Application Note: 30126

Fast GC/HRMS Quantification of 16 EC Priority PAH Components

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Key Words

- DFS
- Food Analysis
- HRGC/HRMS
- Quantitation

Introduction

The European Commission Regulation (EC) No 208/2005 of February 4, 2005, which came into force on April 1, 2005, provides maximum levels for benzo[a]pyrene in different groups of food of which have the strongest regulation foods for infants and young children with max. 1.0 µg/kg and smoked meats and smoked meat products with max. 5.0 µg/kg[1].

The best characterized carcinogenic compound benzo[a]pyrene is used as leading substance out of about 250 different compounds which belong to the PAH group. The German revision of the flavour directive of Mai 2, 2006 (Aromenverordnung) regulates the maximum level for benzo[a]pyrene at 0.03 µg/kg for all types of food with added smoke flavourings.

The Commission Recommendation of February 4, 2005 on the further investigation on the levels of polycyclic aromatic hydrocarbons in certain types of food is directed to analyse the levels of 15 PAH compounds which are classified as priority (see Figure 1) and to check the suitability of benzo[a]pyrene as a marker[2].

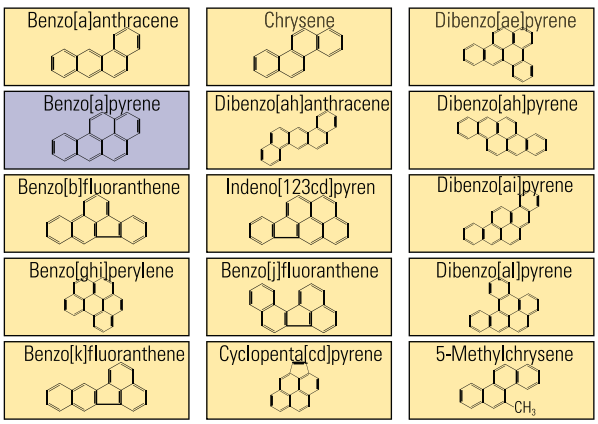

Figure 1: 15 PAH priority compounds classified by the European Commission regulation.

In addition, the Joint FAO/WHO Experts Committee on Food Additives (JECFA) identified the PAH compound benzo[c]fluorene as to be monitored as well[3] see Figure 2.

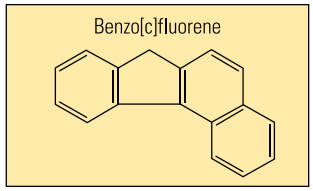

Figure 2: Additional PAH priority compound to be monitored according to JECFA[2].

During GC/MS method setup it turned out that single quadrupole desktop MS instruments could not provide the necessary selectivity at the low decision level[4,5].

The quantitation was done using isotope dilution technique by the addition of isotopically labeled and fluorinated standards before extraction, as well as for the determination of the response factors of all PAH under investigation, see Table 3. Recovery values have been determined by the addition of three ^{2}H labelled compounds, see Table 4.

Analytical Method

The clean-up applied use three steps of pressurized solvent extraction (PSE) for extraction of the lipophilic substances, followed by size exclusion chromatography for the separation from higher molecular substances, and finally a solid phase extraction to remove polar substances.

Experimental Conditions

All measurements were carried out on the Thermo Scientific DFS High Resolution GC/MS system coupled to a Thermo Scientific TRACE GC Ultra™ gas chromatograph equipped with a split/splitless injector. Samples were injected using the Thermo Scientific TriPlus™ Autosampler.

Pressurized solvent extraction (PSE): The homogenized sample (4-6 g meat product, 1-2 g spice) was levigated with the same amount of the drying material poly(acrylic acid), a partial sodium salt-graft-poly(ethylene oxide).

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The resulting material was poured into 33-mL cells, which were locked with glass microfiber filters at the outlet end of the extraction cells. 50 µL of a PAH standard mixture containing the isotope labelled (^{13}C and ^{2}H) and fluorinated PAH compounds were added. The extraction was performed using an ASE 200 unit (Dionex, Sunnyvale, USA) and carried out with n-hexane at 100 °C and 100 bar with a static time of 10 min. The flush volume was 60% and the purge time 120 s. Two static cycles were accomplished. The solvent of the extract was evaporated in a water bath (40 °C) using a nitrogen stream.

