Fast and Accurate GC/MS Testing for EPA and EU Polycyclic Aromatic Hydrocarbons (PAHs) for Food and Environmental Applications,

GC column Choices and Method Optimization

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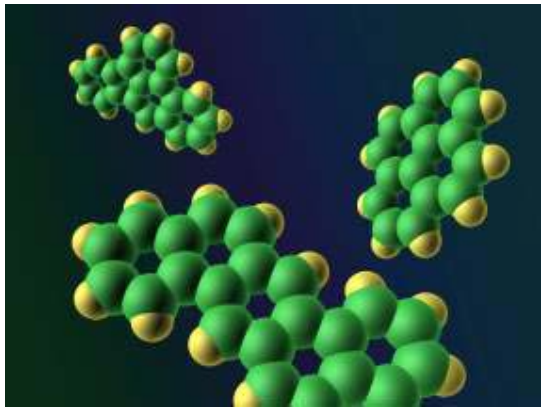
Agilent Technologies

PAHs

Polycyclic Aromatic Hydrocarbons

- Generated by pyrolysis of organic matter
- Two or more condensed aromatic rings
- PAH Group: ca. 660 compounds
- Carcinogenic properties
- GC-FID, GC-MS, GCxGC, LC-UV, LC-Fl



Consumer safety, PAH in food stuffs

- Food Stuffs
  - Fats, edible oils + fats containing foods (biscuits, chocolate)
  - Baby food manufacturers and suppliers
  - All “smoked” products
    - Fish
    - Smoke flavored products
    - Smoked or charcoal grilled meat products
    - Smoke flavoring manufacturing
  - Fresh food
    - Fish, Shellfish (closely related to marine env. PAH monitoring)

Priority PAHs in Food and Environment

	16 EPA	EU PAH (15+1)	EU PAH8	EU PAH4	AOAC	TEF
Napthalene	x				x	0.001
Acenaphthylene	x					0.001
Acenaphtene	x					0.001
Fluorene	x				x	0.001
Phenanthrene	x				x	0.001
Anthracene	x				x	0.01
Fluoranthene	x				x	0.001
Pyrene	x				x	0.001
Benzo[a]anthracene	x	x	x		x	0.1
Chrysene	x	x	x	x	x	0.01
Benzo[b]fluoranthene	x	x	x	x	x	0.1
Benzo[k]fluoranthene	x	x	x		x	0.1
Benzo[j]fluoranthene		x				0.1
Benzo[a]pyrene	x	x	x	x	x	1
Indeno[123cd]pyrene	x	x	x		x	0.1
Dibenzo[ah]anthracene	x	x	x	x	x	1
Benzo[ghi]perylene	x	x	x		x	0.01
Dibenzo[al]pyrene		x				10
Dibenzo[ai]pyrene		x				10
Dibenzo[ah]pyrene		x				10
Dibenzo[ae]pyrene		x				1
Cyclopenta[cd]pyrene		x				0.1
Methylchrysene		x				
Benzo[c]fluorene		x				

Environmental

EU Food stuffs

EU Food stuffs New

Seafood

High conc.

Low conc.

Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food. The EFSA Journal (2008) 724

FDA's " List of Nine-PAHs"

Criteria for Re-opening Areas Closed from Oil Spills Based on 160 g/day Seafood Consumption and Concentrations of Chemical Contaminants in Seafood

Chemical[1]	Level of Concern (ppm)	Basis[2]
Napthalene	20	EPA RfD; 70 kg bw; 160 g/day consumption
Fluorene	20	EPA RfD; 70 kg bw; 160 g/day consumption
Anthracene/phenanthrene	150	EPA RfD; 70 kg bw; 160 g/day consumption
Fluoranthene	0.15	10^{-6} Cancer risk estimate = 0.02B(a)P equivalency
Pyrene	0.025	10^{-6} Cancer risk estimate = 0.13B(a)P equivalency
Benz(a)anthracene	0.2	10^{-6} Cancer risk estimate = 0.014B(a)P equivalency
Chrysene	0.25	10^{-6} Cancer risk estimate = 0.013B(a)P equivalency
Benzo(a)pyrene	0.003	10^{-6} Cancer risk estimate = (34ng/p/d)(70/5yr)/160 g seafood/p/d

[1] Includes alkylated homologues, specifically C-1, C-2, C-3, C-4 napthalenes; C-1, C-2, C-3 fluorenes; C-1, C-2, C-3 anthracenes/phenanthracenes; C-1, C-2 pyrenes

EU Perspective

Recent scientific opinions of the EFSA Panel on Contaminants in the Food Chain have lead to the adoption of alternative and more limited selection of the PAHs for risk characterization of food

Oral carcinogenicity data are only available for benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene,benzo(ghi)perylene, chrysene, dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene.

The EFSA concluded that these eight PAHs (PAH8), either individually or in combination are the best indication of the toxicity of PAHs in food

Recently, the sum of benzo(a)pyrene, chrysene, benz(a)anthracene and benzo(b)fluoranthene (PAH4) have been adopted as a suitable indicator for the occurrence and toxicity of PAHs in food.

From EU PAH (15+1) to EU PAH4 subset

Past – 2010:

- EU regulation on levels of only benzo(a)pyrene (BaP) in foodstuffs
- FAO/WHO concluded 13 PAHs are genotoxic and carcinogenic
- EFSA reviewed 10000 results for PAHs (EU PAH 15+1) in different food commodities on occurrence and toxicity
- EFSA concluded that BaP alone is not a suitable marker for PAHs in foodstuffs
- Maximum levels for the sum of BaP, benzo(b)fluoranthene, chrysene and benzo(a)anthracene = PAH4
- New PAH4 EU regulation will come into effect 2010 - 2011

