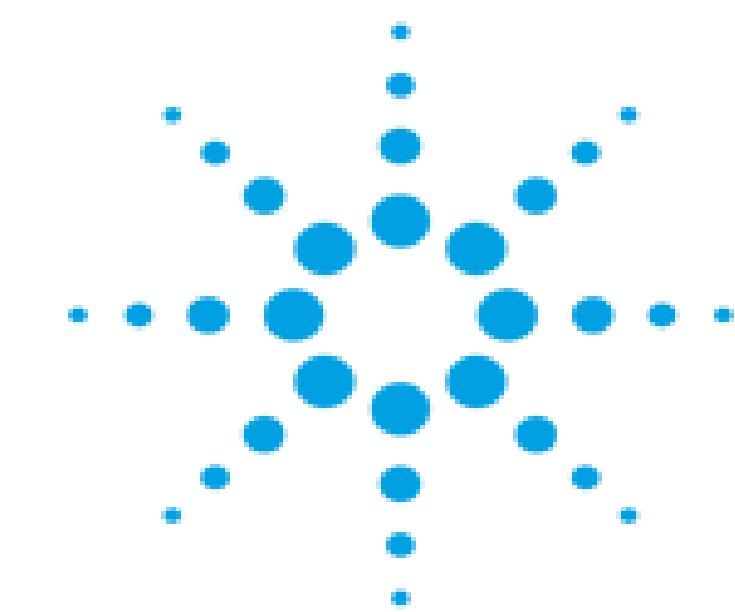


Improved GC/MS Analysis of Priority PAHs in Food Using a Highly Selective GC Column

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Abstract

Polycyclic Aromatic Hydrocarbons (PAHs) comprise of a large group of chemical compounds known to be cancer-causing agents. Some PAHs have been demonstrated to be carcinogenic and mutagenic. The scope of regulated PAHs is under constant change influenced by international advisory committees. Adaptations in regulation set the need for the quantification and therefore separation of PAHs previously not monitored.

For GC/MS analysis some co-eluting PAHs exhibit an identical MS fragmentation pattern. In order to obtain unambiguous identification and more accurate quantification of these PAHs an optimized capillary column selectivity and dedicated liquid phase chemistry is essential.

A new selective liquid phase dedicated for PAH analysis was developed with a unique ability to isolate chrysene from the interfering triphenylene and the simultaneous separation of the three benzo(b,k,j)fluoranthene isomers.

Also other critical peak pairs such as indeno(1,2,3-cd)pyrene, benzo(b)triphenylene and dibenzo(a,h)anthracene can be separated.

The column is highly thermally stable with low bleed characteristics at 350 °C which also enables the elution and detection of the high boiling dibenzopyrenes included in the SCF (Scientific Committee on Food) and EFSA (European Food Safety Authority) PAH priority list. This paper discusses the possibilities offered by this new liquid phase for more accurate reporting of PAHs. Applications are shown illustrating the efficient separation and identification of over 54 PAHs including all (15+1) EU and 16 US EPA priority PAHs as well as interfering isomers for food related matrices in a 30min analysis.

Introduction

Recent scientific opinions of the EFSA Panel on Contaminants in the Food Chain have led 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.

The accurate quantification of either the 16 EPA, PAH(15+1), PAH8 or PAH4 requires the separation of these individual PAHs either via mass ion and/or by chromatographic separation.

Some PAHs have identical mass fragmentation and in addition are difficult to separate on most GC columns. These PAH pairs can be identified as:

1. Phenanthrene/anthracene (Mw 178)
2. Benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(j)fluoranthene (BjF), (Mw 252)
3. Chrysene (CHR), triphenylene (TR), benz(a)anthracene (BaA), cyclopenta(c,d)pyrene (CPP), (Mw 226, 228)
4. Indeno(1,2,3-cd)pyrene (IcP), benzo(b)triphenylene (BtP), dibenzo(a,h)anthracene (DhA), (Mw 276,278)

The GC/MS analysis of PAHs is often performed on columns based on 5% or 50% phenyl modified dimethyl siloxane liquid phases. Although these columns may separate many isomers, they are unable to separate some of the critical peak pairs (figure 1-3).

