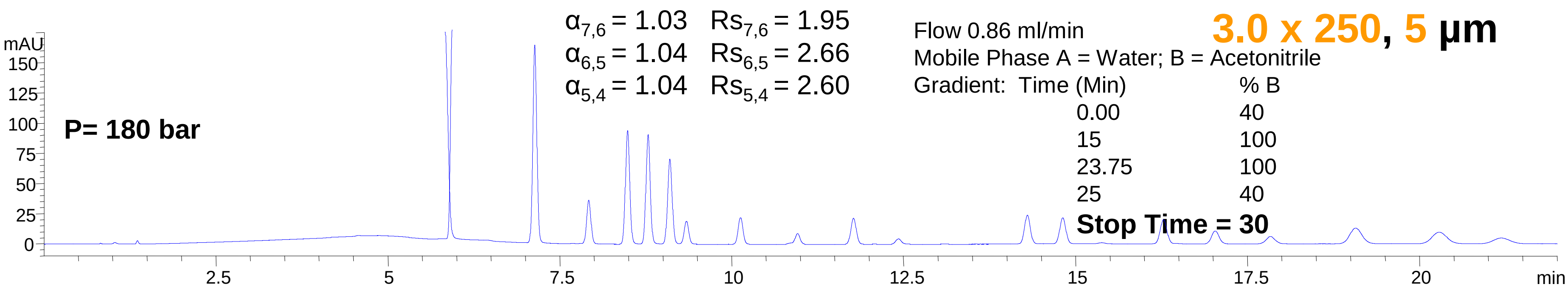
Stop Time = 7
Temp. = 25° C
50 nanogm on Col for each Component
no Mixer & no Pulse Dampener

Throughput = 7 samples/hour
Solvent use = 2.5 ml/sample

Eclipse PAH Scalability is Excellent - Choose column ID, length and particle size for Rs and Time







What Type of Flexibility Do We Have for Other PAH Methods?

Increasing regulation may require more than 16 PAHS be separated.

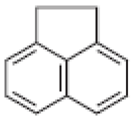
Sub 2-micron columns can enhance resolution for samples with 17 and more PAHs.

- Method with 17 PAHs
- Methods with 18 PAHs
- Methods with 24 PAHs

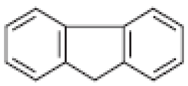
Sub 2-micron columns can speed up separations with samples that have a limited number of PAHs

17 PAHs – PAH's include 5 pairs of Isomers that are Separated by Shape

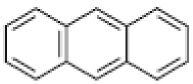
Acenaphthene



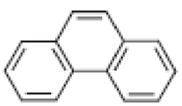
Fluorene



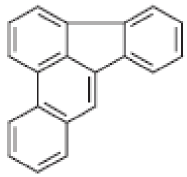
Anthracene



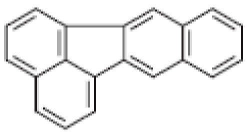
Phenanthrene



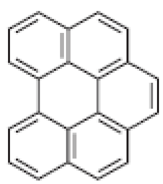
Benzo(b)flouranthene



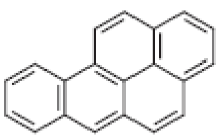
Benzo(k)flouranthene



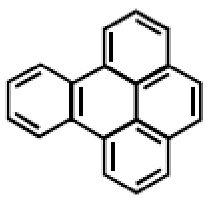
Benzo(g,h,i)perylene



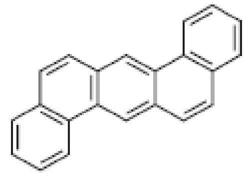
Benzo(a)pyrene



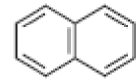
Benzo(e)pyrene



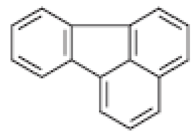
Dibenz(a,h)anthracene



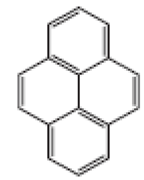
Naphthalene



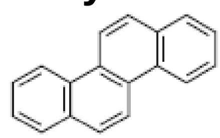
Fluoranthene



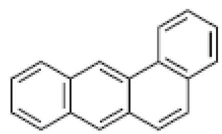
Pyrene



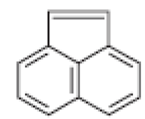
Chrysene



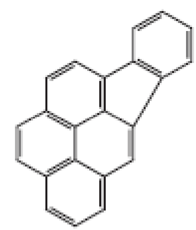
Benz(a)anthracene



Acenaphthylene



Indeno(1,2,3-c,d)pyrene



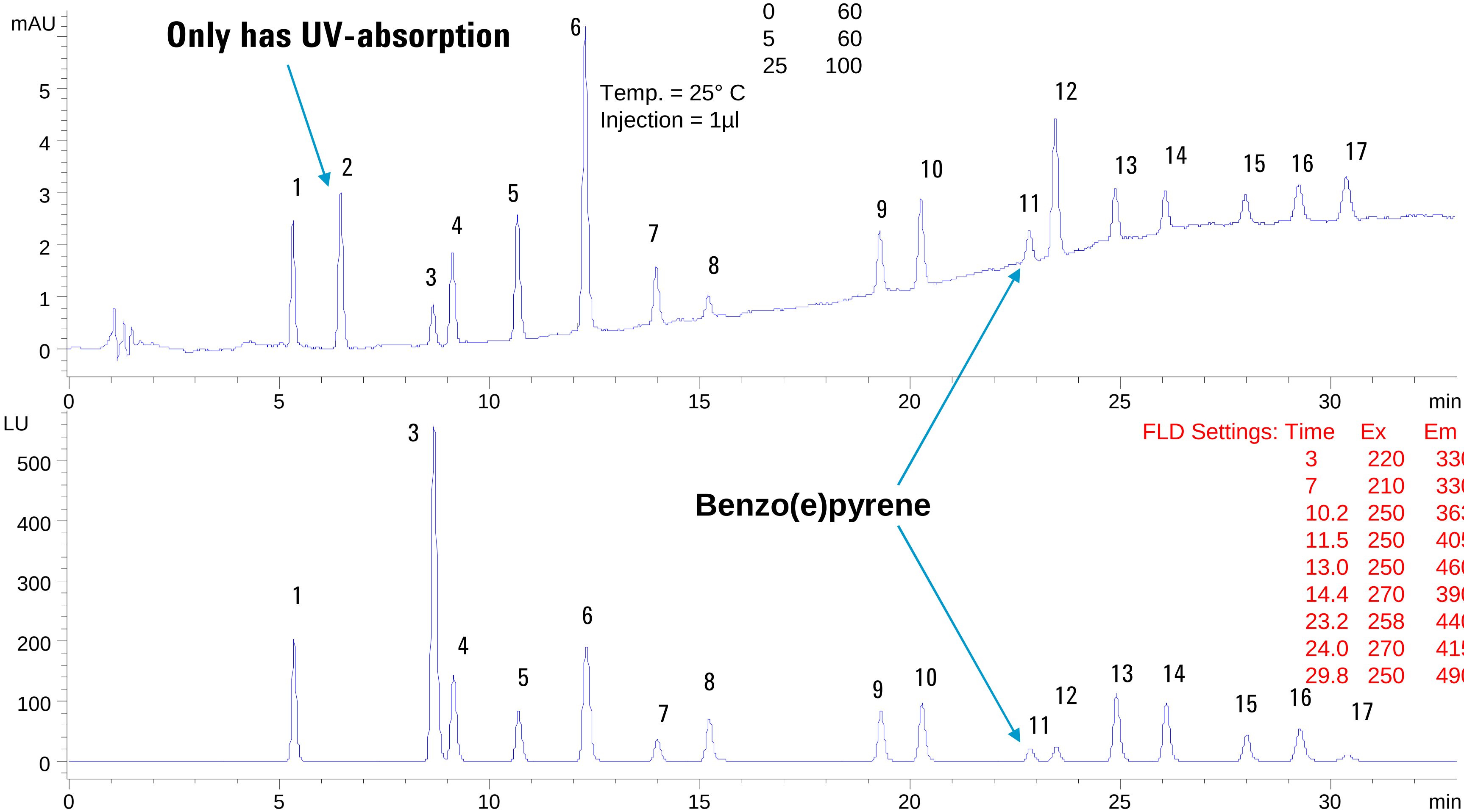
17 PAHs (standard 16 plus B(e)p) on Eclipse PAH 4.6x150mm, 3.5um

Conditions:
DAD 254,4nm No Ref. ; Stop Time = 35min; Flow 1.00 ml/min
Mobile Phase A = Water; B = ACN

Gradient:

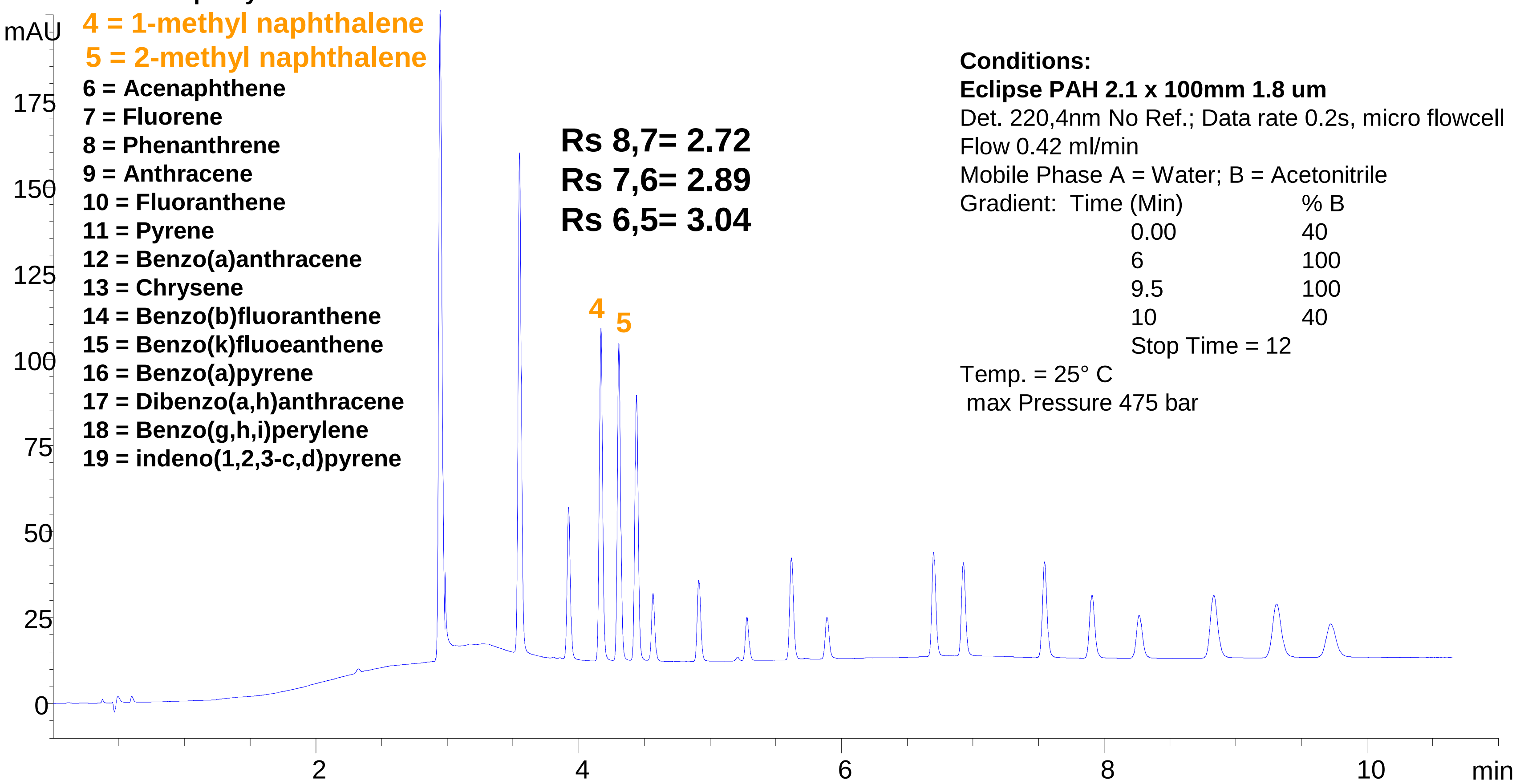
Time (Min)	% B
0	60
5	60
25	100

Temp. = 25° C
Injection = 1µl



FLD Settings: Time	Ex	Em	PMT
3	220	330	10
7	210	330	12
10.2	250	363	12
11.5	250	405	12
13.0	250	460	12
14.4	270	390	12
23.2	258	440	10
24.0	270	415	12
29.8	250	490	12