Gel permeation chromatography (GPC): The evaporated ASE extract was dissolved in 4.5 mL cyclohexane/ethylacetate (50:50 v/v) and filtered through a PTFE filter with a pore size of 1 µm. The GPC column (25 mm i.d.) was filled with Bio-Beads S-X3 (height of filling 42 cm). Samples were eluted at a flow rate of 5 mL/min with cyclohexane/ethylacetate (50:50 v/v) (dump time 0-36 min, collect time 36-65 min). The solvent was removed with a rotary evaporator, and the eluate was dried in a nitrogen stream.

Solid phase extraction (SPE): This clean-up step to remove more polar substances was performed automatically with a modified ASPEC Xli[6]. This system was modified with a fitting rack, teflon funnels and teflon tubes. Silica, dried for 12 h at 550 °C, was deactivated with 15% water. 1 g dried deactivated silica was filled into commercial 8-mL SPE columns (12 mm i.d.). After conditioning of the columns with 3 mL cyclohexane the samples were applied and eluted with 10 mL cyclohexane.

Preparation for GC/MS analysis: The dried eluate of SPE was dissolved in 1 mL isooctane and 50 µL of the PAH recovery standard mixture (benzo[a]anthracene-d_{12} and benzo[a]pyrene-d_{12} in isooctane) and transferred to a 1 mL tapered vial. The sample was carefully concentrated in a nitrogen stream to a volume of about 50 µL.

GC Parameters	
Injector:	Split/splitless, 1 min, 320 °C, 1.5 µL injection volume with Merlin seal
Carrier gas:	He, 0.6 mL/min, const. flow
Column:	TRACE™-50MS, 10 m x 0.1 mm x 0.1 µm
Oven Temp. Program:	140 °C, 1 min
	10 °C/min to 240 °C
	5 °C/min to 270 °C
	30 °C/min to 280 °C
	4 °C/min to 290 °C
	30 °C/min to 315 °C
	3 °C/min to 330 °C
MS Interface Temperature:	transfer line 300 °C ion source 280 °C

Table 1: GC parameters.

MS Parameters	
Ionization:	EI, 45 eV pos.
Scan Mode:	Multiple ion detection mode (MID)
Resolution:	8,000, 10% valley definition
Cycle Time:	0.8 s/scan

Table 2: MS parameters.

PAH	PAH shortform	Exact mass native [u]	PAH ISTD	Exact mass labelled [u]
Benzo[c]fluoren	BcF	216.0939	5-F-BcF	234.0845
Benzo[a]anthracen	BaA	228.0939	$^{13}C_6$-BaA	234.1140
Chrysen	CHR	228.0939	$^{13}C_6$-CHR	234.1140
Cyclopenta[cd]pyrene	CPP	226.0783		
5-Methylchrysene	5MC	242.1096	d_3-5MC	245.1284
Benzo[b]fluoranthene	BbF	252.0939	$^{13}C_6$-BbF	258.1140
Benzo[j]fluoranthene	BjF	252.0939		
Benzo[k]fluoranthene	BkF	252.0939	$^{13}C_6$-BkF	258.1140
Benzo[a]pyrene	BaP	252.0939	$^{13}C_4$-BaP	256.1037
Indeno[123cd]pyren	IcP	276.0939	d_{12}-IcP	288.1692
Dibenzo[ah]anthracene	DhA	278.1096	d_{14}-DhA	292.1974
Benzo[ghi]perylene	BgP	276.0939	$^{13}C_{12}$-BgP	288.1341
Dibenzo[al]pyren	DlP	302.1096	13-F-DlP	320.1001
Dibenzo[ae]pyren	DeP	302.1096	$^{13}C_6$-DeP	308.1297
Dibenzo[ai]pyren	DiP	302.1096	$^{13}C_{12}$-DiP	314.1498
Dibenzo[ah]pyren	DhP	302.1096		

Table 3: Exact masses of PAH and labeled internal standards.

PAH for recovery standard		Exact mass labelled [u]
Benzo[a]anthracen	d_{12}-BaA	240.1692
Benzo[a]pyrene	d_{12}-BaP	264.1692
Benzo[ghi]perylene	d_{12}-BgP	288.1692

Table 4: Exact masses of PAH recovery standards.