Maximum EU PAH levels (BaP) in foodstuffs

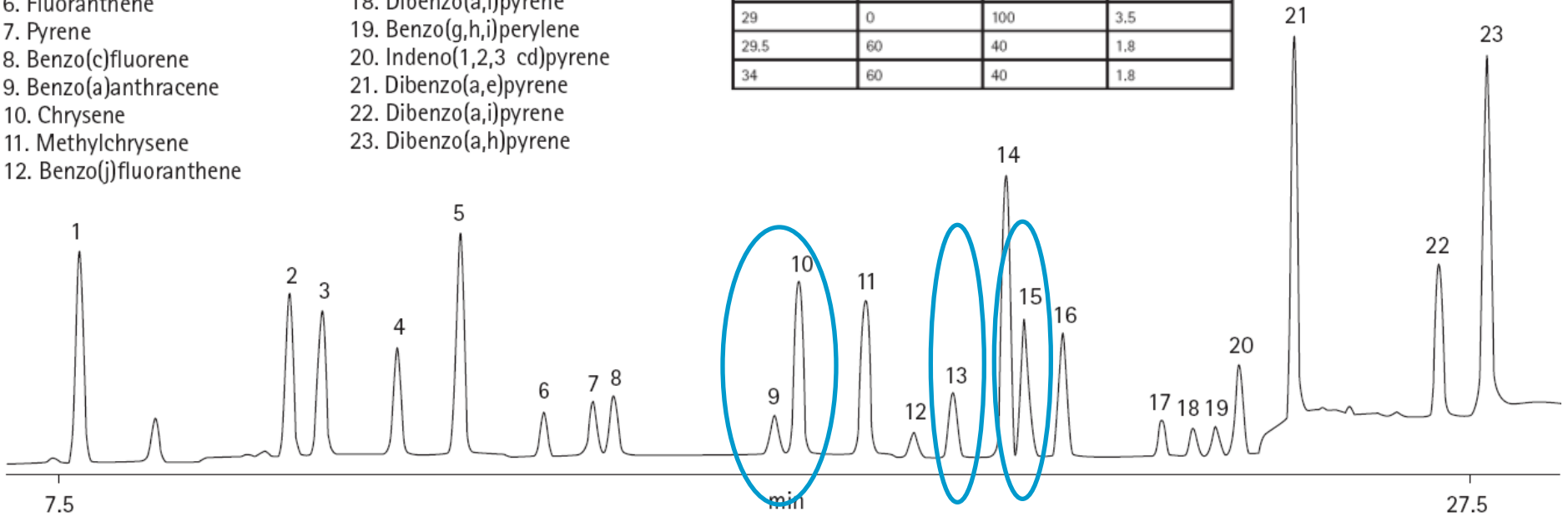
Product	Maximum level µg/kg
Oils and fats for consumption or as ingredient in foods	2.0
Foods for infants and young children	1.0
Baby foods and processed cereal based foods for infants	1.0
Infant formulae, infant milk	1.0
Dietary foods for medical purposes	1.0
Smoked meats and smoked meat products	5.0
Smoked fish, smoked fishery products	5.0
Fish	2.0
Crustaceans, crabs, lobsters, shrimp	5.0
Bivalve molluscs, oysters, mussels, clams	10.0
Cacoa butter	pending

HPLC Alternative PAH's on Pursuit PAH, FLD

KEY

1. Naphthalene
2. Acenaphthene
3. Fluorene
4. Phenanthrene
5. Anthracene
6. Fluoranthene
7. Pyrene
8. Benzo(c)fluorene
9. Benzo(a)anthracene
10. Chrysene
11. Methylchrysene
12. Benzo(j)fluoranthene
13. Benzo(b)fluoranthene
14. Benzo(k)fluoranthene
15. Benzo[a]pyrene d^{12}
16. Benzo(a)pyrene
17. Dibenzo(a,h)anthracene
18. Dibenzo(a,l)pyrene
19. Benzo(g,h,i)perylene
20. Indeno(1,2,3 cd)pyrene
21. Dibenzo(a,e)pyrene
22. Dibenzo(a,i)pyrene
23. Dibenzo(a,h)pyrene

HPLC Gradient Profile			
Time (min)	% H_2O	% ACN	Flow (mL/min)
0	60	40	1.8
4	60	40	1.8
23	10	90	1.8
23.5	10	90	2.0
24	5	95	2.0
24.5	0	100	3.0
25	0	100	3.5
29	0	100	3.5
29.5	60	40	1.8
34	60	40	1.8



Triphenylene?

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GC Analysis PAHs

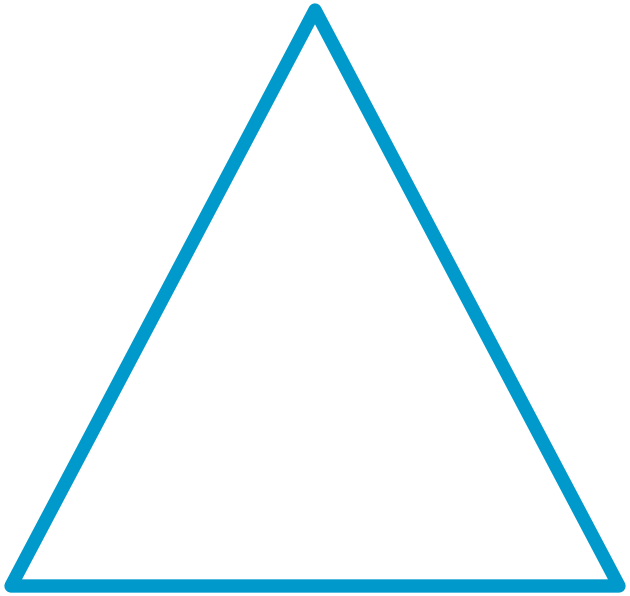
Demanding GC analysis of PAH's

- Similar chemical structures
  - Difficult chromatographic separations
- Same mass
  - Difficult to differentiate on mass (little and identical fragmentation)
- High boiling compounds
  - High GC column elution temperatures
  - Long analysis times
  - Injector discrimination effects

What is needed of GC column for PAH Analysis?