PAH Analysis for Florida Administrative Code 17.700



Conditions:
Eclipse PAH 2.1 x 100mm 1.8 um
Det. 220,4nm No Ref.; Data rate 0.2s, micro flowcell
Flow 0.42 ml/min
Mobile Phase A = Water; B = Acetonitrile

Gradient:

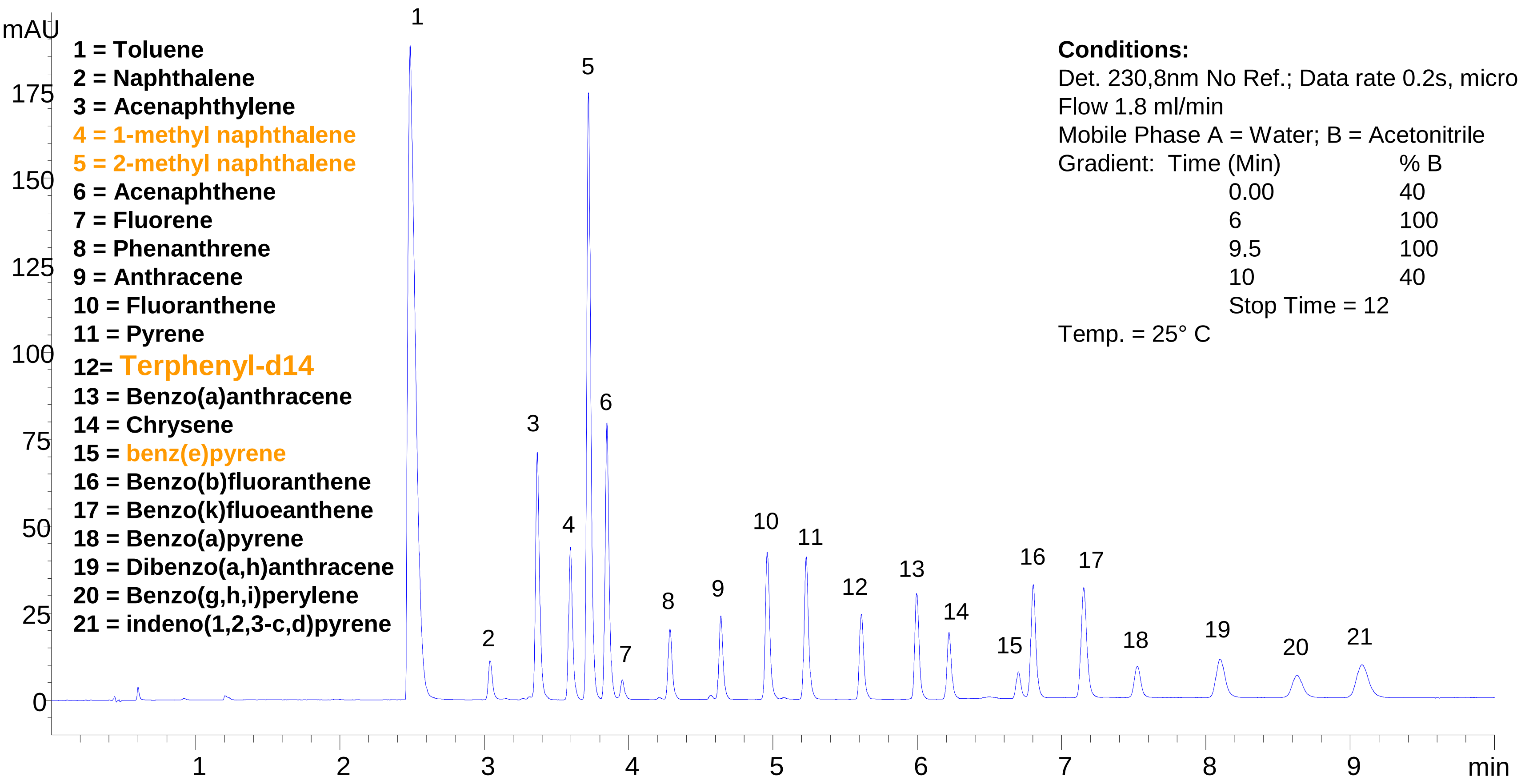
Time (Min)	% B
0.00	40
6	100
9.5	100
10	40

Stop Time = 12
Temp. = 25° C
max Pressure 475 bar

Florida PAH mix contains two other PAHs in addition to the EPA 16 priority pollutants

“PAH 20” mix

Eclipse PAH 4.6x 100mm, 1.8 um



1 = Toluene
2 = Naphthalene
3 = Acenaphthylene
4 = 1-methyl naphthalene
5 = 2-methyl naphthalene
6 = Acenaphthene
7 = Fluorene
8 = Phenanthrene
9 = Anthracene
10 = Fluoranthene
11 = Pyrene
12= Terphenyl-d14
13 = Benzo(a)anthracene
14 = Chrysene
15 = benz(e)pyrene
16 = Benzo(b)fluoranthene
17 = Benzo(k)fluoeanthene
18 = Benzo(a)pyrene
19 = Dibenzo(a,h)anthracene
20 = Benzo(g,h,i)perylene
21 = indeno(1,2,3-c,d)pyrene

Conditions:
Det. 230,8nm No Ref.; Data rate 0.2s, micro flowcell
Flow 1.8 ml/min
Mobile Phase A = Water; B = Acetonitrile

Gradient: Time (Min)	% B
0.00	40
6	100
9.5	100
10	40

Stop Time = 12
Temp. = 25° C

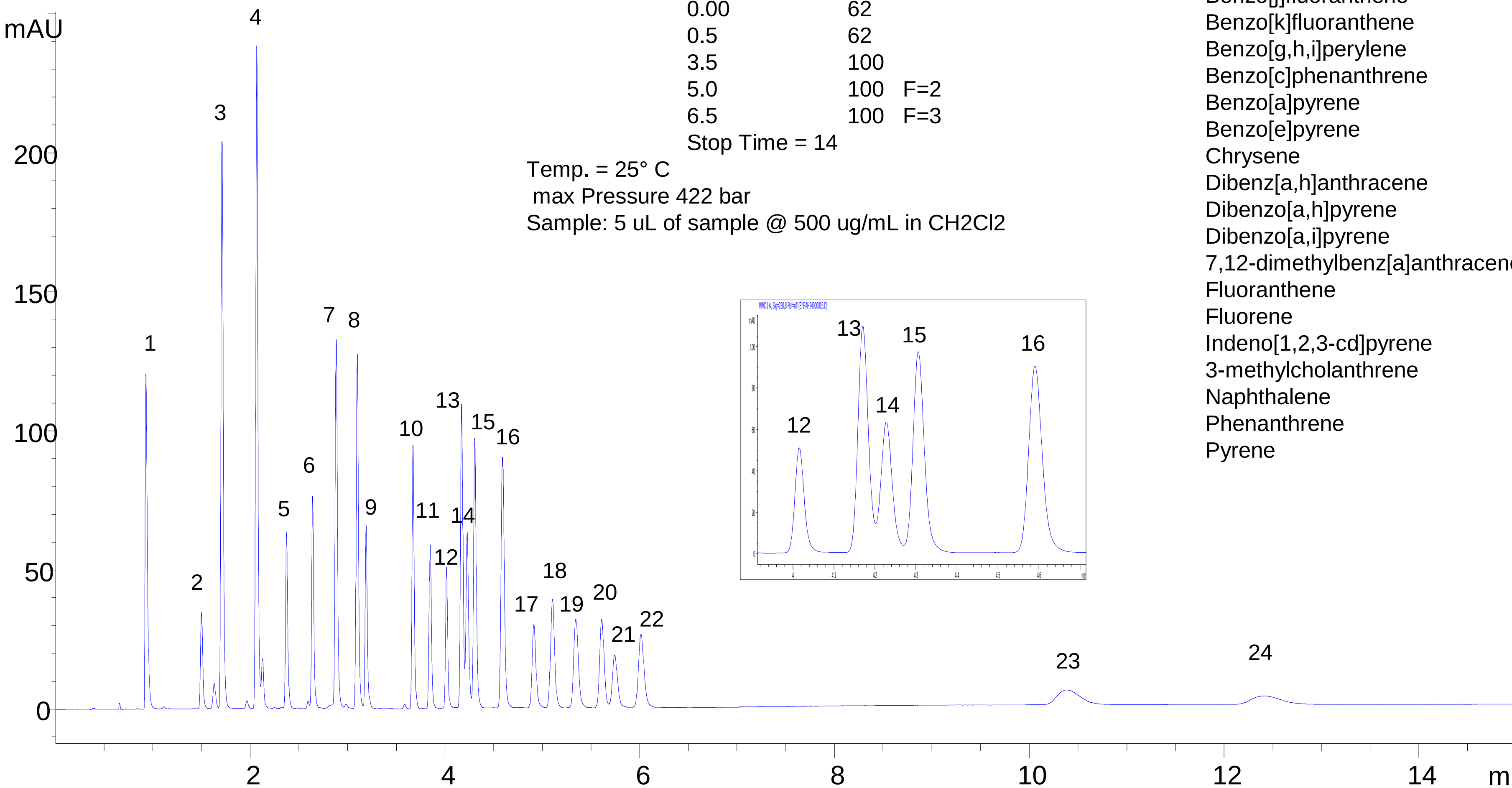
RRHT Quebec Ministry of Environment PAH Std.
(24 PAHs)

Conditions:
Eclipse PAH 4.6 x 100mm 1.8 um
Det. 230,8nm No Ref.; Data rate 0.2s, micro flowcell
Flow 2.0 ml/min- 3 mL/ min
Mobile Phase A = Water; B = Acetonitrile

Gradient: Time (Min)	% B	
0.00	62	
0.5	62	
3.5	100	
5.0	100	F=2
6.5	100	F=3

Stop Time = 14
Temp. = 25° C
max Pressure 422 bar
Sample: 5 uL of sample @ 500 ug/mL in CH2Cl2

Acenaphthene
Acenapththylene
Anthracene
Benz[a]anthracene
Benzo[b]fluoranthene
Benzo[j]fluoranthene
Benzo[k]fluoranthene
Benzo[g,h,i]perylene
Benzo[c]phenanthrene
Benzo[a]pyrene
Benzo[e]pyrene
Chrysene
Dibenz[a,h]anthracene
Dibenzo[a,h]pyrene
Dibenzo[a,i]pyrene
7,12-dimethylbenz[a]anthracene
Fluoranthene
Fluorene
Indeno[1,2,3-cd]pyrene
3-methylcholanthrene
Naphthalene
Phenanthrene
Pyrene



Low Pressure Quebec Ministry of Environment PAH Std. (24 compounds)

Conditions:

Eclipse PAH 4.6 x 150mm 3.5 um

Det. 230,8nm No Ref.; Data rate 0.2s, micro flowcell

Flow 2.0 ml/min- 3 mL/ min

Mobile Phase A = Water; B = Acetonitrile

Gradient:

