GC/MS analysis of 16 EPA and (15+1) EU PAHs in salmon using a Agilent J&W Select PAH GC column

Application Note

Authors

Johan Kuipers and John Oostdijk
Agilent Technologies, Inc.

Claudia Schulz, Ansgar Ruthenschroer,
Holger Fritz
Eurofins WEJ Contaminants GmbH

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) comprise of a large group of chemical compounds known to be cancer-causing agents. They may occur in food either by uptake from the environment or are generated during food processing. 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 separation of PAHs has previously not been monitored. Benzo[a]pyrene was thought to be a suitable marker of the occurrence and effect of carcinogenic PAHs in food and is until now the only regulated one. The sum of benzo[a]pyrene, chrysene, benz[a]anthracene and benz[b]fluoranthene (PAH4) has been recommended by EFSA as suitable indicator. An amendment of the EU regulation towards PAH in 2010 is announced. The accurate quantification of either EPA or EU priority (15+1) PAHs requires the separation of these individual PAHs either via mass ion and/or by chromatographic separation. 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. The presented application shows the ability to isolate all EPA and EU priority PAHs (15+1) by a single GC/MS analysis in SIM mode using the Select PAH column thus improving accuracy and reliability of data for the determination of PAH toxicity levels in food.

The Select PAH GC column introduces a novel, high selectivity stationary phase for accurate analysis of PAHs, providing a single solution for PAH analysis separating all relevant isomers, thereby avoiding false positives and inaccurate results. The selective liquid phase has the unique ability to isolate chrysene from the interfering triphenylene and simultaneously separates 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 dibenz[a,h]anthracene can be separated. The Select PAH column shows a highly thermally stable profile with low bleed characteristics at 350 °C which enables the elution and low threshold detection of the high boiling dibenzopyrenes included in the EFSA PAH priority list. This application note illustrates the possibilities offered by this new liquid phase for more accurate reporting of PAHs including all (15+1) EU and 16 US EPA priority PAHs as well as interfering isomers for a salmon matrix in a 30 min GC-MS analysis.

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PAH analysis in salmon using Select PAH

The PAH separation performance of the Select PAH column for GC/MS in SIM mode was verified for a salmon sample. The sample used was a certified material which was additionally spiked with EU priority PAHs as well as triphenylene as important interference. The PAH concentration range was variable from 1 ppb up to 7 ppb.

Sample preparation

An initial weight of 2 g homogenized sample material was used followed by the addition of a deuterated internal standard solution. For saponification, 100 mL of a 1 M solution of methanolic potassium hydroxide solution were added to the weighted food samples. After complete saponification the PAHs were extracted several times with a total of 150 mL cyclohexane. The combined organic extract was dried and concentrated. A further clean-up was carried out using a fully automated gel permeation chromatograph (GPC) being directly coupled to an evaporation unit in the same analytical system. Dichloromethane was used as the eluent. The collected fraction containing the PAHs was concentrated and analyzed by GC-MS.

Results and discussion

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. Benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[j]fluoranthene (BjF), (Mw 252)

2. Chrysene (CHR), triphenylene (TR), benz[a]anthracene (BaA), cyclopenta[c,d]pyrene (CPP), (Mw 226, 228)

3. Indeno[1,2,3-cd]pyrene (IcP), benzo[b]triphenylene (BtP), dibenzo[a,h]anthracene (DhA), (Mw 276,278)

4. Benzo[b]fluorene (BbF) and benzo[c]fluorene (BcF), (Mw 216)

Figures 2-5 illustrate the excellent separation that can be obtained on the Select PAH column for the four critical peak pairs. The 50% valley separation between triphenylene and chrysene allows unambiguous quantification of chrysene (figure 3), included in the EU priority PAH as well as in the PAH4. The benz[a]anthracene (Mw 228) is adequately isolated from interfering masses. At the same time an optimal separation of benzo[b]fluoranthene, benzo[k]fluoranthene and benzo[j]fluoranthene (shown in figure 4) can be achieved. Figure 5 demonstrates the good peak shape and therefore

good sensitivity for the highly toxic dibenzopyrenes and coronene eluting at higher oven operating temperatures. The sample preparation using GPC fractionating limits the level of background matrix interference benefitting both method accuracy at the low ppb level and GC column longevity.