RT	Exact mass [min]	Function [u]	Dwell time [ms]
8:50	216.09375	native	82
	218.98508	lock	2
	226.07830	native	82
	228.09383	native	82
	234.08450	native	82
	234.11400	native	82
	240.16920	native	82
	263.98656	cali	6
13:00	218.98508	lock	2
	242.10960	native	82
	245.12840	native	82
	252.09390	native	82
	256.10730	native	82
	258.11400	native	82
	263.98656	cali	6
	264.16920	native	82
19:00	263.98656	lock	2
	276.09390	native	74
	278.10960	native	74
	288.13410	native	74
	292.19740	native	74
	313.98340	cali	6
22:00	263.98656	lock	2
	302.10960	native	120
	308.12970	native	120
	313.98340	cali	6
	314.13980	native	120
	320.10010	native	120

Table 5: MS parameters with MID descriptor for PAH Fast GC/HRMS data acquisition.

Results

The initial use of a 50% phenyl capillary column of 60 m length (60 m x 0.25 mm x 0.25 µm, at constant pressure) provided the required chromatography resolution for the various isomers. The necessary retention time of more than 90 min turned out to be not appropriate for a control method with high productivity.

The application of fast GC column technology reduced the required retention by 3/4 to only 25 min maintaining the necessary isotope resolution, see Figure 3. The critical separation components are shown in detail in Figure 4 a-c. For all components the fast GC method provides a robust peak separation for a quantitative peak integration.

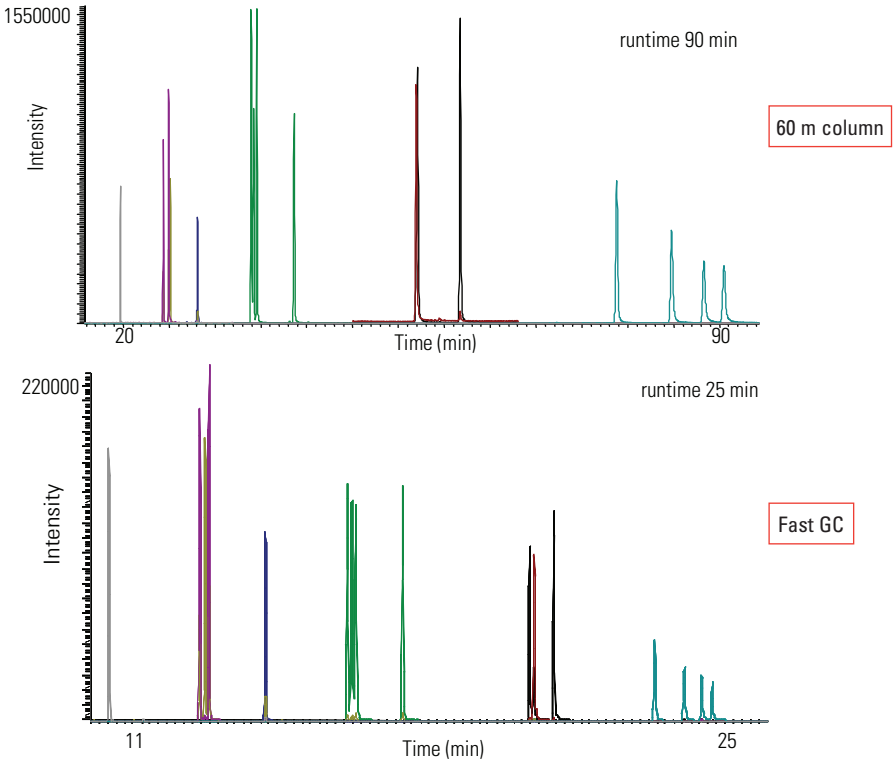


Figure 3: Top: Regular GC separation, 60 m column, > 90 min retention time. Bottom: Fast GC separation, 10 m column, approx. 25 min retention time.