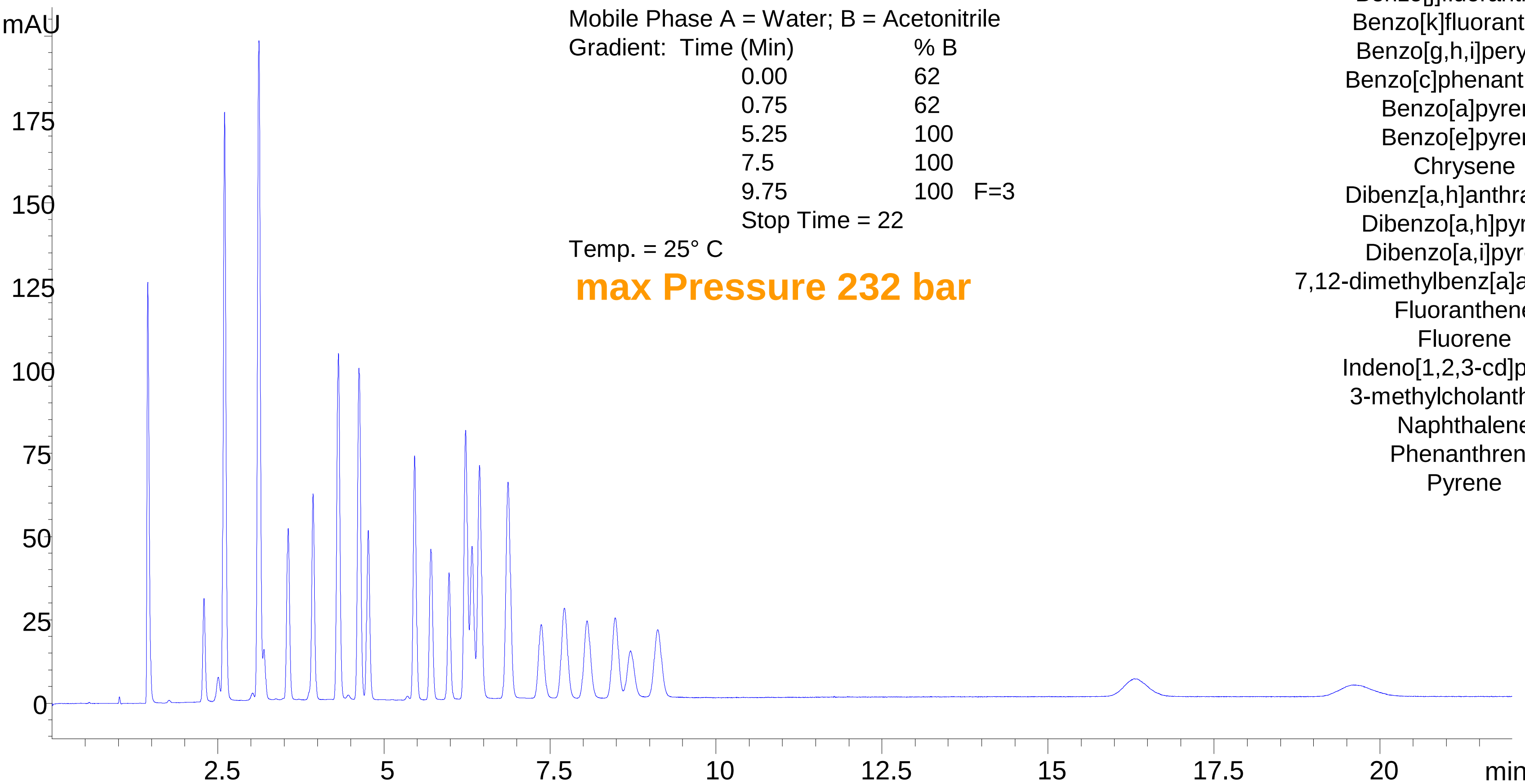
Time (Min)	% B	
0.00	62	
0.75	62	
5.25	100	
7.5	100	
9.75	100	F=3

Stop Time = 22

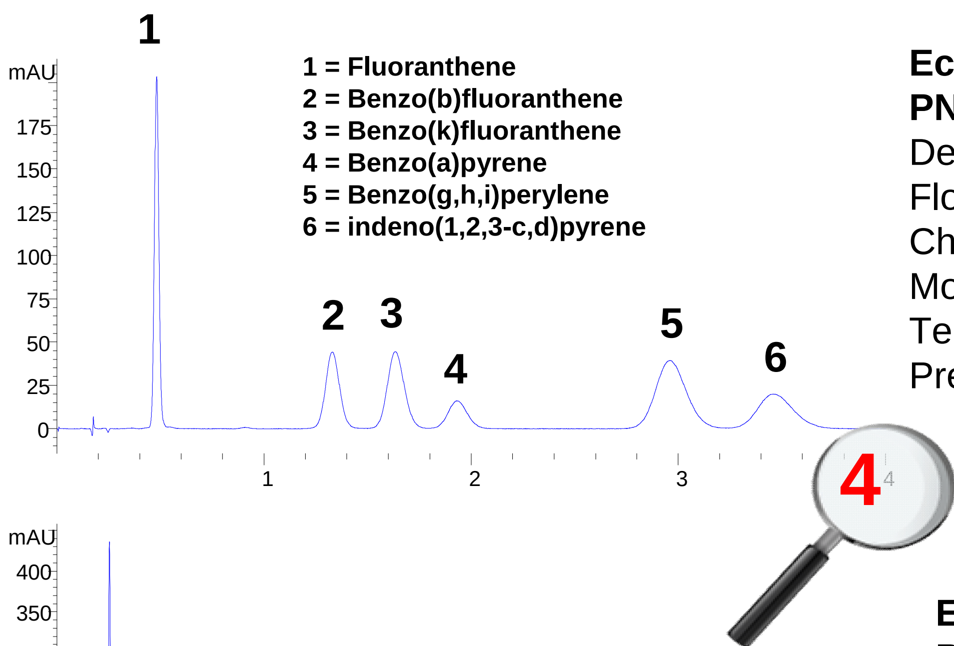
Temp. = 25° C

max Pressure 232 bar

Acenaphthene
Acenapththylene
Anthracene
Benz[a]anthracene
Benzo[b]fluoranthene
Benzo[j]fluoranthene
Benzo[k]fluoranthene
Benzo[g,h,i]perylene
Benzo[c]phenanthrene
Benzo[a]pyrene
Benzo[e]pyrene
Chrysene
Dibenz[a,h]anthracene
Dibenzo[a,h]pyrene
Dibenzo[a,i]pyrene
7,12-dimethylbenz[a]anthracene
Fluoranthene
Fluorene
Indeno[1,2,3-cd]pyrene
3-methylcholanthrene
Naphthalene
Phenanthrene
Pyrene



Rapid Resolution PAH Screening Column
(In accordance with European Community Directive 80/778/EEC which calls for rapid isocratic screening of water samples for these six PAHs)



Eclipse PAH, 4.6 x 50 mm, 3.5 µm
PN 959943-918
Det. 220,4nm
Flow 2.5 ml/min
Channel A = Water; B = Acetonitrile
Mobile Phase 92% B
Temp. = 20° C, low vol heat sink
Pressure: 105 bar

Eclipse PAH, 4.6 x 30 mm, 1.8 µm
PN 959931-918
Det. 220,4nm
Flow 2.5 ml/min
Channel A = Water; B = Acetonitrile
Mobile Phase 95% B
Temp. = 20° C, low vol heat sink
Pressure: 147 bar

Review of PAH Column with Environmental Samples

1. Sample preparation is required for most environmental samples – sample must be concentrated/extracted for good results
2. Must look for interferences in the actual samples – spike samples to check for selectivity
3. Examine results with UV and FLD

Sample preparation

SPE: AccuBOND C18, 500mg, 3ml (PN:188-1350)