Productivity
High S/N ratio's

Analysis Time



Selectivity

Accurate reporting
Reduced bias

Robustness

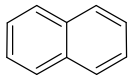
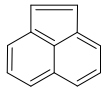
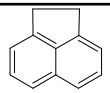
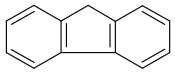
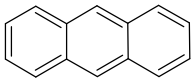
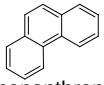
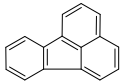
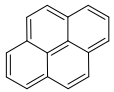
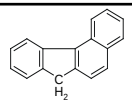
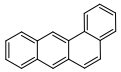
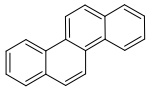
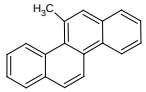
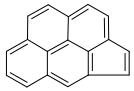
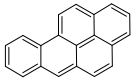
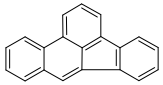
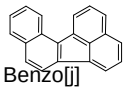
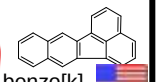
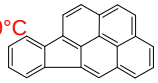
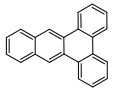
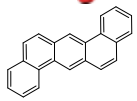
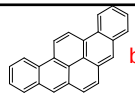
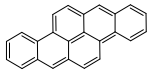
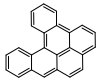
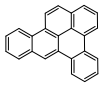
Long lifetime
Increased system uptime

The PAH GC Column

PAH GC Column

- Selective for small PAH structural differences
  - Phenyl/arylene substituted siloxanes
    - π/π stacking interactions & electrostatic supported mechanisms
  - Liquid crystals
    - Limited Max T 285°C, no 5/6-ring elution
- High temperature stability
  - Note EPA and EU regulation differences
  - 6 ring PAHs eluting > 320°C
  - High stability of siloxane liquid phases
- Column durability, longevity
- Low Bleed Characteristics
- Fast Analysis

PAHs: Critical Isomers EU and EPA

	MW	26 PAH target compounds			
2 ring	128	 bp 218°C naphthalene			
3 ring	152, 154	 acenaphthylene	 acenaphthene		
	166, 178	 fluorene	 anthracene	 phenanthrene	
4 ring	202, 216	 fluoranthene	 bp 341°C pyrene	 7H-benzo[c]fluorene	
	228, 242	 benz[a]anthracene	 bp 448°C chrysene	Triphenylene	 5-Methylchrysene
5 ring	226, 252	 cyclopenta[c,d]pyrene	 bp 360°C benzo[a]pyrene	 Benzo[b]fluoranthene	 Benzo[j] fluoranthene  benzo[k] fluoranthene
5/6 ring	276, 278	benzo[ghi]perylene	 bp 530°C indeno[1,2,3-cd]pyrene	 Benzo[b]triphenylene	 dibenz[a,h]anthracene
6 ring	302	 bp 596°C dibenzo[a,i]pyrene	 Dibenzo[a,h]pyrene	 Dibenzo[a,l]pyrene	 Dibenzo[a,e]pyrene

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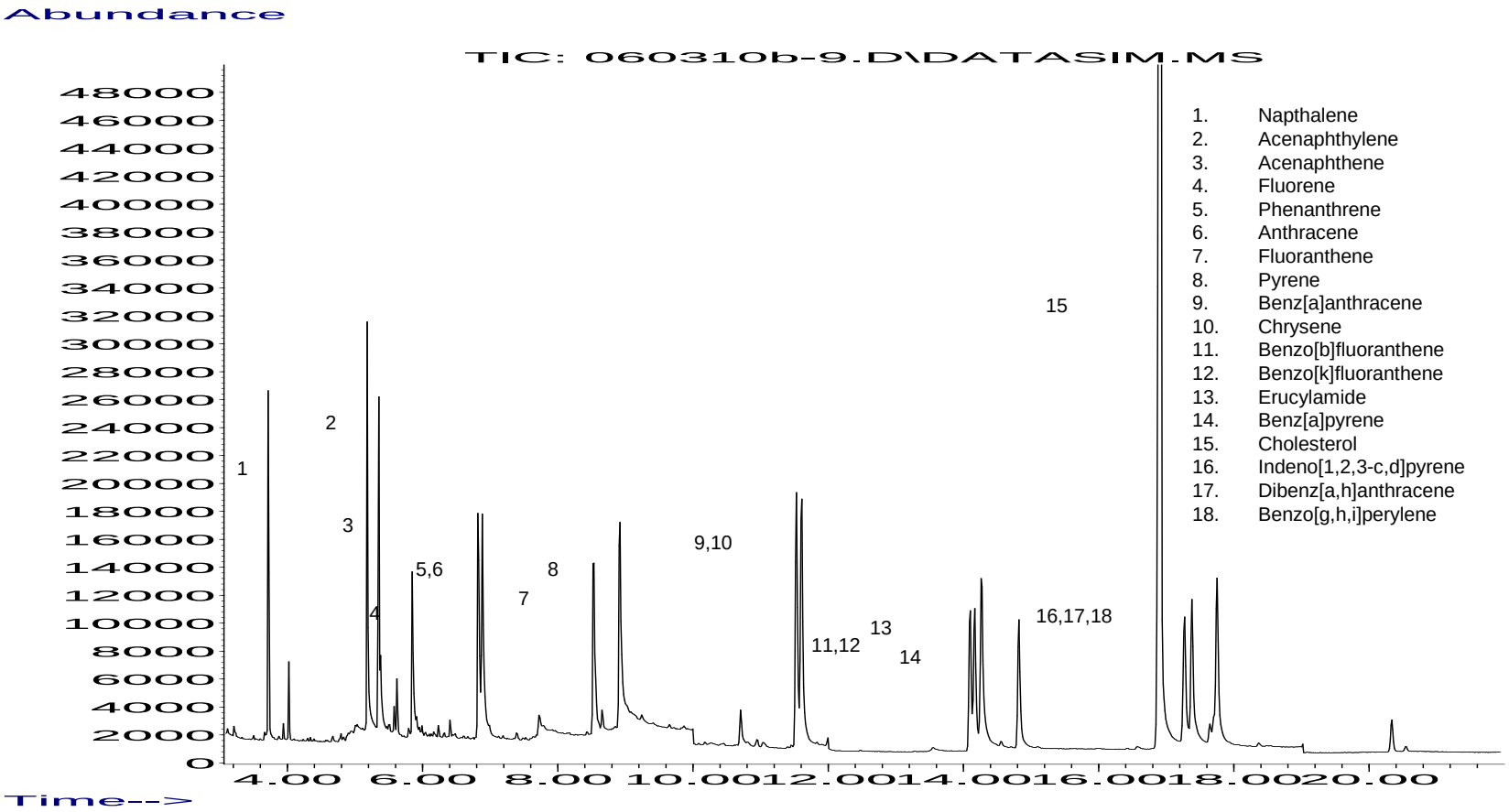
Critical PAH separations in GC/MS