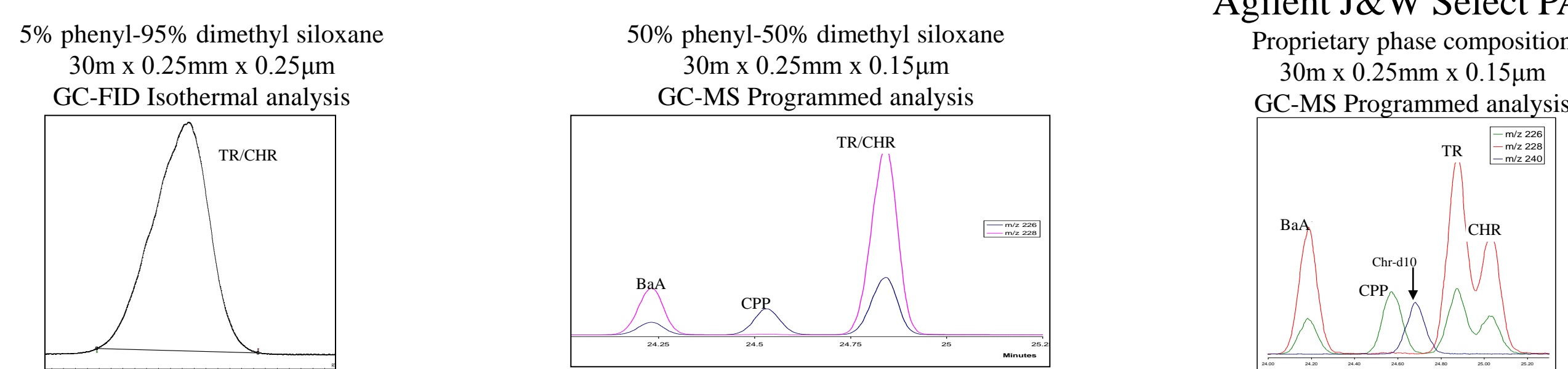


Fig. 1 Separation of chrysene, triphenylene (mw 228)

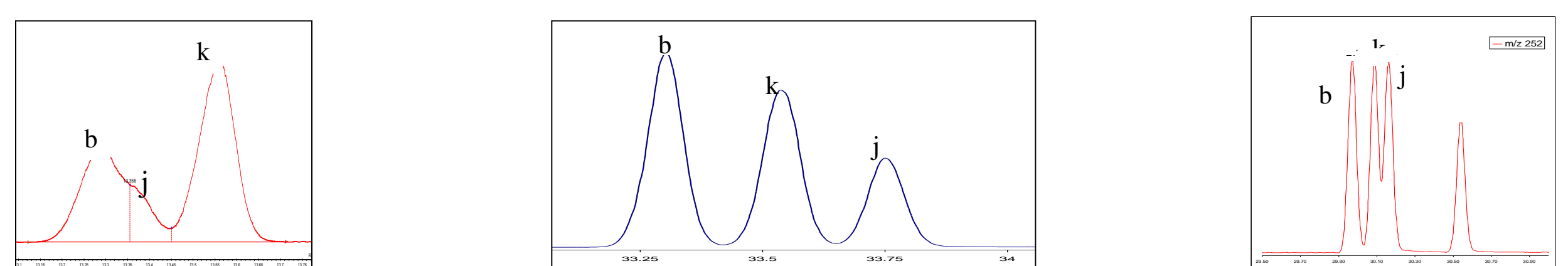


Fig. 2 Separation of benzo(b)fluoranthene, benzo(j)fluoranthene and benzo(k)fluoranthene (Mw 252)

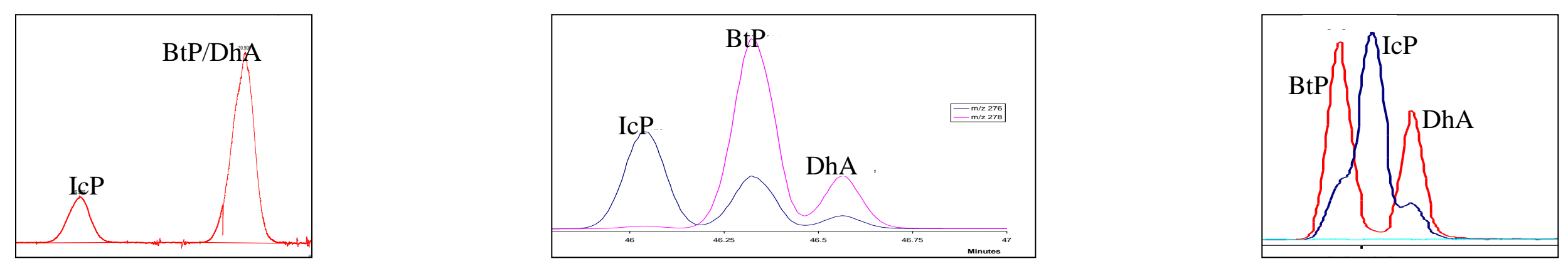


Fig. 3 Separation of indeno(1,2,3-cd)pyrene, benzo(b)triphenylene, dibenzo(a,h)anthracene (Mw 276,278)