Sample filter: 0.2 um filter

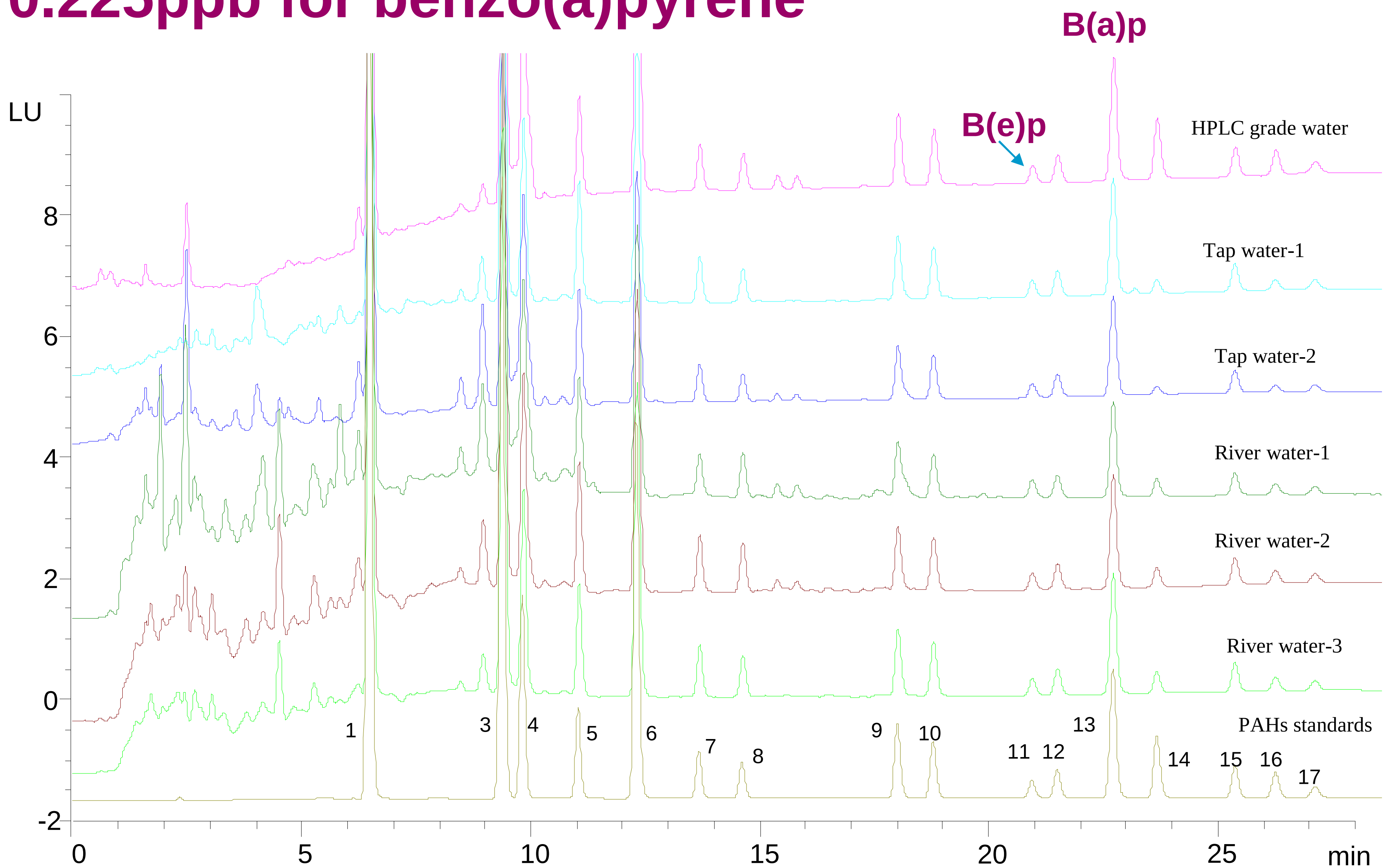
Zymark AutoTrace Extractor

Blank: 1L pure water + 200ml methanol

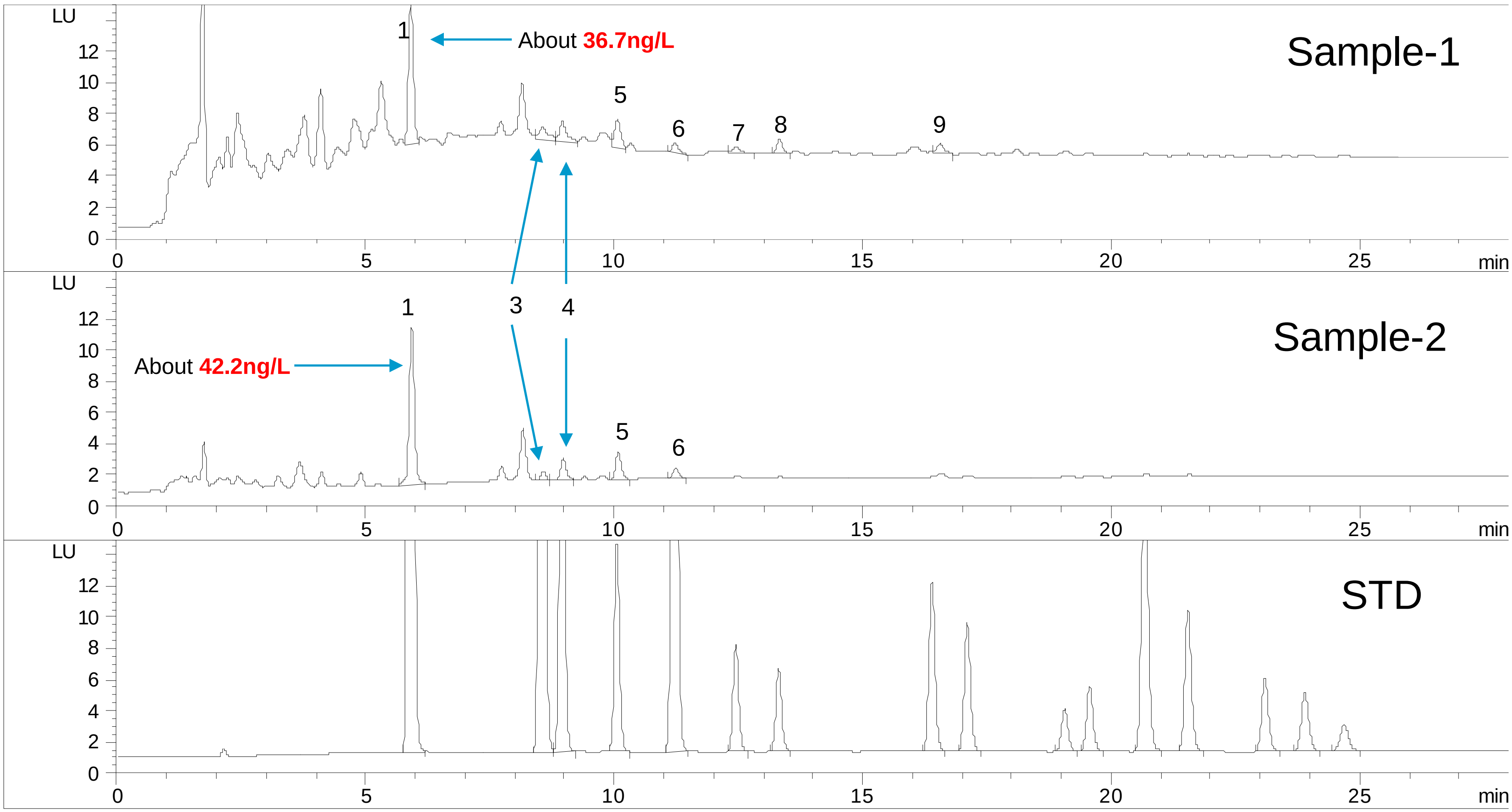
Recovery sample: 1L pure water + 200ml methanol+ 10ul stock STD mixture

Drinking water: 1L water + 200ml methanol

Water samples spiked with PAHs standards
0.225ppb for benzo(a)pyrene



Sample of Drinking Water – PAHs Separated on Eclipse PAH and Detection with FLD



HPLC conditions for Water samples

Column: Eclipse PAH, 4.6x150mm, 3.5um

DAD 254,4nm No Ref. or 220,4nm No Ref. ; Stop Time = 28min; Flow 1.5 ml/min

Mobile Phase A = Water; B = ACN

Gradient:

Time (Min)	% B
0	50
2	50
22	100

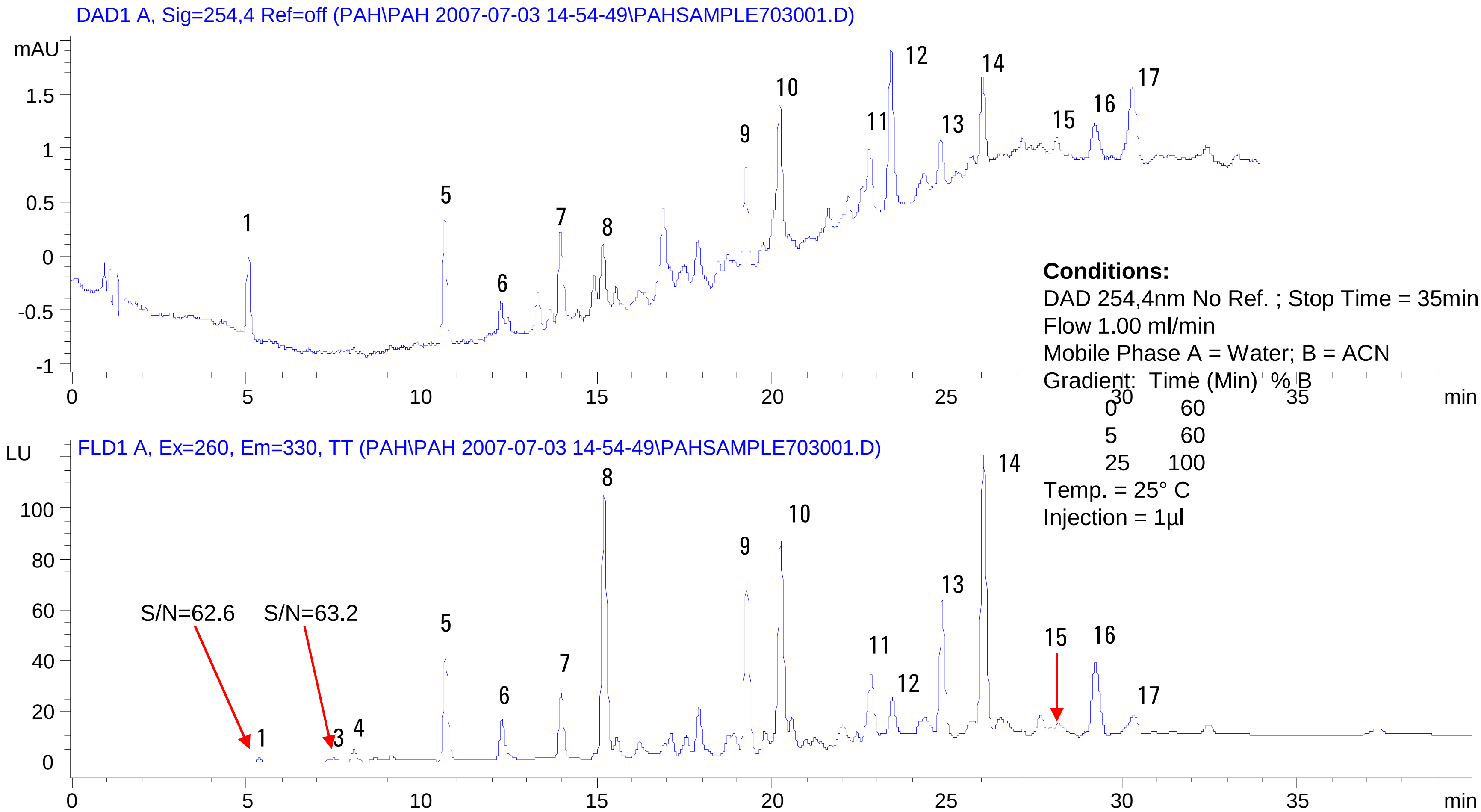
Temp. = 25° C

Injection = 2µl

FLD Settings:
PMT=12

Time	Ex	Em
0	220	330
7	210	330
9.6	250	363
10.7	250	405
12	250	460
13	270	400
20.2	270	415
24.3	250	490

Environmental sample - soil (4.6 x 150mm, 3.5um)



Conditions:
DAD 254,4nm No Ref. ; Stop Time = 35min;
Flow 1.00 ml/min
Mobile Phase A = Water; B = ACN
Gradient: Time (Min) % B

Time (Min)	% B
0	60
5	60
25	100

Temp. = 25° C
Injection = 1µl

Total 14 PAHs are detected with UV-254 in soil sample and all are detected with FLD except Acenaphthylene(2).

Life Test Eclipse PAH 2.1 x 50mm, 1.8um

Overlay of Runs # 2, 500, 900 injections

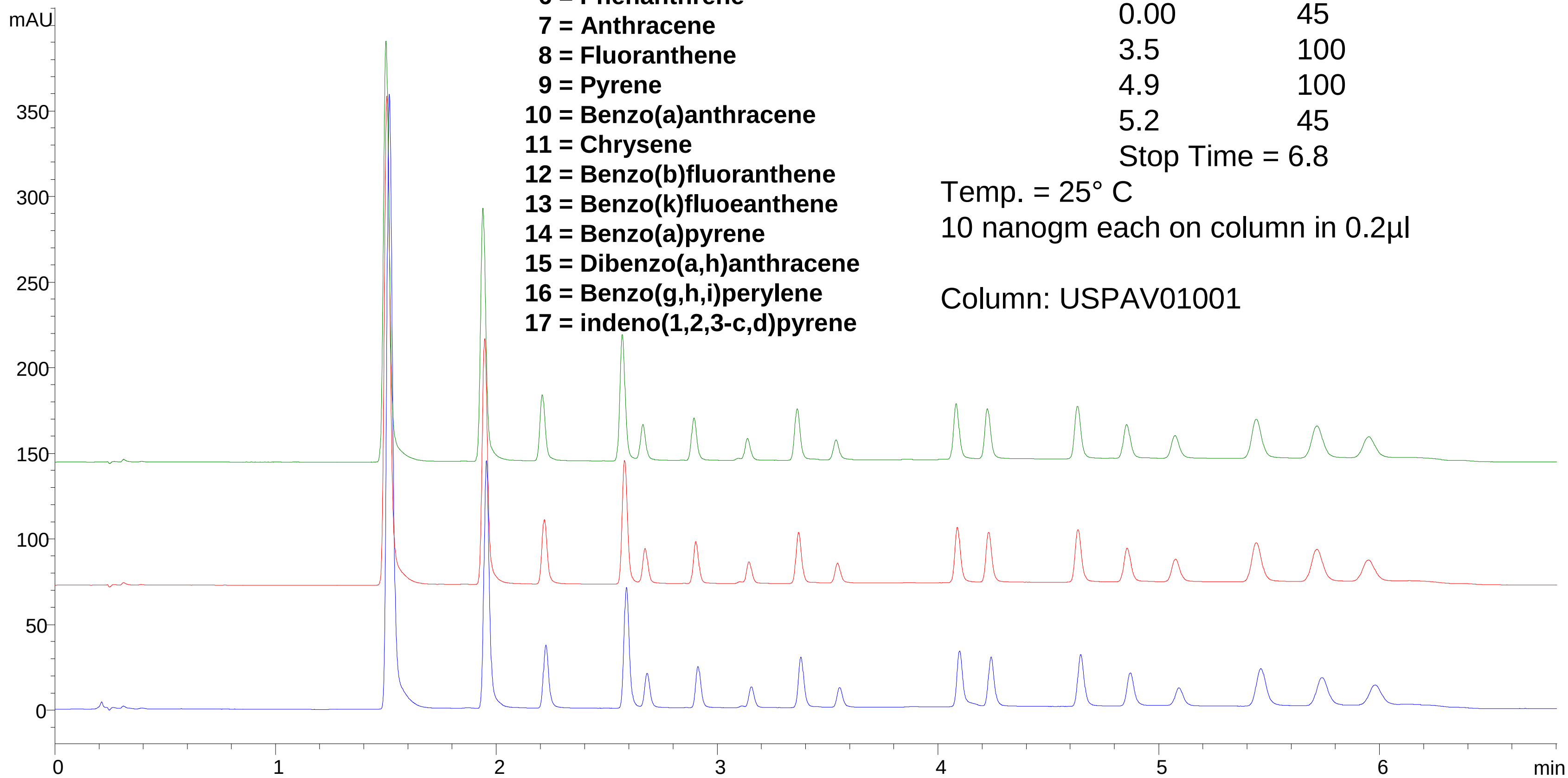
1 = Toluene
2 = Naphthalene
3 = Acenaphthylene
4 = Acenaphthene
5 = Fluorene
6 = Phenanthrene
7 = Anthracene
8 = Fluoranthene
9 = Pyrene
10 = Benzo(a)anthracene
11 = Chrysene
12 = Benzo(b)fluoranthene
13 = Benzo(k)fluoeanthene
14 = Benzo(a)pyrene
15 = Dibenzo(a,h)anthracene
16 = Benzo(g,h,i)perylene
17 = indeno(1,2,3-c,d)pyrene

Conditions:
Det. 220,4nm No Ref.; Data rate 0.2s, micro flowcell
Flow 0.417 ml/min
Mobile Phase A = Water; B = Acetonitrile

Gradient: Time (Min)	% B
0.00	45
3.5	100
4.9	100
5.2	45

Stop Time = 6.8
Temp. = 25° C
10 nanogm each on column in 0.2µl

Column: USPAV01001



Lifetime is good – this is an example up to 1000 injections RRHT Columns are packed well

Long Life of Eclipse PAH 2.1 x50 mm, 1.8 um

1 = Toluene
2 = Naphthalene
3 = Acenaphthylene
4 = Acenaphthene
5 = Fluorene
6 = Phenanthrene
7 = Anthracene
8 = Fluoranthene
9 = Pyrene
10 = Benzo(a)anthracene
11 = Chrysene
12 = Benzo(b)fluoranthene
13 = Benzo(k)fluoeanthene
14 = Benzo(a)pyrene
15 = Dibenzo(a,h)anthracene
16 = Benzo(g,h,i)perylene
17 = indeno(1,2,3-c,d)pyrene

Agilent 1200SL
DAD 220,4nm No Ref. DAD
Stop Time = 7.0min
Flow 0.417 ml/min
Mobile Phase A = Water; B = Acetonitrile

Gradient: Time (Min)	% B
0.00	45
3.5	100
4.9	100
5.2	45

Stop Time = 6.8
Temp. = 25° C
no Mixer or Pulse Dampener

Run	$Rs_{5,4}$
Run 5000	1.75
Run 4000	1.81
Run 3000	1.97
Run 2000	1.95
Run 1000	2.00
Run 1	2.09

Fluorescence Detectors and the PAH column

Many PAHs have very strong fluorescence and it is the preferred detector for many of the analytes. Methods will commonly use the UV and FLD in series.