The data shown in table 2 proves a good repeatability for the applied method with an LOQ of either 0.5 ppb or 1 ppb for all compounds. Standard deviations and variation coefficients for the repeatability are within the limits generally required. Furthermore, the linearity was determined for all compounds with standards at 10 different levels ranging from 1 ng/mL up to 400 ng/mL. For all compounds, a coefficient of determination of equal or more than 0.995 can be achieved. The accuracy for all PAH is between 74% up to 117%, and demonstrates the accurate measurement with no overestimation due to insufficient separation of the relevant PAH.

Conditions

Technique:	GC/MS
Column:	Select PAH, 15m x 0.15mm, df = 0.10µm (p/n CP7461)
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 (3.6 min)
Carrier Gas:	Helium, constant flow 1.2 mL/min
Injector:	PTV, large volume injection
Detector:	MSD, EI at 70eV in SIM mode, ion source 300 °C, transfer line 300 °C
Inj. Volume:	50 µL

Table 1. Peak Identification for Figure 1

Peak	MW	Compound	EPA 610	EU priority PAHs	CAS
1	136	Naphthalene-d8			1146-65-2
2	128	naphthalene	x		91-20-3
3	142	2-methylnaphthalene			91-57-6
4	142	1-methylnaphthalene			90-12-0
5	152	acenaphthylene	x		208-96-8
6	164	Acenaphthene-d10			15067-26-2
7	154	acenaphthene	x		83-32-9
8	166	fluorene	x		86-73-7
9	188	Phenanthrene-d10			1517-22-2
10	178	phenanthrene	x		85-01-8
11	178	anthracene	x		120-12-7
12	202	fluoranthene	x		206-44-0
13	202	pyrene	x		129-00-0
14	216	benzo[a]fluorene			238-84-6
15	216	benzo[b]fluorene			243-17-4
16	216	7H-benzo[c]fluorene			205-12-9

Peak	MW	Compound	EPA 610	EU priority PAHs	CAS
17	234	benzo[b]naphto[2,1-d]thiophene			239-35-0
18	226	Benzo[g,h,i]fluoranthene			203-12-3
19	228	Benzo[c]phenanthrene			195-19-7
20	228	benz[a]anthracene	x	x	56-55-3
21	226	Cyclopenta[c,d]pyrene		x	27208-37-3
22	240	Chrysene-d10			1719-03-5
23	228	Triphenylene			217-59-4
24	228	chrysene	x	x	218-01-9
25	242	6-methylchrysene			1705-85-7
26	242	5-methylchrysene		x	3697-24-3
27	252	Benzo[b]fluoranthene	x	x	205-99-2
28	252	benzo[k]fluoranthene	x	x	207-08-9
29	252	Benzo[j]fluoranthene		x	205-82-3
30	252	Benzo[a]fluoranthene			203-33-8
31	252	Benzo[e]pyrene			192-97-2
32	252	benzo[a]pyrene	x	x	50-32-8
33	264	Perylene-d12			1520-96-3
34	252	Perylene			198-55-0
35	268	3-methylcholanthrene			56-49-5