The set benz(a)anthracene, cyclopenta(c,d)pyrene, chrysene and triphenylene, has different mass ions of m/z 226 and 228. The quantification of chrysene is often biased due to its coelution with triphenylene (fig. 1) The compounds with m/z 228 also contain fragments of m/z 226 and therefore optimized separation on the column is of great importance for accurate analysis.

Also for benzo(b)fluoranthene, benzo(j)fluoranthene and benzo(k)fluoranthene (fig. 2) with the same mass of m/z 252 an optimized separation on the GC column is mandatory.

The triplet indeno(1,2,3-cd)pyrene, benzo(b)triphenylene and dibenzo(a,h)anthracene (fig.3) exhibits m/z ratios of 276 and 278 and requires adequate chromatographic separation.

Figures 1-3 clearly show the ability of the new Agilent J&W Select PAH column to separate all of these critical peak pairs with GC-MS.

Agilent J&W Select for PAH Optimized Liquid Phase Chemistry

The Agilent J&W Select PAH column incorporates new highly selective dedicated selectors for PAH isomer separation. The percentage content of the different selectors was tuned to obtain the most optimal separation for PAH analysis in food and biota matrices. The liquid phase composition was optimized for the separation of the chrysene/triphenylene and benzo(b,k,j)fluoranthene peak pairs. Figure 4 shows a 45 min single run GC/MS analysis (TIC of SIM mode) of 54 PAHs including all EU and EPA regulated PAHs and important critical interfering congeners on a 30m x 0.25mm x 0.15µm column (fig. 10)