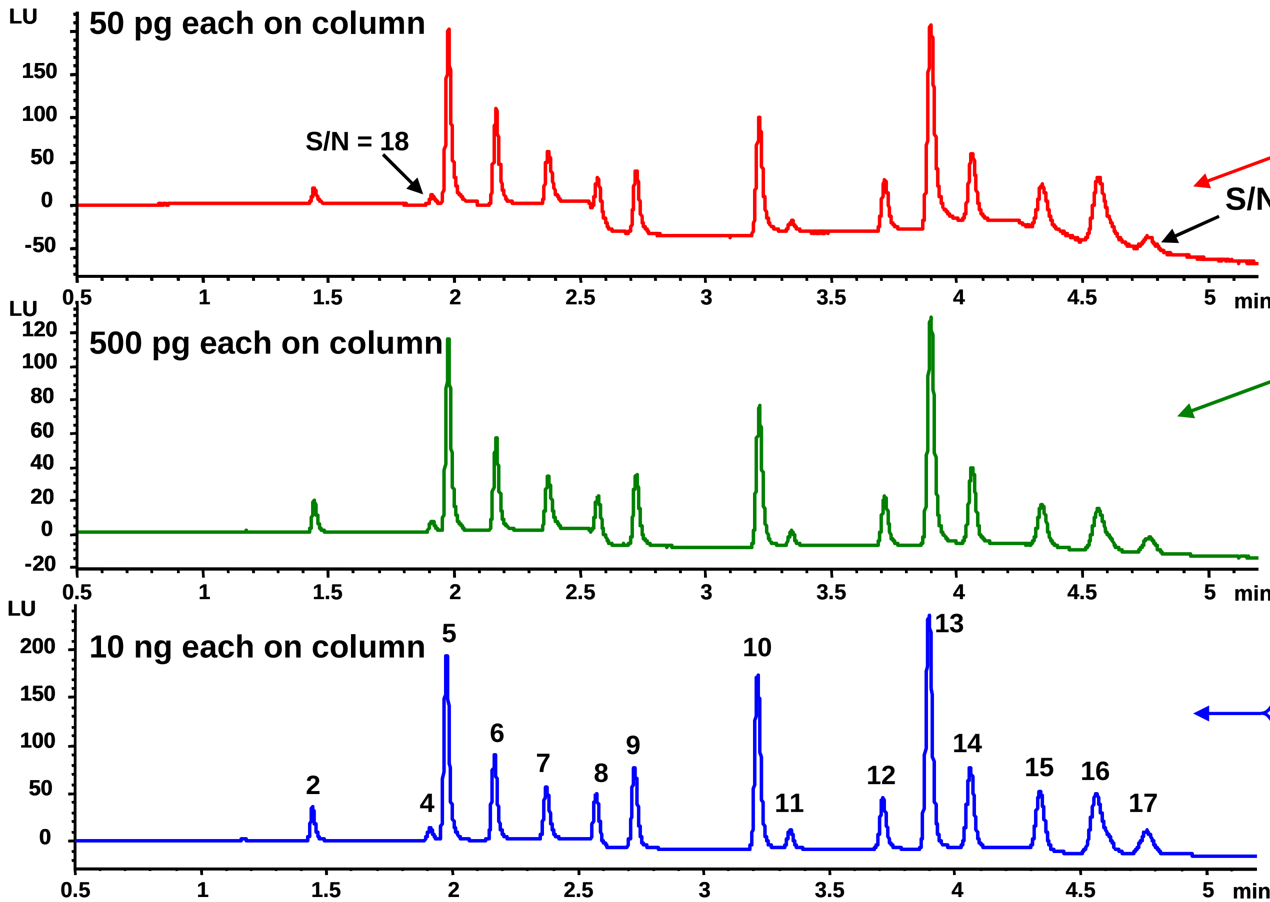
This is well accepted and not a problem. It does require wavelength switching on the FLD.

The 1.8um column can be accommodated on the FLD with fewer wavelength changed during the method.

No compromises are needed with the 3.5 and 5um columns and the FLD.

PAH 610 Mix on 4.6x50mm, 1.8µm PAH Column – Quantitative Sensitivity of FLD detection: S/N ≥ 10

Using a 3.5 or 5um column a wavelength change would be made to detect the last peak.
We avoided it here but still got good sensitivity with the 1.8um column.



For 50 pg on column all of the PMT settings are 6 numbers higher

S/N = 15

Good S/N for trace work!
No specific λ change!

For 500 pg on column all of the PMT settings are 3 numbers higher

Conditions:

FLD Settings:	Time	Ex	Em	PMT
Append all	0.00	224	330	7
baselines	1.75	210	314	7
	2.07	250	368	7
	2.45	252	402	11
	2.63	237	440	10
	3.50	255	420	7
	4.20	234	453	11

Flow 2.00 ml/min
Mobile Phase A = Water; B = Acetonitrile

Gradient:	Time (Min)	% B
	0.00	45
	3.5	100
	4.9	100
	5.2	45

Stop Time = 5.6
Temp. = 25° C
Inject 0.1µl

Methods for the Analysis of Explosives

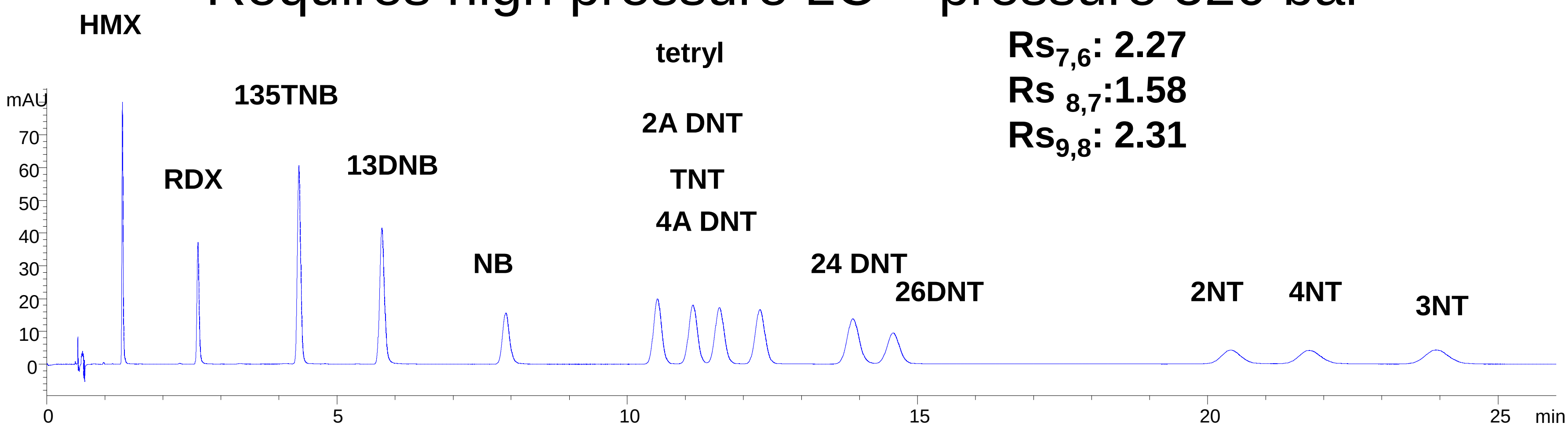
Explosives methods are also typically complex methods.

A confirmatory column is often used for the separations, but isn’t necessary if all explosives can be adequately resolved on one column.

Explosives separations are very temperature sensitive.

EPA 8330 Explosive Standard High-Resolution Separation on Extend-C18

Requires high pressure LC – pressure 520 bar

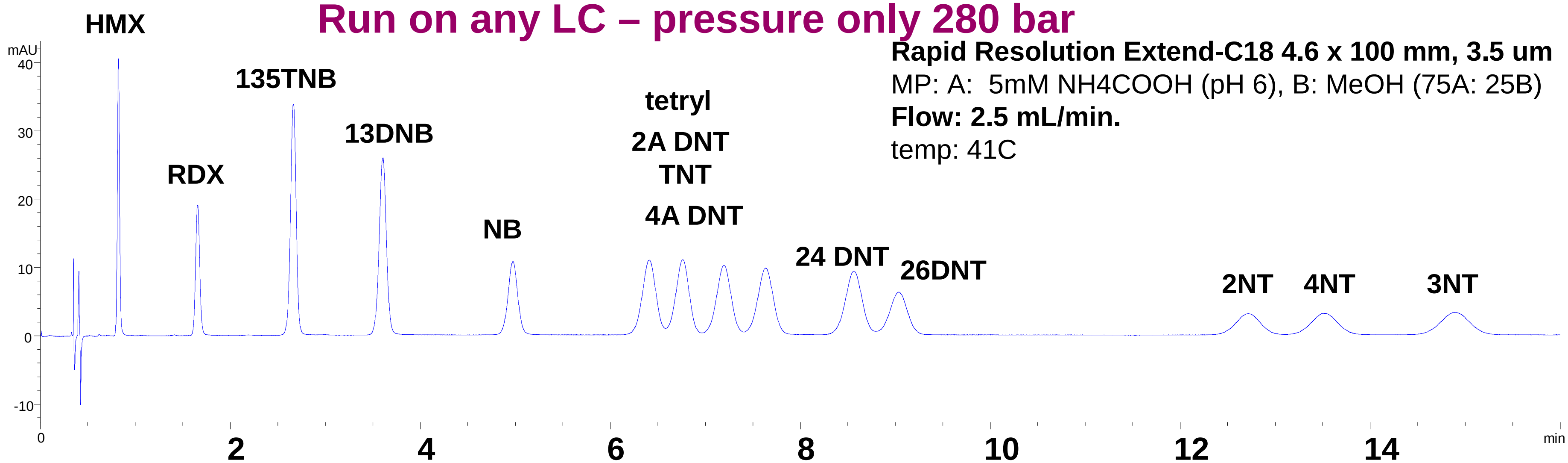


Rapid Resolution HT Extend-C18 4.6 x 100mm, 1.8 um
MP: A: 5mM NH4COOH (pH 6), B: MeOH (75A: 25B)
Flow: 1.7 mL/min.
temp: 41C
2 uL injection x(0.5 ug ea/mL)

14 peaks, no co-elutions – all peaks resolved!