Critical GC PAH separations

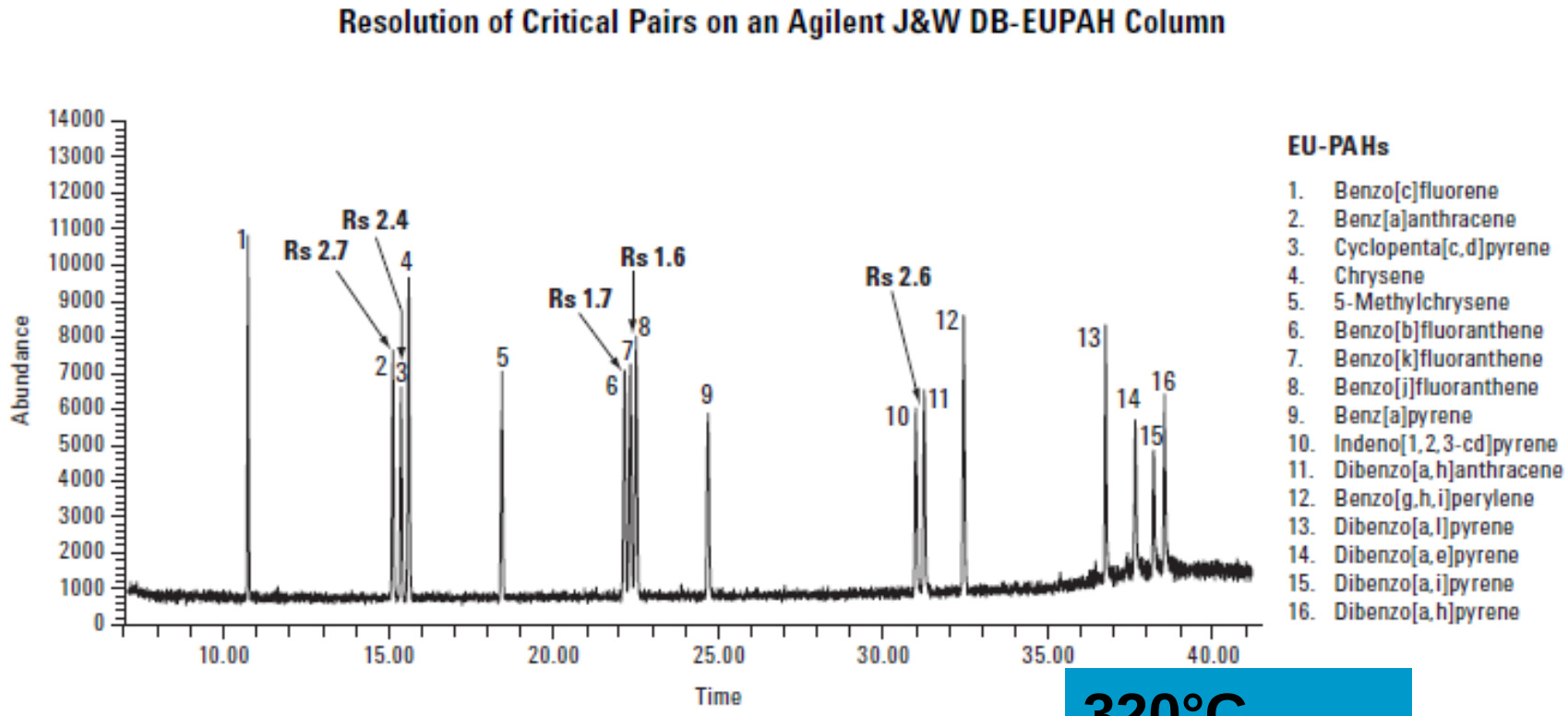
• Phenanthrene/Anthracene (EU(15+1) & EPA)	m/z 178
• Cyclopenta[c,d]pyrene/Chrysene	m/z 226/228
• Chrysene/Triphenylene (EU PAH4)	m/z 228
• 5-methylchrysene/6-methylchrysene (EU (15+1))	m/z 242
• Benzo(b,k,j)fluoranthenes (EU PAH4, EPA)	m/z 252
• Indeno(1,2,3-CD)pyrene	m/z 276/278
Benzo(b)triphenylene	
Dibenzo(a,h)anthracene	
• Dibenzopyrenes/Coronene and benzo[b]perylene	MW 300/302

125 ppb EPA PAHs extracted from Swai fish using QuEChERS
DB-5ms 20m 0.18mm 0.18µm
GC/MS SIM TIC



1. Napthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benz[a]anthracene
10. Chrysene
11. Benzo[b]fluoranthene
12. Benzo[k]fluoranthene
13. Erucylamide
14. Benz[a]pyrene
15. Cholesterol
16. Indeno[1,2,3-c,d]pyrene
17. Dibenz[a,h]anthracene
18. Benzo[g,h,i]perylene

Critical pairs on J&W DB-EUPAH, TIC



320°C
Low bleed!