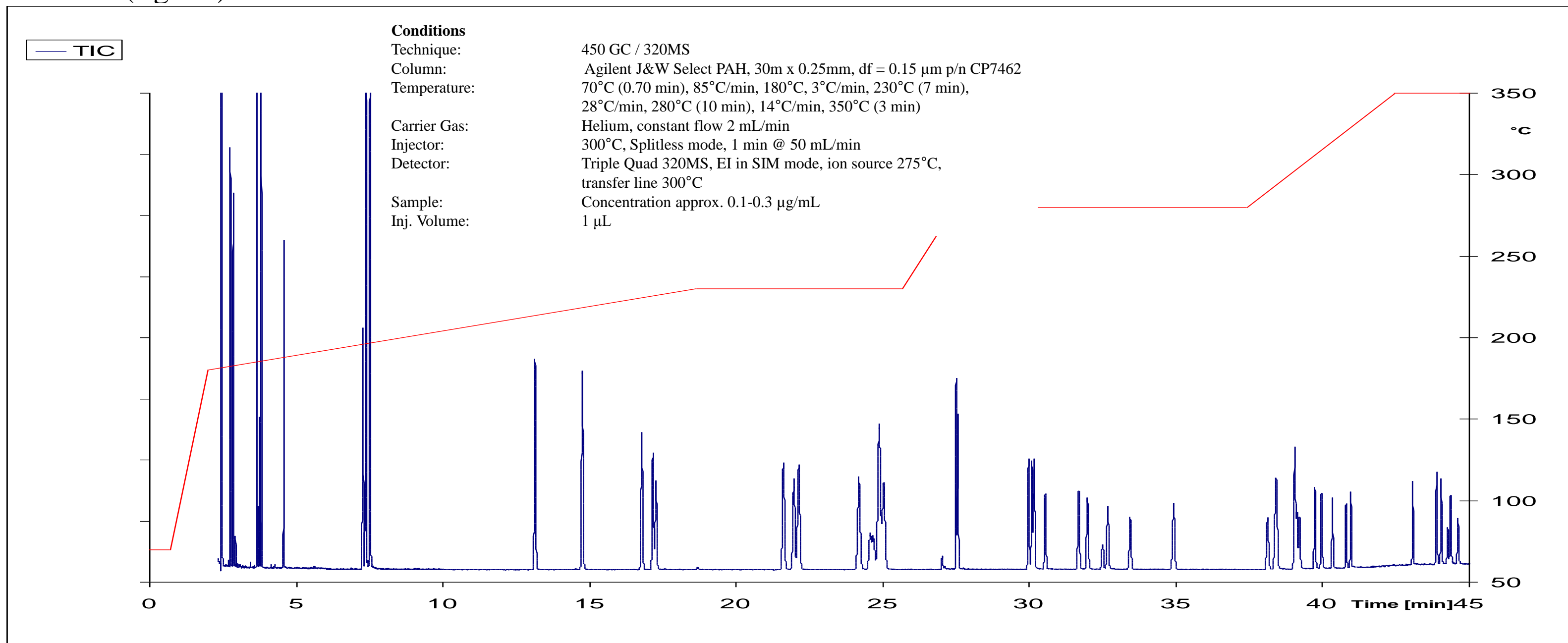


Fig. 4 GC/MS analysis (TIC of SIM mode) of 54 PAHs on 30m x 0.25mm x 0.15µm Select PAH column

The high thermal stability of the Agilent J&W Select PAH liquid phase is evident from the low baseline rise at 350°C (fig 4). Oven temperatures of 325°C are required to elute the high molecular weight 5 – 6 ring benzopyrenes. Shorter analysis times of 30 min can be achieved on a high resolution 15m x 0.15mm x 0.10µm Select PAH.

Peak number	MW	Compound	EPA 610	SCF and EFSA PAHs (15+1)	CAS
1	136	Naphthalene-dt			1146-65-2
2	128	naphthalene	x		81-20-3
3	152	2-methylnaphthalene			31-57-6
4	142	1-methylnaphthalene			90-12-0
5	152	acenaphthylene	x		208-96-3
6	164	Acenaphthene-d10			16087-26-2
7	154	acenaphthene	x		83-32-9
8	166	fluorene	x		86-73-7
9	188	phenanthrene-d10			1811-22-2
10	178	phenanthrene	x		85-01-8
11	178	anthracene	x		120-12-7
12	202	fluoranthene	x		208-98-0
13	202	pyrene	x		129-00-0
14	216	benzo(b)fluorene			238-64-0
15	216	benzo(b)fluorene			243-17-4
16	216	7H-benzo(c)fluorene			205-12-9
17	234	benzo(b)naphtho(2,1-d)pyrene			239-35-0
18	226	benzo(a,b)fluoranthene			203-12-3
19	228	benzo(k)fluoranthene	x		186-119-7
20	228	benzo(a)anthracene	x		125-28-5
21	226	Cyclopenta(c,d)pyrene	x		27208-37-3
22	226	Indeno(1,2,3-cd)pyrene	x		1719-00-5
23	228	triphenylene	x		217-59-4
24	228	chrysene	x		218-01-9
25	242	6-methylchrysene			1705-85-7
26	242	1-methylchrysene			3597-24-3
27	252	benzo(b)fluoranthene	x		205-99-2
28	252	benzo(k)fluoranthene	x		207-08-0
29	252	benzo(j)fluoranthene	x		203-33-8
30	252	benzo(a)anthracene	x		182-97-2
31	252	benzo(a)pyrene	x		56-32-8
32	252	benzo(a)pyrene	x		150-146-3
33	284	perylene			198-55-0
34	282	perylene			68-49-5
35	298	5,6-benzofluoranthene			216-105-1
37	276	dibenz(a,h)anthracene			226-36-8
38	274	dibenz(a,j)anthracene			274-42-0
39	278	dibenz(a,i)anthracene			224-41-1
40	292	dibenz(a,h)anthracene O14			13260-98-1
41	278	benzo(b)triphenylene	x		215-58-7
42	276	indeno(1,2,3-cd)pyrene	x		183-38-5
43	278	dibenz(a,h)anthracene	x		53-70-3
44	278	benzo(b)triphenylene			214-17-5
45	278	picene			213-46-7
46	276	benzo(b)triphenylene	x		191-24-2
47	276	dibenzodimethylchrysene			191-25-0
48	287	7H-benzo(c,g)carbazole			194-59-2
49	302	dibenz(a)pyrene	x		181-30-0
50	302	dibenz(a)pyrene	x		182-65-4
51	300	coronene			181-07-1
52	302	benzo(b)pyrene			197-70-6
53	302	benzo(b)pyrene			182-65-4
54	302	dibenz(a)pyrene	x		189-84-0

Fig.5 EPA and SCF/EFSA polycyclic aromatic hydrocarbons and interfering PAH isomers

PAH analysis in food matrices using Agilent J&W Select PAH

The performance of the Select PAH column for GC/MS in SIM mode was verified for salmon and smoke flavor. Salmon was spiked with a mixture of EPA and EU regulated PAHs as well as triphenylene as important interference. The PAH concentration range was variable with < 0.5 ppb up to 10 ppb for benzo(a)pyrene.