| Peak	MW	Compound	EPA 610	EU priority PAHs	CAS
36	330	9,10-diphenylanthracene			216-105-1
37	279	dibenzo[a,h]acridine			226-36-8
38	279	dibenzo[a,j]acridine			224-42-0
39	278	dibenz[a,j]anthracene			224-41-9
40	292	Dibenzo[a,h]anthracene D14			13250-98-1
41	278	Benzo[b]triphenylene			215-58-7
42	276	indeno[1,2,3-cd]pyrene	x	x	193-39-5
43	278	dibenz[a,h]anthracene	x	x	53-70-3
44	278	Benzo[b]chrysene			214-17-5
45	278	Picene			213-46-7
46	276	benzo[ghi]perylene	x	x	191-24-2
47	276	Dibenzo[def,mno]chrysene			191-26-4
48	267	7Hdibenzo[c,g]carbazole			194-59-2
49	302	Dibenzo[a,l]pyrene		x	191-30-0
50	302	Dibenzo[a,e]pyrene		x	192-65-4
51	300	coronene			191-07-1
52	302	Benzo[b]perylene			197-70-6
53	302	Dibenzo[a,i]pyrene		x	189-55-9
54	302	Dibenzo[a,h]pyrene		x	189-64-0
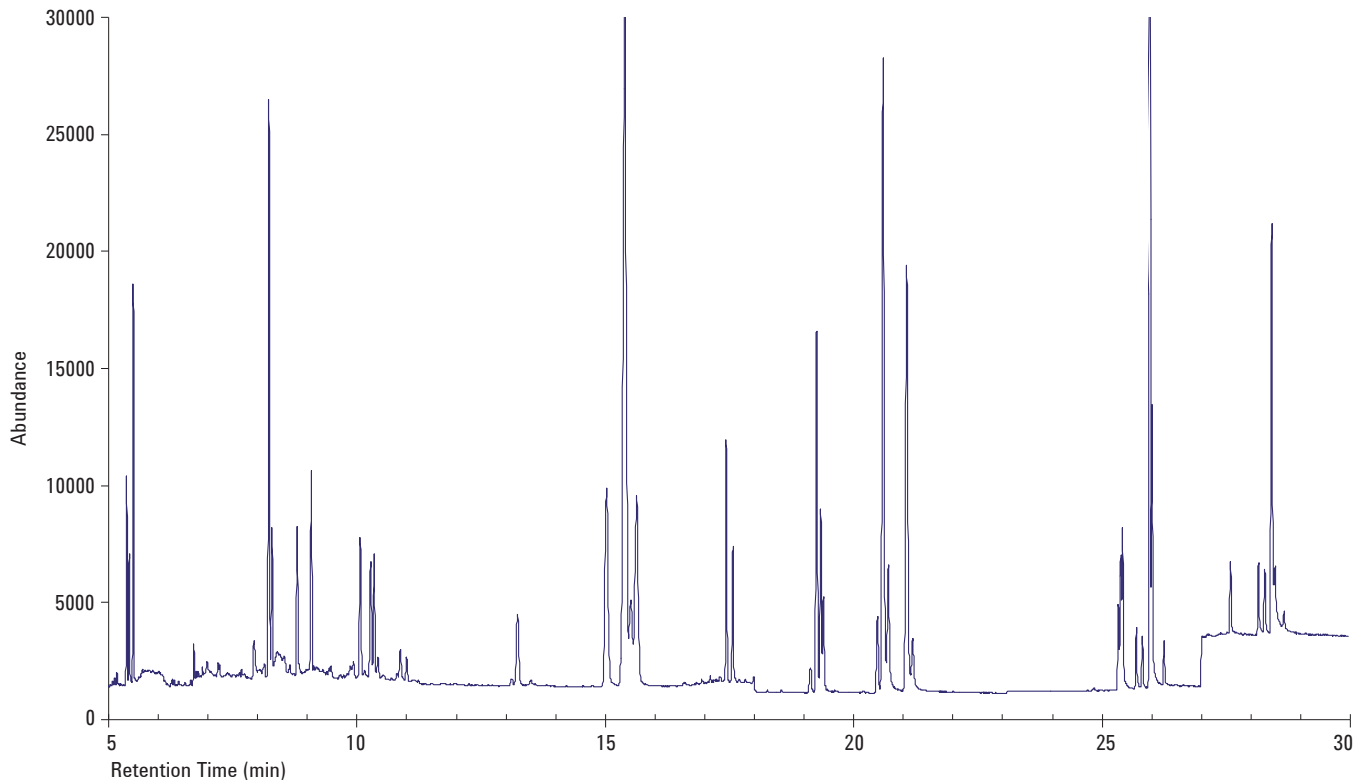
Figure 1. GC/MS analysis (TIC) of PAHs in salmon using Select PAH column, 15 m x 0.15 mm x 0.10 µm