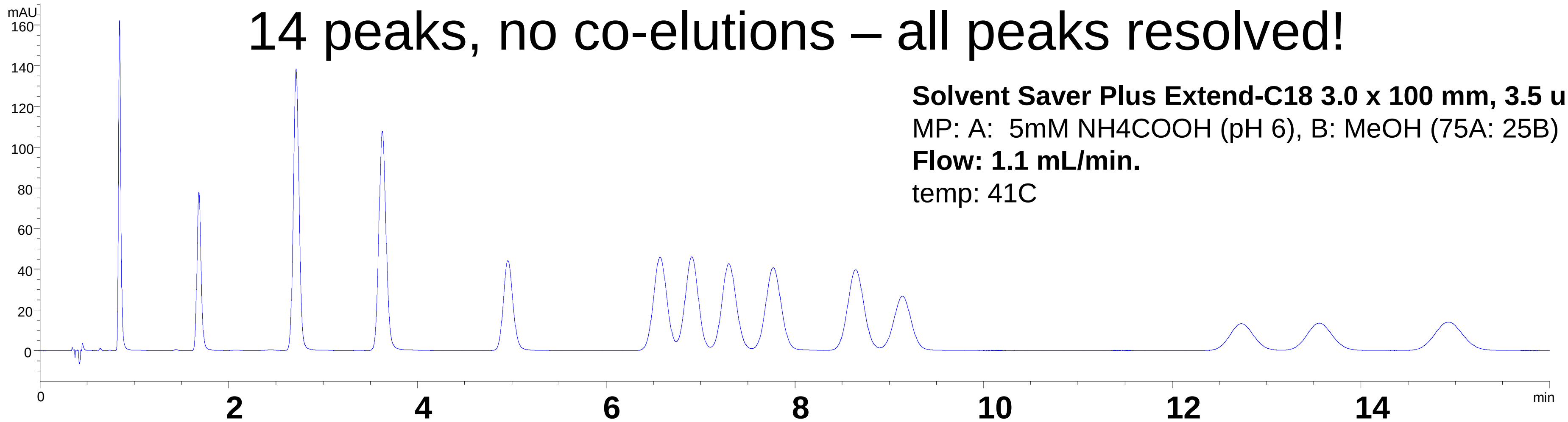
Rapid Resolution options for EPA 8330 Explosive Standard on Extend-C18

Run on any LC – pressure only 280 bar



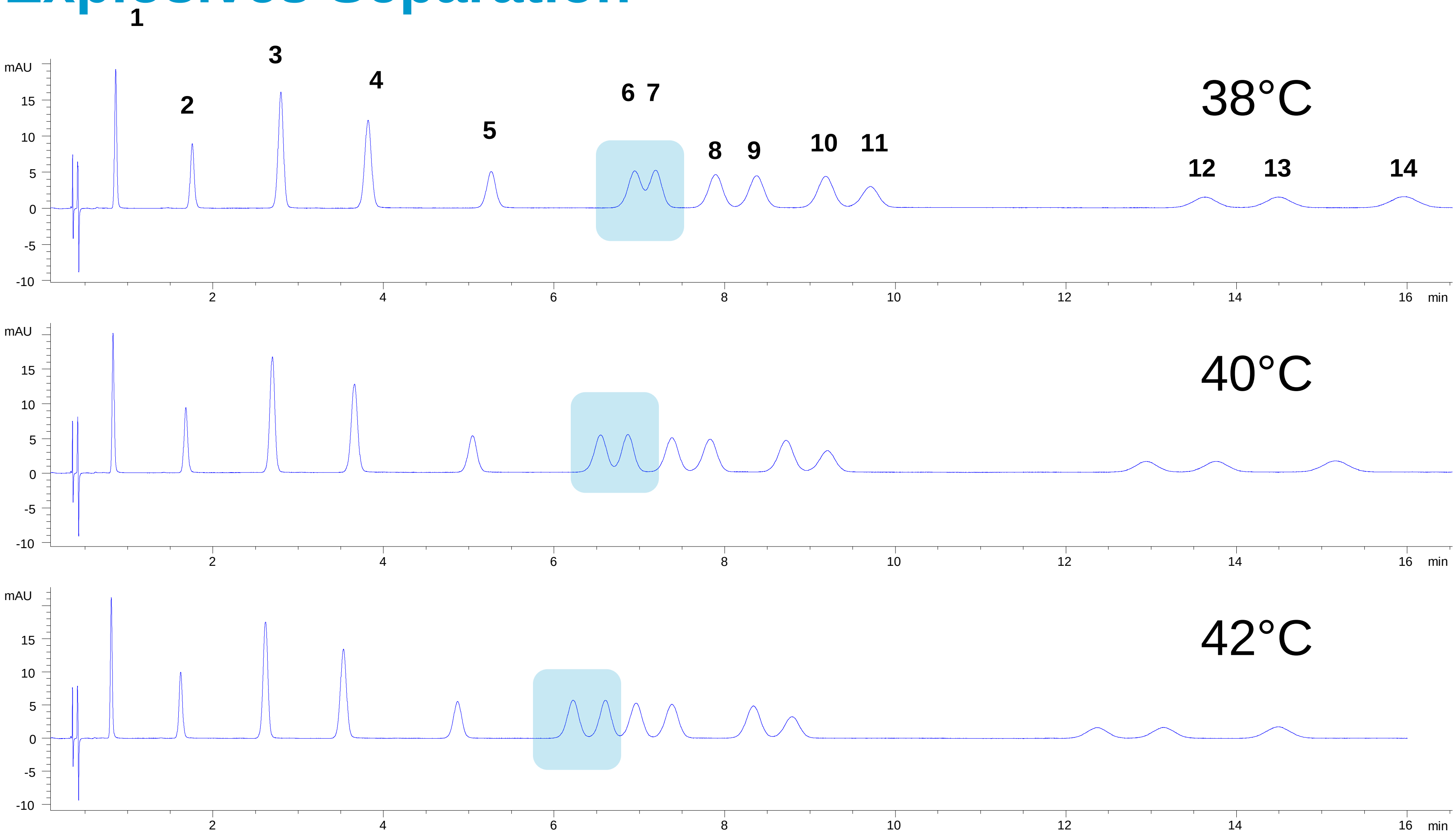
Rapid Resolution Extend-C18 4.6 x 100 mm, 3.5 um
MP: A: 5mM NH4COOH (pH 6), B: MeOH (75A: 25B)
Flow: 2.5 mL/min.
temp: 41C

14 peaks, no co-elutions – all peaks resolved!



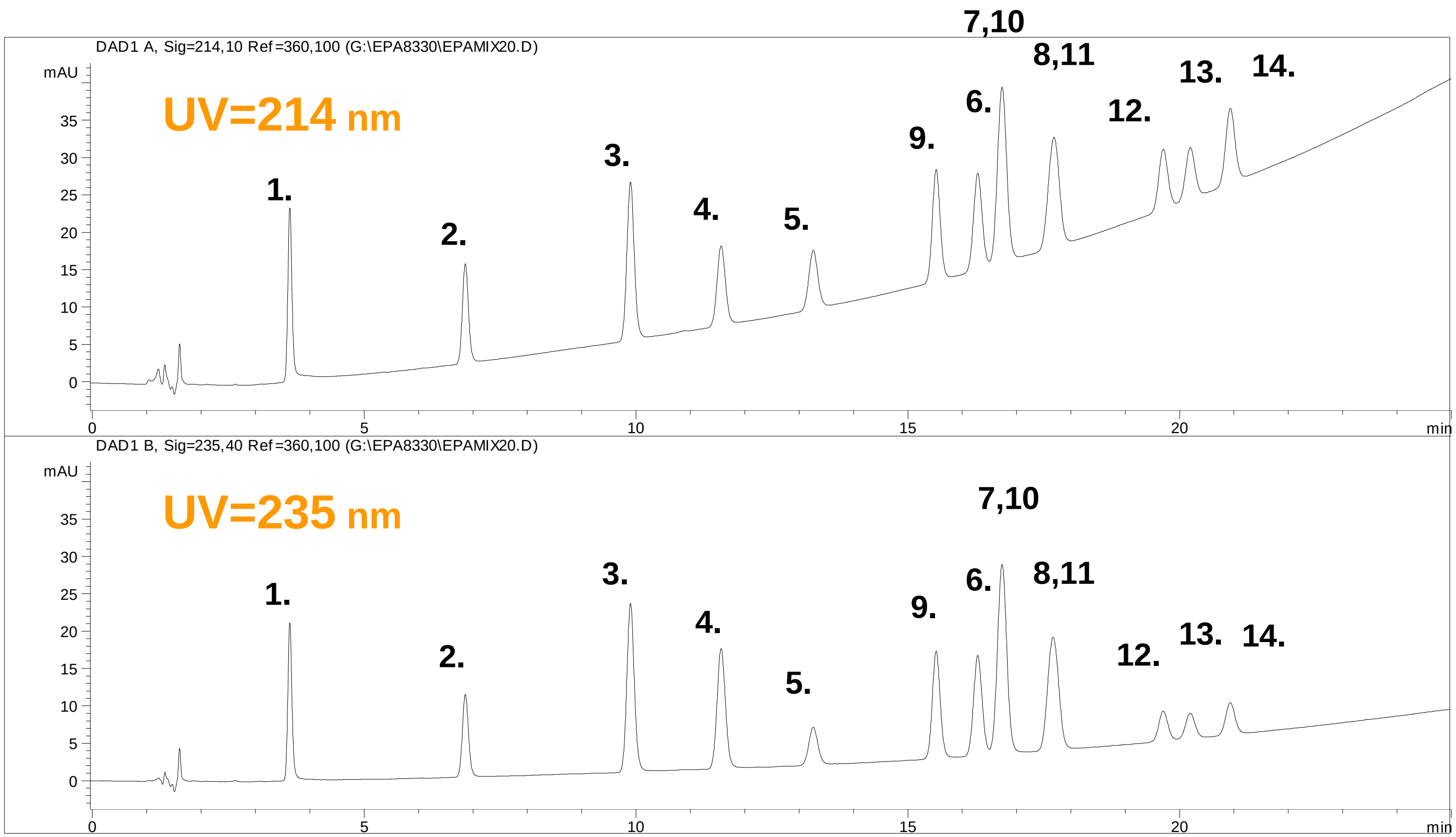
Solvent Saver Plus Extend-C18 3.0 x 100 mm, 3.5 um
MP: A: 5mM NH4COOH (pH 6), B: MeOH (75A: 25B)
Flow: 1.1 mL/min.
temp: 41C

Temperature optimizes critical pair resolution in Explosives Separation



HPLC Analysis of EPA-8330 Explosives on ZORBAX Eclipse XDB-C18

2 Co-elutions using Eclipse XDB-C18



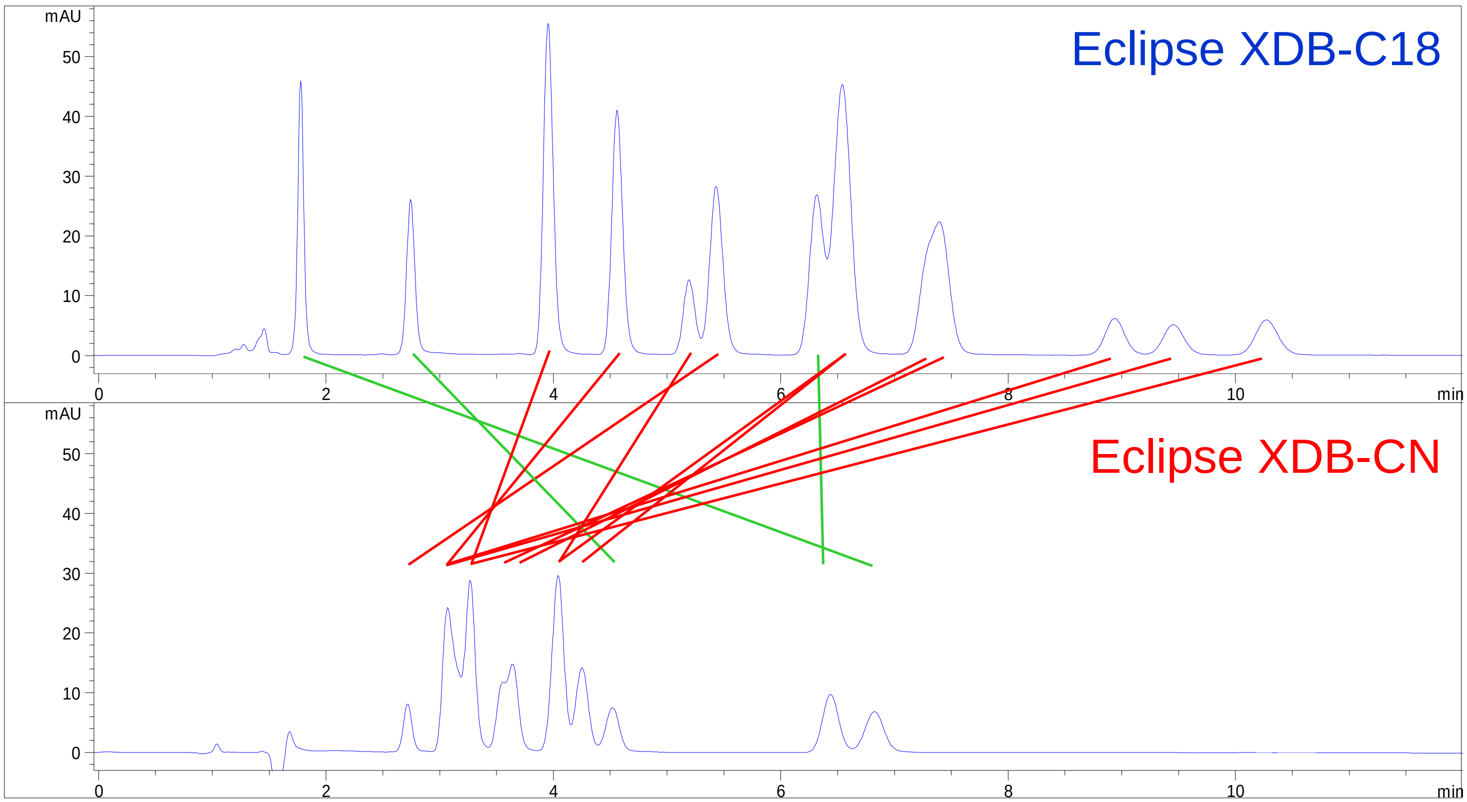
EXPLOSIVES
(5ng/µL in ACN)