Sample preparation

For saponification a solution of potassium hydroxide in methanol was added to the homogenised and weighted food samples. After completed saponification the PAHs were extracted with cyclohexane. The extract was concentrated and a further clean-up was carried out using a fully automated gel permeation chromatography (GPC) being directly coupled to an evaporation unit in the same analytical system (TACS, LC-Tech, Dorfen, Germany). Dichloromethane was used as eluent. The collected fraction containing the PAH was concentrated and analysed by GC-MS

Figure 10 illustrates the difference in PAH elution pattern and separation between a 5% phenyl column and the Select PAH column for spiked EPA and EU PAHs in salmon.

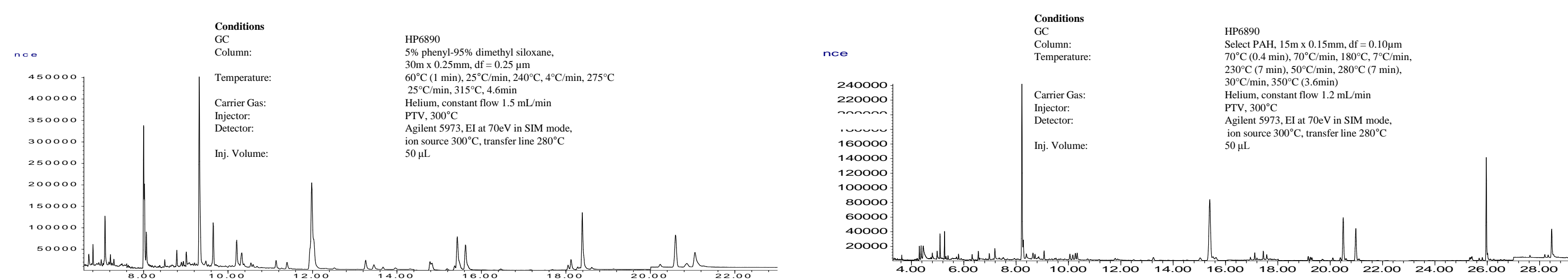


Fig. 10 GC/MS analysis (TIC) of PAHs in salmon on 5% phenyl-95% dimethyl siloxane and Select PAH columns

PAH analysis in salmon matrix using Agilent J&W Select PAH

Separation characteristics of a 5% phenyl-95% dimethyl siloxane and Select PAH column (fig. 11 – 15) in SIM mode for salmon oil illustrate the high degree of separation detail on the Select PAH column and subsequent reduced biased quantification of all EU- priority PAHs.