Table 2. Repeatability data of 32 spiked PAHs in salmon by GPC clean-up and GC/MS analysis

| Analyte	EPA	EU	Average (ppb)	SD (ppb)	Spiked Value (ppb)	Variation Coefficient VC (%)	Trueness (%)
Phenanthrene	x		6.6	1.41	6.70	21.5	98
Anthracene	x		3.2	0.42	2.70	13.1	117
Fluoranthene	x		2.7	0.20	2.90	7.3	95
Pyrene	x		3.5	0.71	3.50	20.0	101
Benzo[a]anthracene	x	x	3.7	0.26	3.70	7.1	99
Chrysene	x	x	3.6	0.29	3.40	8.0	106
Benzo[b]fluoranthen	x	x	3.2	0.36	3.60	11.1	89
Benzo[k]fluoranthen	x	x	1.2	0.17	1.30	14.1	94
Benzo[a]pyrene	x	x	1.6	0.12	1.70	7.0	97
Indeno[1,2,3-cd]pyrene	x	x	1.6	0.22	1.50	14.1	103
Dibenzo[a,h]anthracene	x	x	1.2	0.17	1.30	14.1	95
Benzo[g,h,i]perylene	x	x	1.5	0.11	1.60	7.4	94
Benzo[e]pyrene			1.9	0.14	2.00	7.1	96
Perylene			2.0	0.13	2.00	6.3	99