20m x 0.18mm x 0.14µm

Column, bleed and S/N ratio's

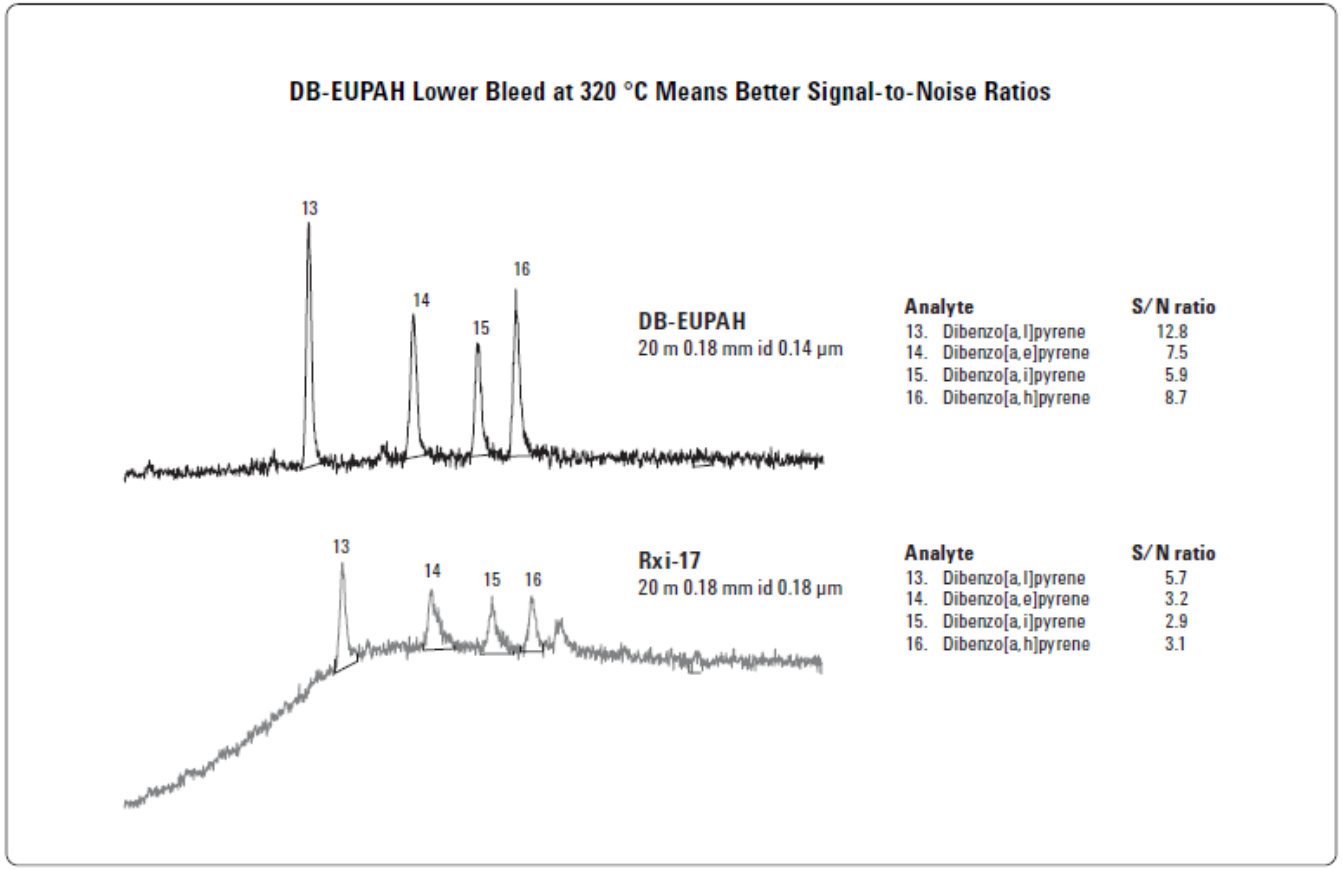
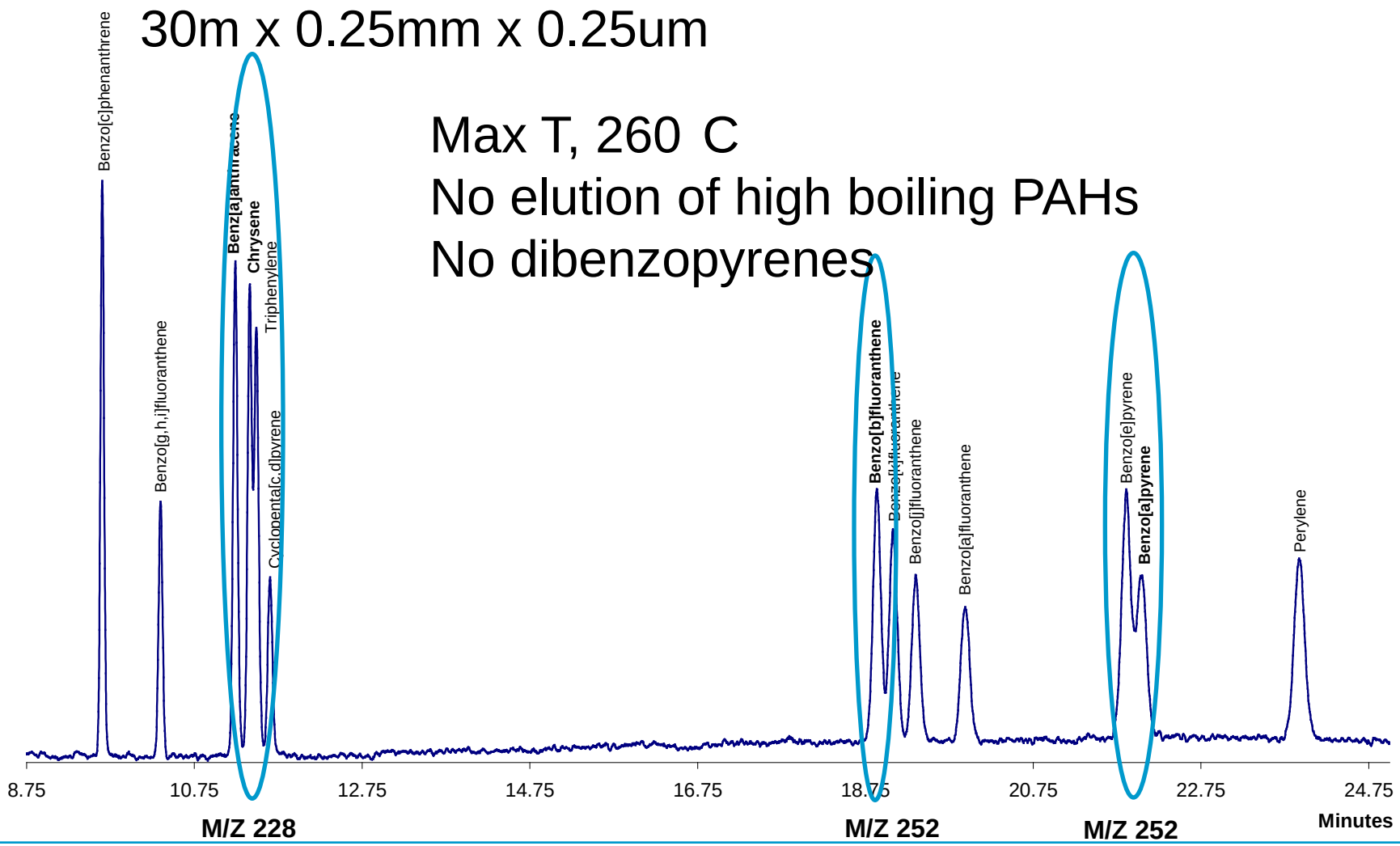