1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. Nitrobenzene
6. 2,4,6-Trinitrotoluene
7. 2-Amino -4,6-dinitrotoluene
8. 2,4-Dinitrotoluene
9. Tetryl
10. 4-Amino -2,6-dinitrotoluene
11. 2,6-Dinitrotoluene
12. 2-Nitrotoluene
13. 4-Nitrotoluene
14. 3-Nitrotoluene

Sample: EPA-8330 Explosives (5ng/µL each); **Injection:** (4µL); **Column:** Zorbax Eclipse XDB-C18, 4.6 x 100mm, 3.5µm, (P/N:961967-902)
Mobile Phase: A=H2O, B=Methanol; **Gradient:** 26–40%B in 10min, 40–55%B in 10min, 55–70%B in 10min, 70–26%B in 1min; Total=31min.
Flow rate: 0.72 mL/min; **Temperature:** 38°C; **Detection:** UV (Sig=214,10nm, Ref=360,100nm; Sig=235,40nm, Ref=360,100nm)

HPLC Analysis of EPA-8330 Explosives on Eclipse XDB-C18 confirmed on Eclipse XDB-CN

Dramatic changes in elution order occur with CN, but resolution not adequate



EXPLOSIVES
(5ng/µL in ACN)

1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. Nitrobenzene
6. 2,4,6-Trinitrotoluene
7. 2-Amino -4,6-dinitrotoluene
8. 2,4-Dinitrotoluene
9. Tetryl
10. 4-Amino -2,6-dinitrotoluene
11. 2,6-Dinitrotoluene
12. 2-Nitrotoluene
13. 4-Nitrotoluene
14. 3-Nitrotoluene

Sample: EPA8330 Explosives (5ng/µL each); **Injection:** (4µL);
Columns: Zorbax (4.6 x 100mm, 3.5µm); Eclipse XDB-C18 P/N:961967-902) Eclipse XDB-CN (P/N:961967-902)
Mobile Phase: A=H2O, B=Methanol; **Isocratic:** 50:50 (A:B);
Flow rate: 0.72 mL/min; **Temp.:** 38°C; **Detection:** UV (Sig=235,40nm, Ref=360,100nm)

Pesticides - Another Sample You Can Use with the Sub 2-um RRHT Columns

The following example shows pesticides on RRHT columns.

The complex mixture can be separated easily, but this is not a specific regulatory method.

Analysis of Pesticides with Extend-C18 RRHT

Method Parameters (Pesticides)

MS: G6410 QQQ

ESI (+)
Mass Range: 100-500 amu
Scan Time: 300 ms
Drying gas: 350 ^{o}C
Gas Flow: 9 L/min
Nebulizer: 40 psi
Capillary: 3500 V

LC: 1200

Mobile A: 0.1% Formic Acid in water
add NH_4OH to pH 5.5
Mobile B: ACN
Flow rate: 0.3 mL/min
Column:ZORBAX Extend-C18
Rapid Resolution HT
2.1 x 100 mm, 1.8 µm
Column: 40 ^{o}C
1 µL injection

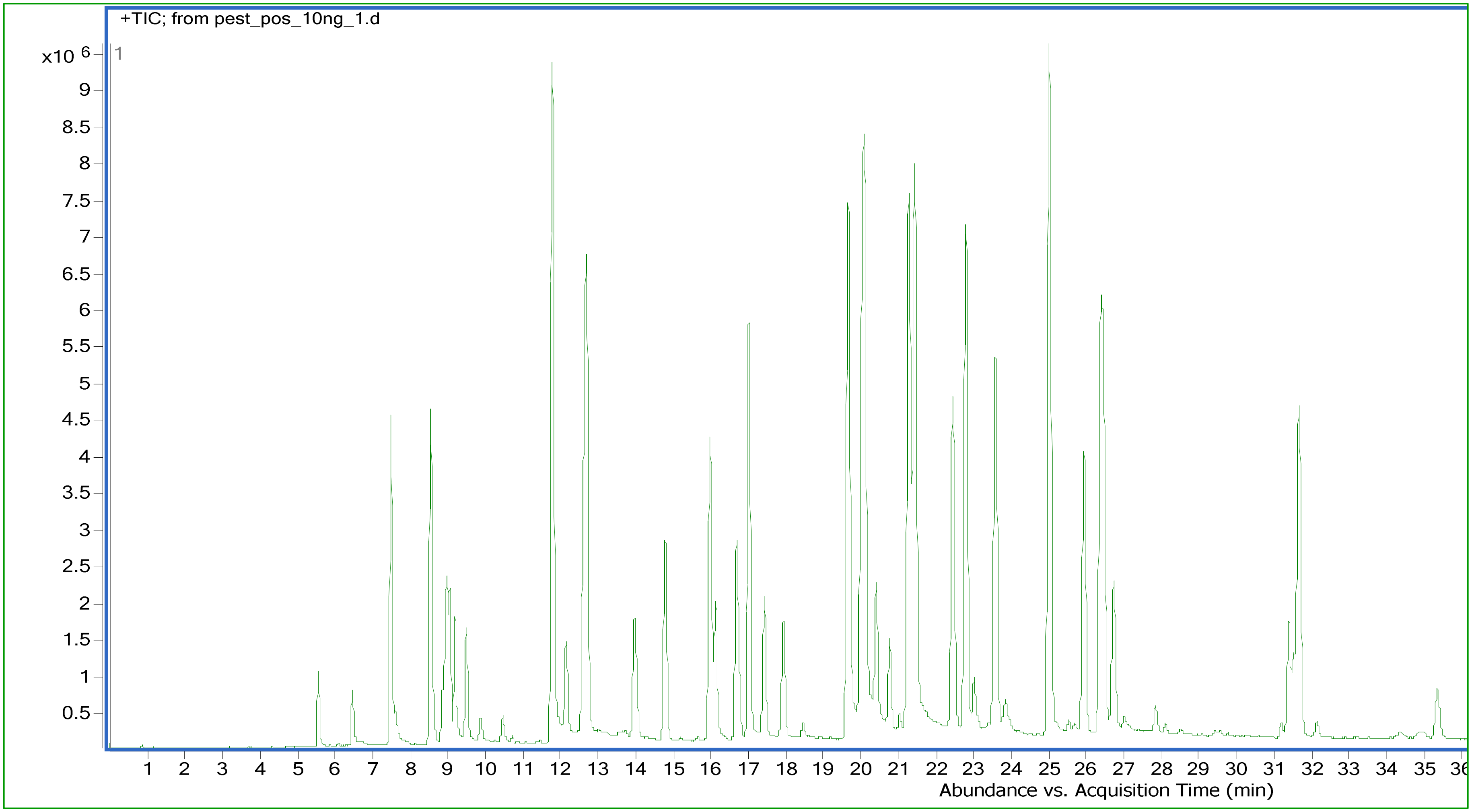
FAST run		SLOW run	
Time	B(%)	Time	B(%)
0	0	0	0
15	100	55	100
20	100	60	100
21.5	0	61.5	0

MRM Method Parameters, Pesticides (ESI+), slow run

Name	RT	precur.	Quant	Qual	Col V.	Dwell	Seg.	Name	RT	precur.	Quant	Qual	Col V.	Dwell	Seg.
3(4 chlorophenyl)methyl/urea	15.99	185	128	93	10	60	4	Fluometuron	21.44	233	72	168	20	80	5
3-Keto Carbofuran	16.69	236	179	151	10	60	4	Hydroxy-Atrazine	11.38	198	156	114	20	80	3
3-OH Carbofuran	12.69	238	163	181	10	80	3	Imazaquin	14.79	312	267	252	20	60	4
Aldicarb	16.14	116	89	70	5	60	4	Imazethapyr	12.61	290	177	69	30	80	3
Aldicarb Sulfone	8.56	223	76	86	5	100	2	Imidacloprid	12.79	256	175	209	15	80	3
Aldicarb Sulfoxide	7.49	207	89	132	5	300	1	Linuron	26.75	249	160	182	15	120	7
Atrazine	21.44	216	174	96	20	80	5	Metalaxyl (Apron)	22.81	280	220	192	10	150	6
Bendiocarb (Ficam)	19.99	224	167	109	5	80	5	Methiocarb	26.36	226	169	121	5	120	7
Benomyl	11.76	192	160	132	30	80	3	Methomyl	8.98	163	88	106	5	100	2
Bensulfuron	25.10	411	149	182	15	120	7	Metsulfuron methyl	17.44	382	167	199	15	60	4
Bromacil	17.44	261	205	162	20	60	4	Neburon	31.67	275	57	88	20	120	8
Caffeine	9.05	195	138	110	15	100	2	Nicosulfuron (Accent)	16.79	411	182	213	15	60	4
Carbaryl	21.24	202	145	127	15	80	5	Norflurazon	23.60	304	284	160	30	150	6
Carbofuran	20.08	222	165	123	10	80	5	Oryzalin	31.20	347	288	305	10	120	8
Chlorimuron ethyl	27.85	415	186	213	10	120	8	Oxamyl (Vydate)	8.87	237	72	90	10	100	2
Cycloate	35.37	216	83	154	15	120	8	Propham	23.87	138	120	92	10	150	6
Desethyl Atrazine	12.71	188	146	79	15	80	3	Propiconazole (Tilt)	31.40	342	156	69	20	120	8
Desisopropyl Atrazine	9.51	174	68	104	30	100	2	Propoxur (Baygon)	19.68	210	111	168	5	80	5
Desisopropyl Desethyl Atrazine	3.17	142	86	57	15	300	1	Siduron	25.94	233	137	94	15	120	7
Diphenamid	25.03	240	134	167	20	120	7	Siduron isomer	26.45	233	137	94	15	120	7
Diuron	22.45	233	72	160	20	150	6	Sulfometuron, methyl ester	20.42	365	150	199	15	80	5
Fenuron	11.75	165	72	92	15	80	3	Tebuthiuron	17.02	229	127	116	15	60	4
Flumetsulam	13.99	326	129	262	20	60	4	Terbacil	17.94	161	144	88	15	60	4