Anthanthrene			1.9	0.12	2.00	6.6	93
Coronene			1.8	0.31	2.00	16.7	92
Dibenzo[a,l]pyrene		x	2.3	0.25	2.00	10.8	115
Dibenzo[a,i]pyrene		x	1.9	0.14	2.00	7.6	93
Dibenzo[a,h]pyrene		x	1.8	0.19	2.00	10.7	91
Dibenzo[a,e]pyrene		x	1.9	0.29	2.00	15.2	96
Cyclopenta[c,d]pyrene		x	1.5	0.09	2.00	5.8	74
Methylchrysene		x	2.3	0.25	2.00	11.1	114
Benzonaphthothiophen			2.1	0.30	2.00	14.6	104
Benzo[c]fluorene		x	2.1	0.34	2.00	15.8	107
Triphenylene			1.6	0.12	2.00	7.6	81
Benzo[j]fluoranthene		x	2.1	0.17	2.00	8.3	104
Benzo[b]triphenylene			1.8	0.30	2.00	16.7	90
Picene			2.3	0.18	2.00	7.6	116
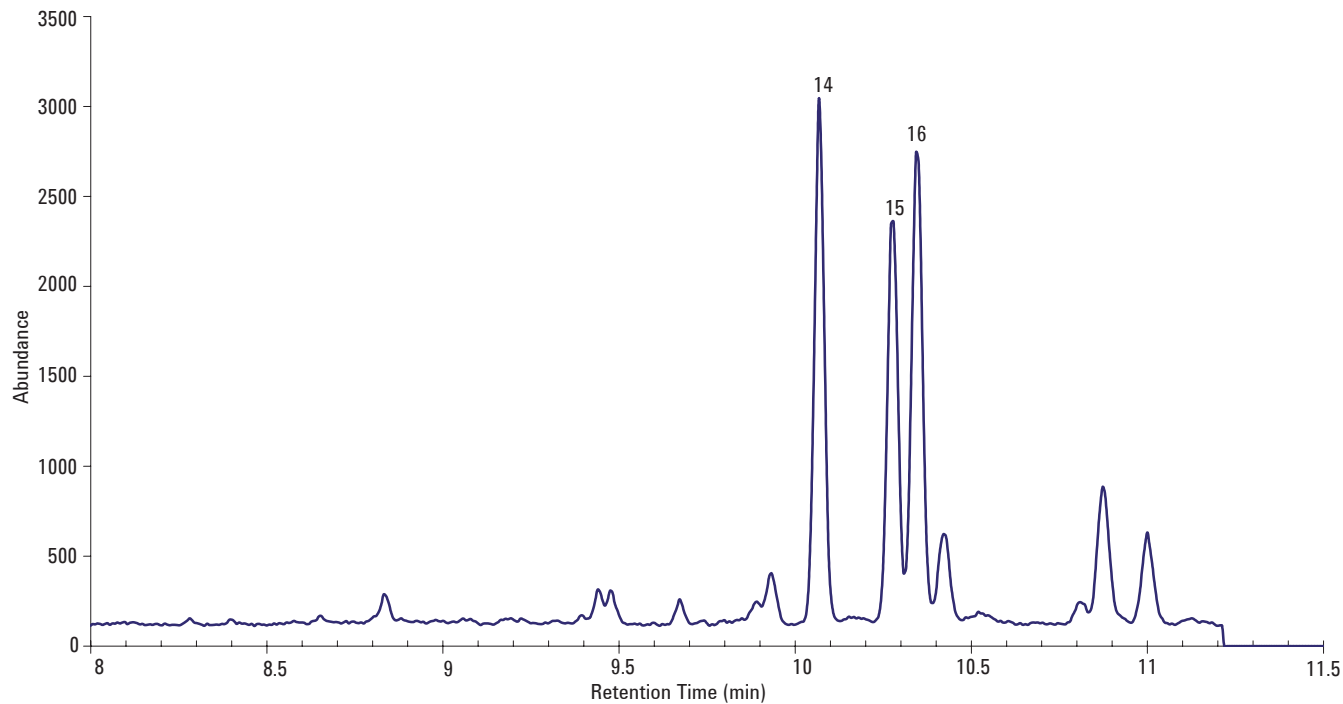
Figure 2. Separation of benzo(b)fluorene (15) and 7H-benzo(c)fluorene (16), m/z 216
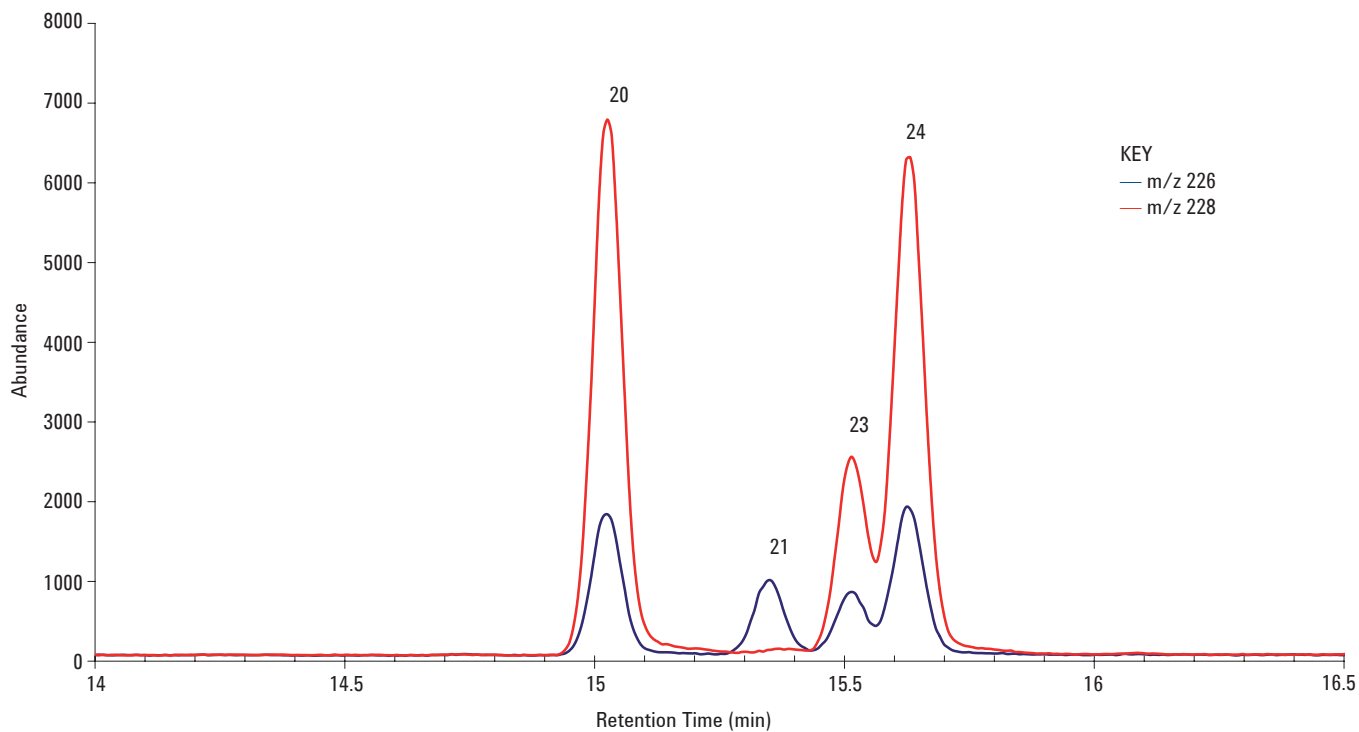