Figure 3. Overlaid TICs of 2.5 ng on-column loading injections of 15+1 EU priority PAH standard on Agilent J&W DB-EUPAH and Rxi-17. Chromatographic conditions are listed in Table 1.

DB-EUPAH: Low bleed, higher accuracy

EU PAH4 on high cyano content siloxane



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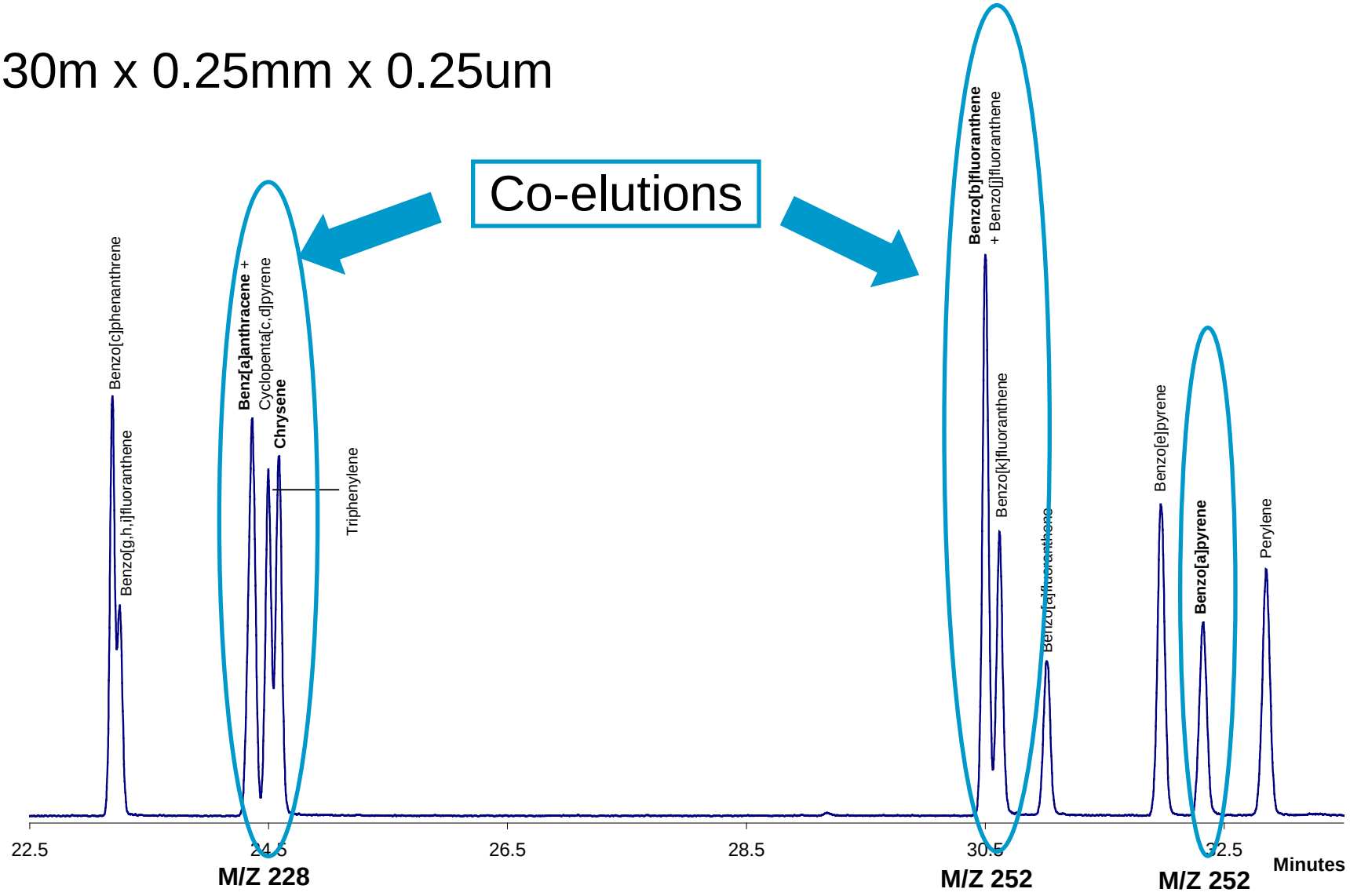
PAH4 on Rxi-XLB