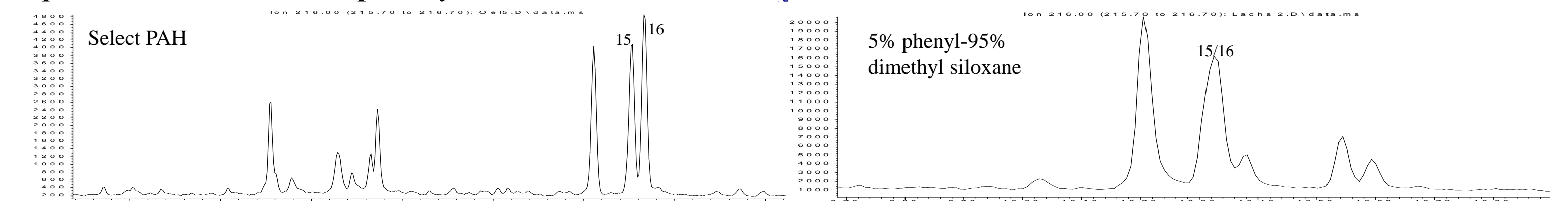


Fig. 11 Separation of benzo(b)fluorene (15) and 7H-benzo(c)fluorene (16), Mw 216

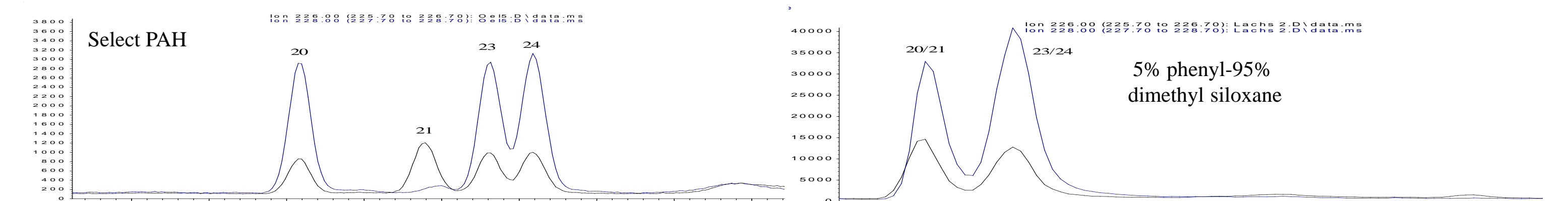


Fig. 12 Separation of benz(a)anthracene (20), cyclopenta(c,d)pyrene (21), chrysene (24) and interfering triphenylene (23), Mw 226,228

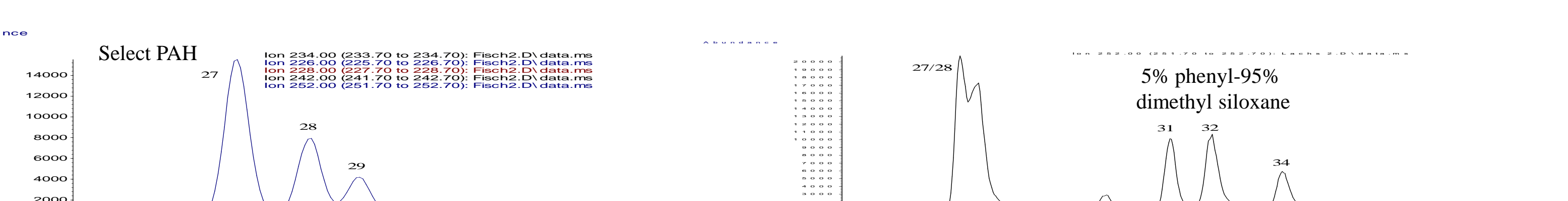


Fig. 13 Separation of benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(j)fluoranthene (BjF), (Mw 252)

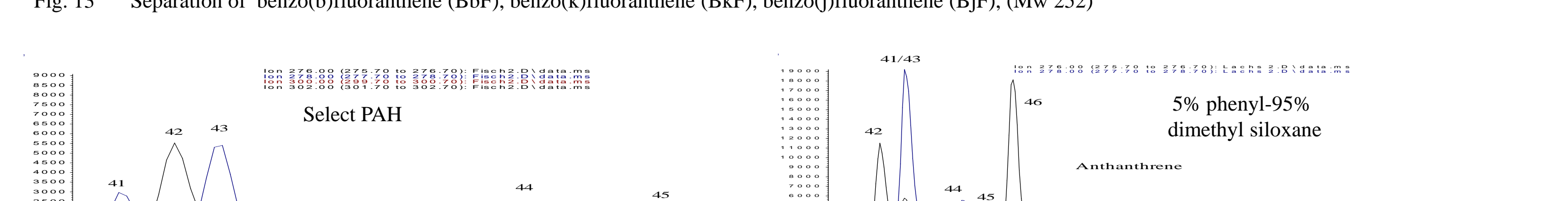


Fig. 14 Separation of benzo(b)triphenylene (41), indeno(1,2,3-cd)pyrene (42), dibenz(a,h)anthracene (43) and interfering benzo(b)chrysene (44), picene (45), Mw 276,278