Figure 3. Separation of benz[a]anthrancene (20), cyclopenta[c,d]pyrene (21), chrysene (24) and interfering triphenylene (23), m/z 226 (blue), m/z 228 (red)
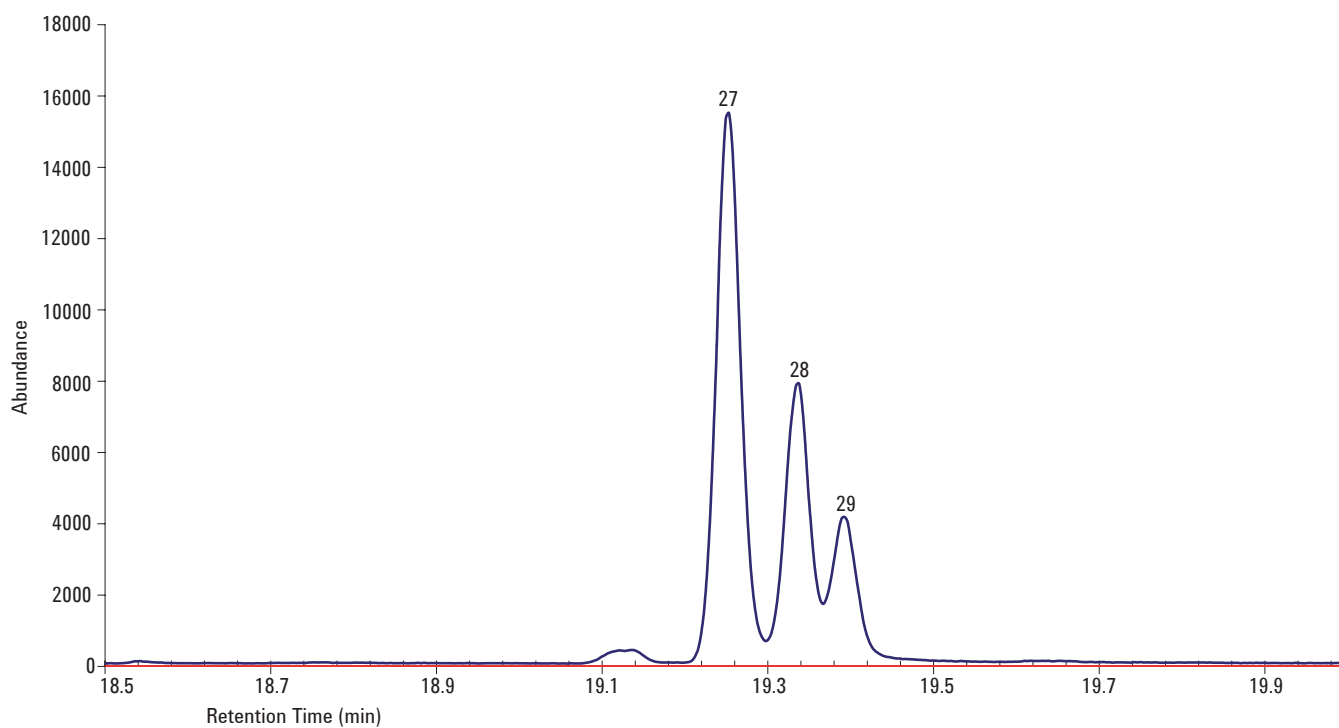