Fragmentor = 100 V

36-minute “Slow” Run – Providing Maximum Resolution with Extend-C18 RRHT

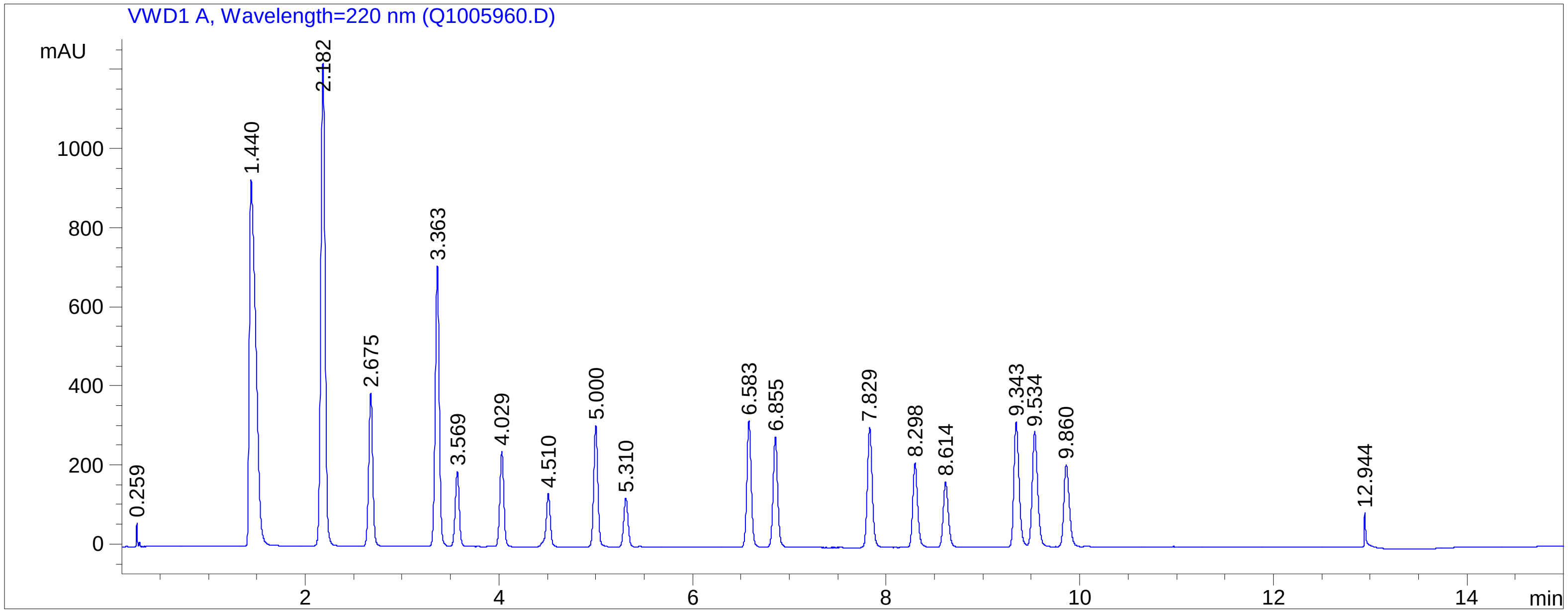


Conclusions

RRHT columns, with sub 2-um particles, provide the technology to improve environmental separations at lower cost.

Many column dimension choices exist allowing substantial reductions in analysis time, to complete more samples in less time

Manufacturing Test of 16 PAHs for Every Eclipse PAH Column - Example of RRHT, 4.6 x 50mm, 1.8um



Conditions

QA Gradient Profile for PAH analysis (5, 3.5 and 1.8 micron)

Column dimension: 4.6 x 100 (5 micron)
4.6 x 100 (3.5 micron)
4.6 x 50 (1.8 micron)

Solvent A: water

Solvent B: acetonitrile

Flow rate: 2 mL/min

Injection volume: 1 microliter

Detector: UV – 220nm, 4 nm bandwidth: reference off

Temperature 25C

Data acquisition time: 25 minutes (15 minutes for 1.8 micron)

Analysis cycle time: 30 minutes (15 minutes for 1.8 micron)

Note: Run gradient blank before sample analysis

Standard: PAH Mixture – Agilent Part Number 8500-6035

5 and 3.5 micron, 4.6 x 100

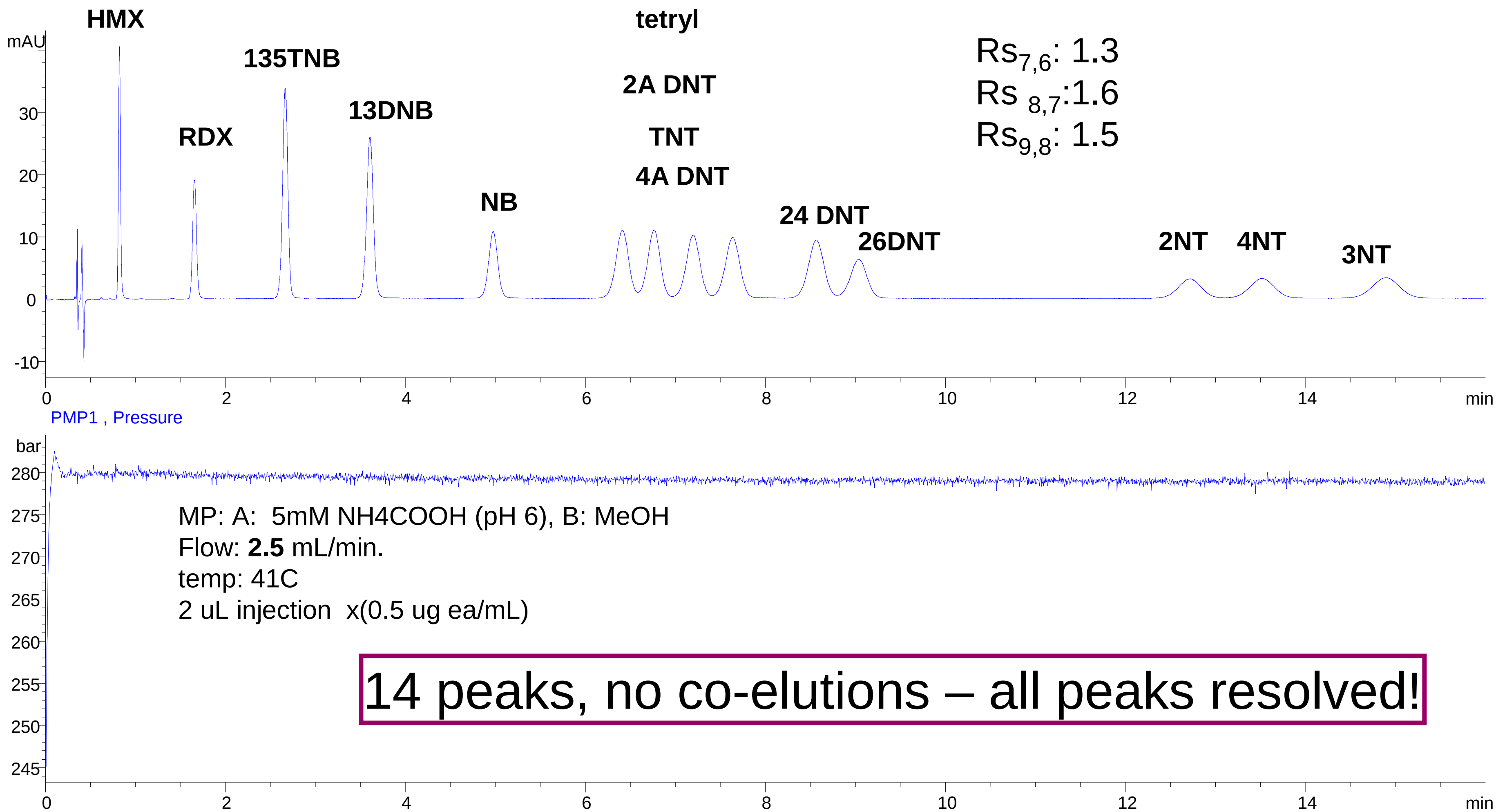
TIME	Percent B
0.00	40
0.66	40
20.00	100
25.00	100
27.00	40
30.00	40

1.8 micron, 4.6 x 50

TIME	Percent B
0.00	40
0.33	40
10.00	100
12.50	100
13.50	40
15.00	40

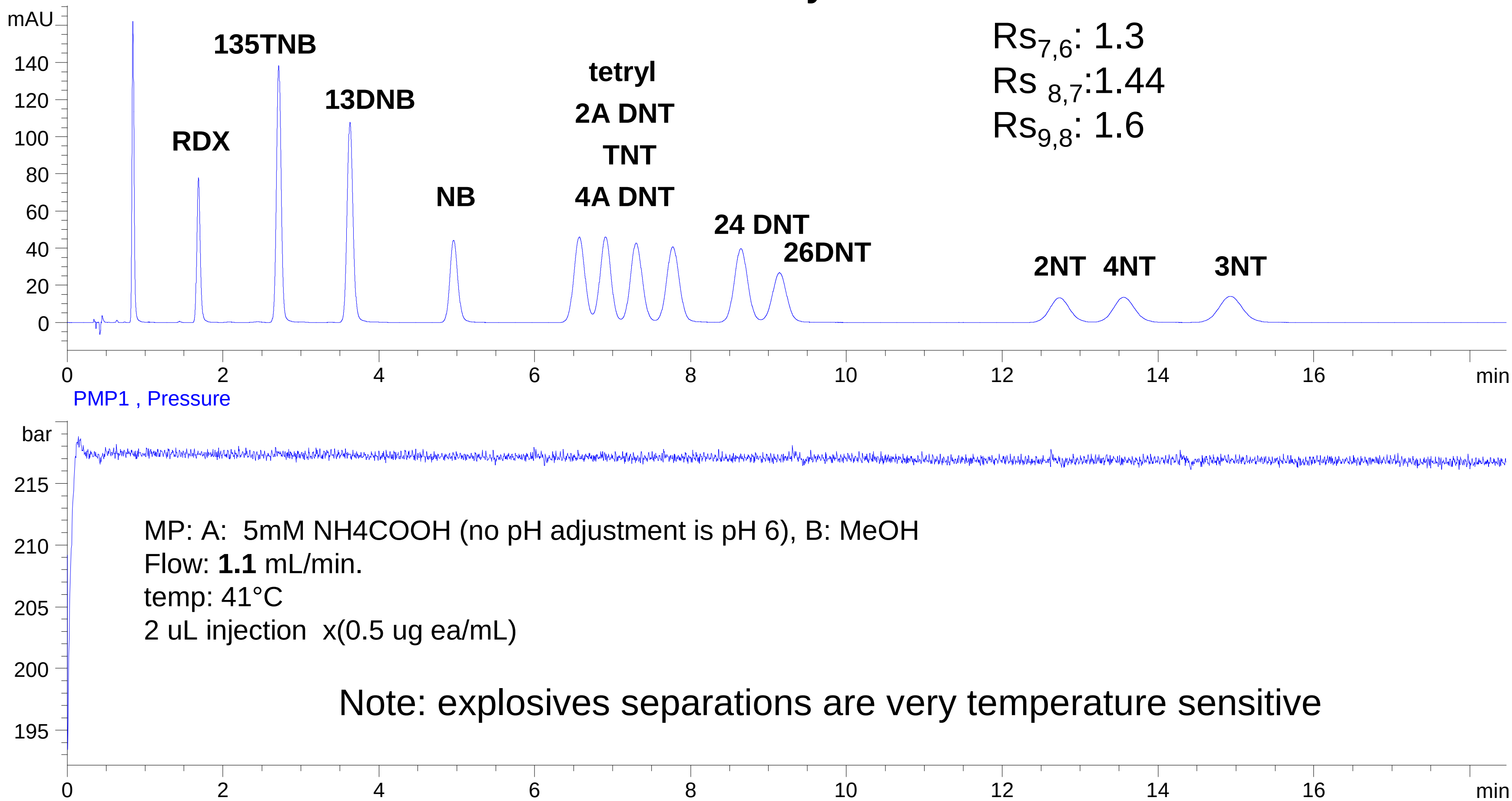
Elution order:
acetone (from sample solvent)
naphthalene
acenaphthylene
acenaphthene
fluorene
phenanthrene
anthracene
fluoranthene
pyrene
benz(a)anthracene
chrysene
benzo(b)fluoranthene
benzo(k)fluoranthene
benzo(a)pyrene
dibenz(a,h)anthracene
benzo(ghi)perylene
indeno(1,2,3-cd)pyrene

Low Pressure EPA 8330 Explosive Standard 0.1µg each on Rapid Resolution 4.6 x 100 mm, 3.5 µm Extend-C18



Solvent Saver EPA 8330 Explosive Standard 0.1µg each on Rapid Resolution 3.0 x 100 mm, 3.5 µm Extend-C18

3.0 mm ID saves solvent in comparison to a 4.6 mm ID column
Pressure 220 bar – run on any LC



MP: A: 5mM NH4COOH (no pH adjustment is pH 6), B: MeOH
Flow: **1.1** mL/min.
temp: 41°C
2 uL injection x(0.5 ug ea/mL)

Note: explosives separations are very temperature sensitive