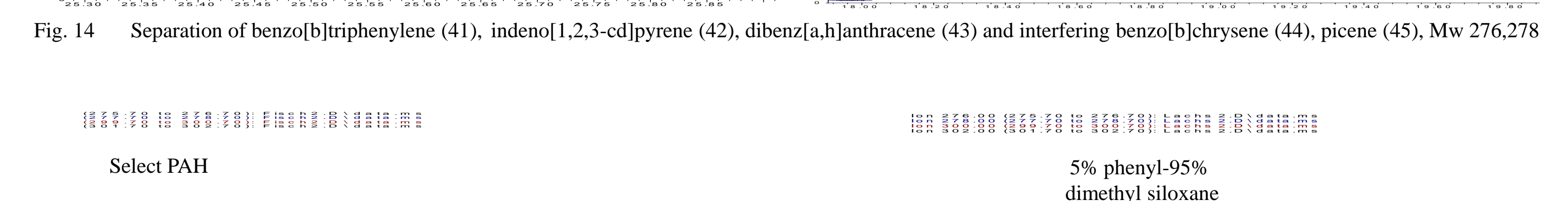


Fig. 15 Elution of the dibenzopyrenes (Mw 302) at 325-350°C (Varian PAH) and 315°C (5% phenyl-95% dimethyl siloxane)

Analysis of smoke flavor using the Agilent J&W Select PAH column

Figure 16 give separation details of chrysene/triphenylene in the PAHs analysis of smoke flavor using the Select PAH column. The separation is not baseline but allows adequate quantification of chrysene. Figure 17 shows the difference in reported chrysene concentration levels between an analysis on a 5% phenyl-95% dimethyl siloxane and Select PAH as well as other important PAHs.

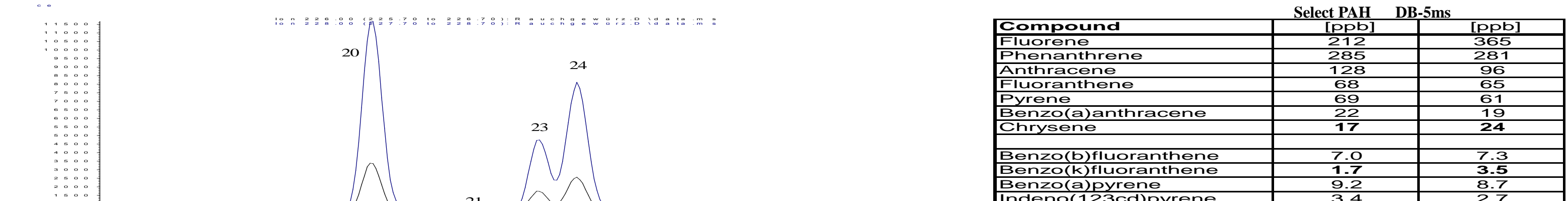


Fig. 16 Separation of benz(a)anthracene (20), cyclopenta(c,d)pyrene (21), chrysene (24) and interfering triphenylene (23), Mw 226,228

Compound	Select PAH (ppb)	DB-5ms (ppb)
Fluorene	212	365
Phenanthrene	286	281
Anthracene	128	96
Fluoranthene	68	65
Pyrene	69	63
Benzo(a)anthracene	22	19
Chrysene	17	24
Benzo(b)fluoranthene	7.0	7.3
Benzo(k)fluoranthene	4.7	3.5
Benzo(a)pyrene	9.2	8.7
Indeno(1,2,3-cd)pyrene	3.4	2.7
Dibenz(a,h)anthracene	< 0.5	< 0.5
Benzo(ghi)perylene	2.4	2.4
Benzo(e)pyrene	6.9	7.7
Perylene	1.4	< 1
Anthracene	1.3	< 1
Coronene	< 1	< 1
Dibenz(a)pyrene	< 1	< 1
Dibenz(a)pyrene	< 1	< 1
Dibenz(a)pyrene	< 1	< 1
Cyclopenta(c,d)pyrene	0.5	5.4
Methylchrysene	1.2	1.4
Benzo(a)anthracene	2.1	2.1
Benzo(ghi)perylene	2.4	2.4

Fig. 17 Smoke flavor, PAH concentration levels

Conclusion

The emphasis of recent years in consumer safety has led to more stringent regulations for the toxic PAHs in food. So far, the complete separation of all 16 EPA and (15+1) EU priority PAHs and their interferences was impossible using currently available GC columns of the 5ms or 17ms type. The separation of critical peak pairs on the Agilent J&W Select PAH such as chrysene/triphenylene is a unique feature. The column also elutes the high molecular weight dibenzopyrenes as sharp peaks with low background noise and good signal-to-noise.

The presented applications show the isolation all EPA and EU priority PAHs by a single GC/MS analysis in SIM mode using the Agilent J&W Select PAH column thus improving accuracy and reliability of data for the determination of PAH toxicity levels in food.

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