Figure 4. Separation of benzo[b]fluoranthene (27), benzo[k]fluoranthene (28), benzo[j]fluoranthene (29), (m/z 252)
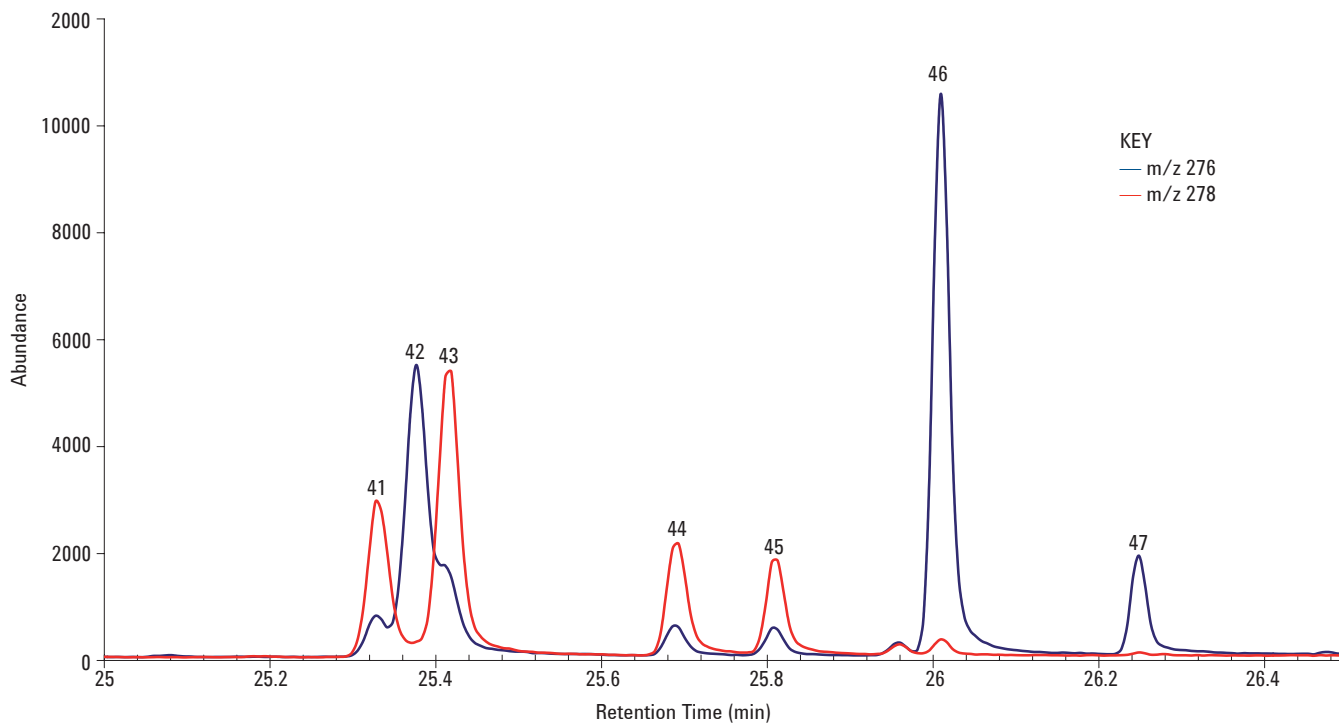