30m x 0.25mm x 0.25um



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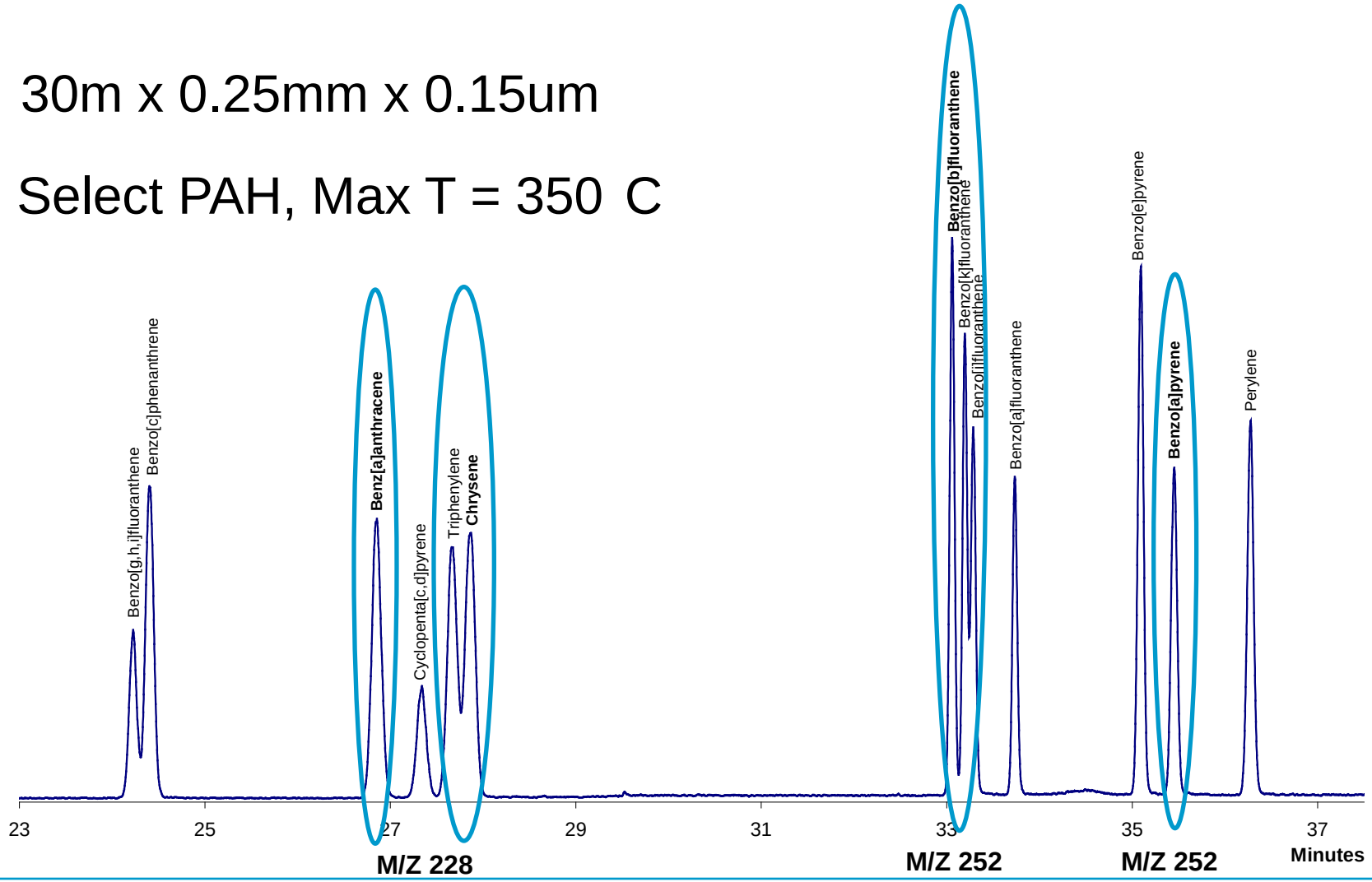
PAH4 on Select PAH

30m x 0.25mm x 0.15um

Select PAH, Max T = 350 C



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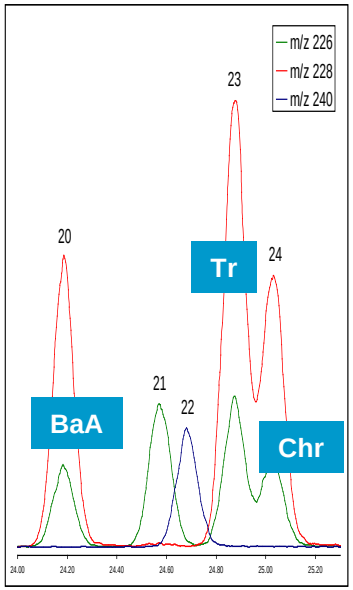
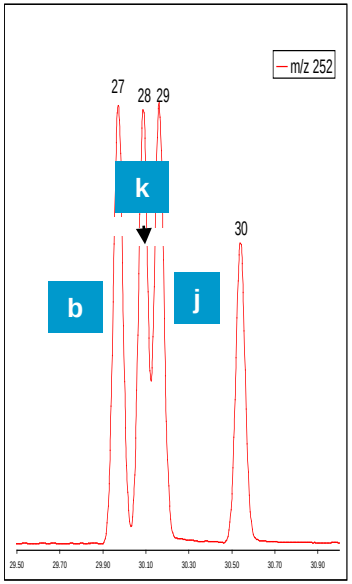
Agilent J&W Select PAH

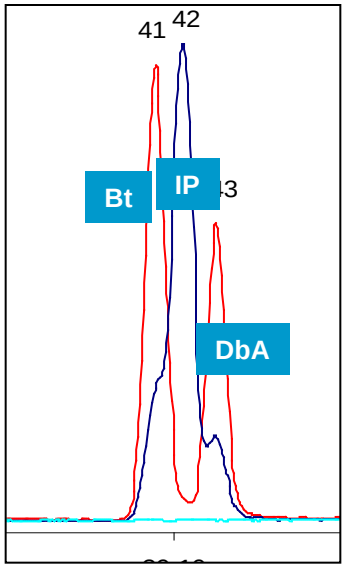
Siloxane liquid phase with PAH selector building blocks



Chrysene/Triphenylene/Benz(a)anthracene/CPP
(Mw 226,228)



Benzo(b,k,j)fluoranthenes
(Mw 252)