Figure 5. Separation of benzo[b]triphenylene (41), indeno[1,2,3-cd]pyrene (42), dibenz[a,h]anthracene (43),benzo[b]chrysene (44), picene (45), benzo[ghi] perylene (46), dibenzo[def,mno]chrysene (47) m/z 276 (blue), m/z 278 (red)
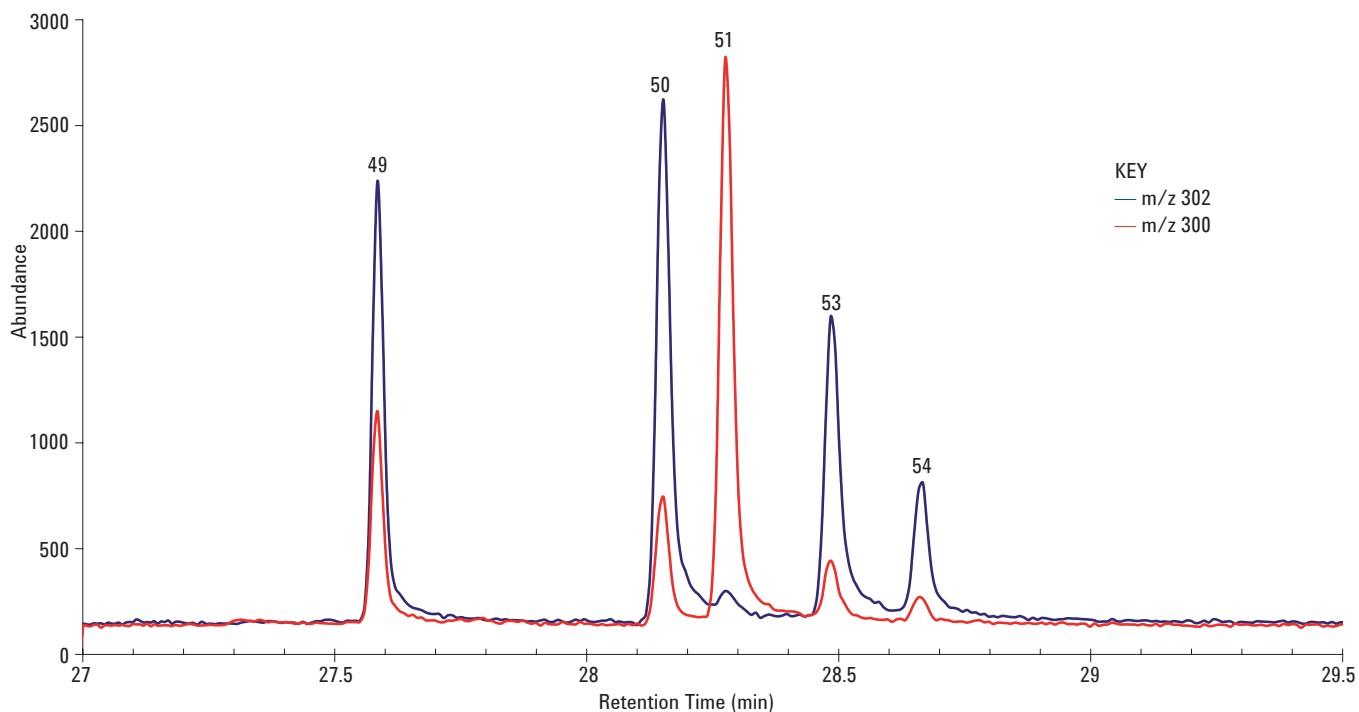
Figure 6. Elution of the dibenzopyrenes (m/z 302, blue) and coronene (51, m/z 300, red) at 325 – 350 °C

Conclusions

The emphasis of recent years in consumer safety in the EU has lead to more stringent regulations for the toxic PAHs in food. So far, complete separation of all (15+1) EU priority PAHs and their interferences on a single column was not possible using currently available GC columns. The combined separation of chrysene/triphenylene and the three benzofluoranthene isomers is a unique feature of the Select PAH column and impossible on other commercially available columns. The column also elutes the high molecular weight dibenzopyrenes as sharp peaks with low column background and good signal-to-noise. The presented application shows the ability of the Select PAH column to isolate all EPA and EU priority PAHs by a single GC/MS analysis in SIM mode. The column offers a higher degree of accuracy and reliability for identification and quantification of PAHs in food.

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