Indeno(1,2,3-cd)pyrene, benzo(b)triphenylene,
dibenzo(a,h)anthracene
(Mw 276,278)

Select PAH average column lifetime, 0.15mm ID

GC	HP6890
Column:	Select PAH, 15m x 0.15mm, df = 0.10µm
Temperature:	70°C (0.4 min), 70°C/min, 180°C, 7°C/min, 230°C (7 min), 50°C/min, 280°C (7 min), 30°C/min, 350°C (3.6min)
Carrier Gas:	Helium, constant flow 1.2 mL/min
Injector:	**PTV, 300°C**
Detector:	Agilent 5973, EI at 70eV in SIM mode, ion source 300°C, transfer line 280°C
Inj. Volume:	**50 µL**

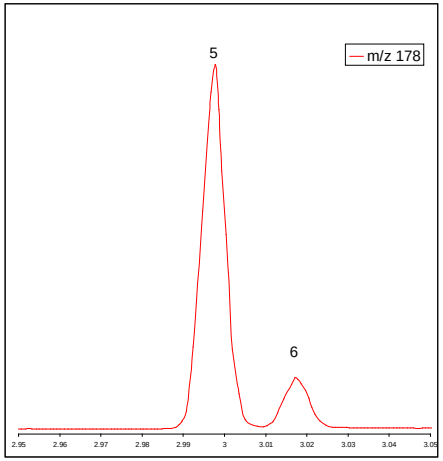
Salmon, olive oil, smoke flavoring matrices GPC clean-up

Serial No	Dimension	# Injections
2214871	15m x 015 x 010	1280
2213791	15m x 015 x 010	870
2214505	15m x 015 x 010	890
2213791	15m x 015 x 010	870
2213468	15m x 015 x 010	1450

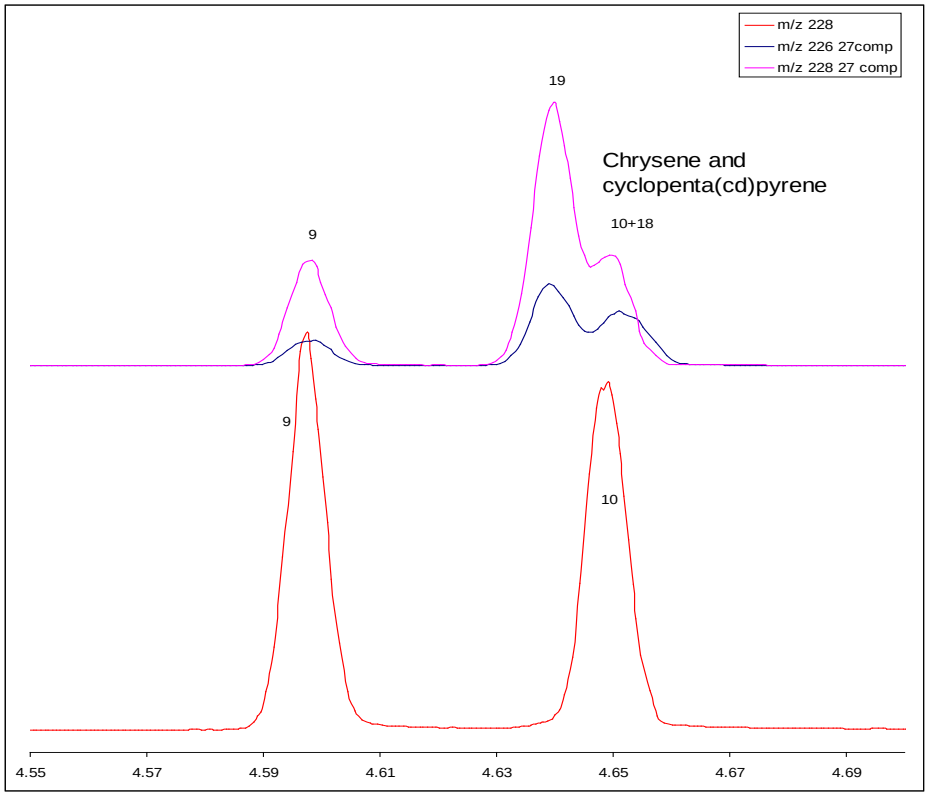
Average lifetime 2 – 4 months, ca. 1000 injections

Data courtesy: Eurofins WEJ, Hamburg

16 EPA PAHs on Select PAH column



Phenanthrene/Anthracene



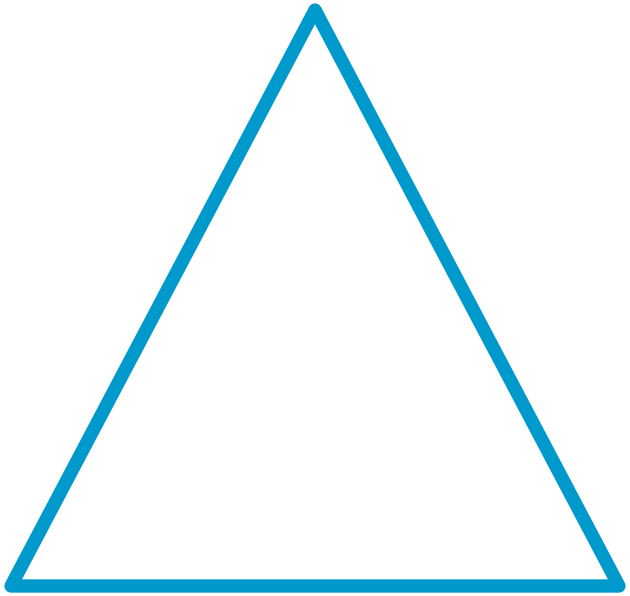
Chrysene/Triphenylene/Benz(a)anthracene

Select PAH Column
- Triphenylene bonus
- b,k,j, Fluoranthenes
- Within 10min Analysis

Selecting your GC column for PAH Analysis?

Smaller ID's
Thinner films

Analysis Time



Selectivity

Select right phase for job

Robustness

Select high temperature stable GC columns

Thank you for your attention