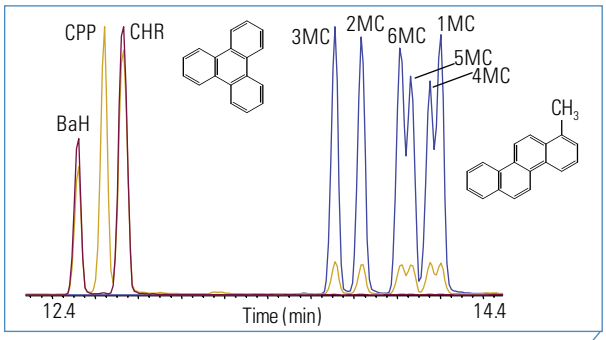


Figure 4a: Detail of isomer separation from Figure 3.

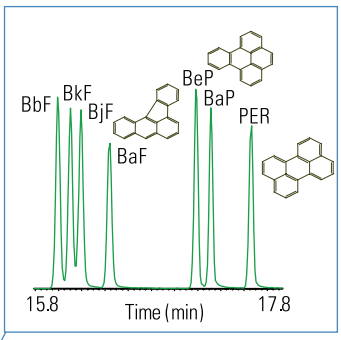
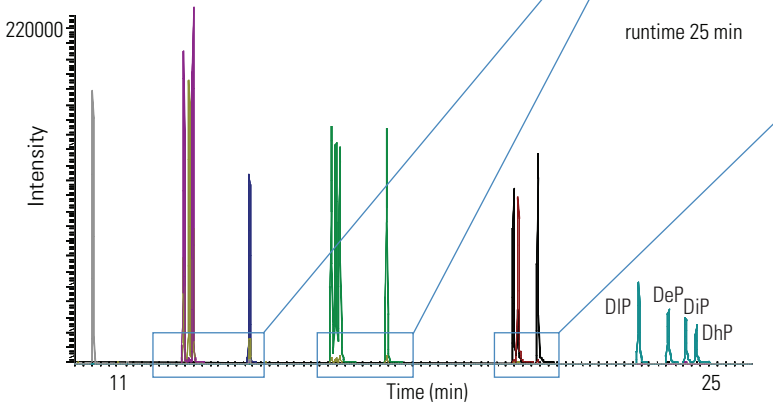


Figure 4b: Detail of isomer separation from Figure 3.



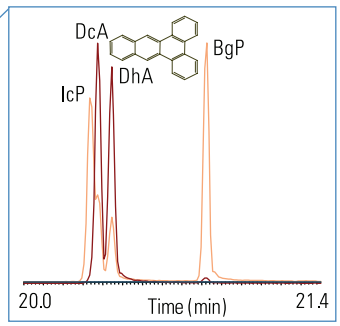


Figure 4c: Detail of isomer separation from Figure 3.

Figure 4: Fast GC separation with zoom.

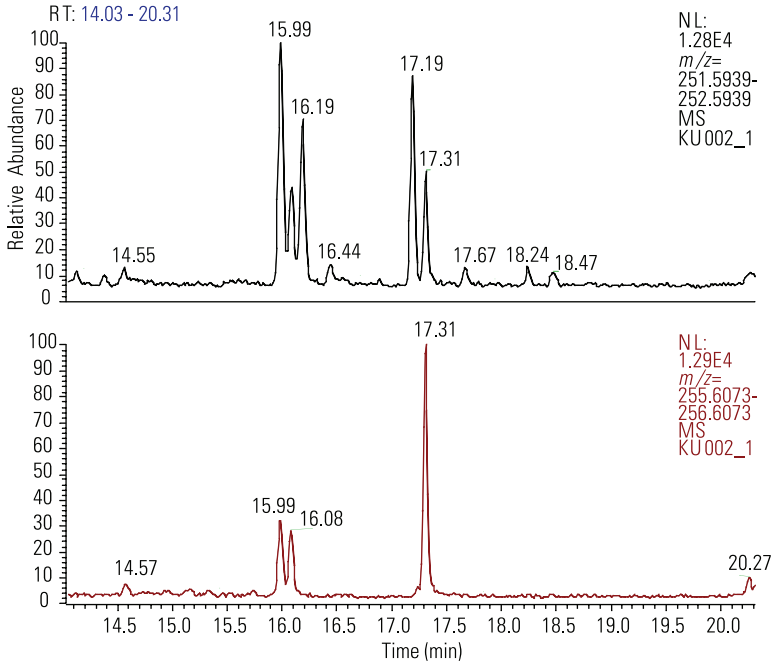


Figure 5: Benzo[a]pyrene determination (RT 17:31 min) in caraway seeds at a level of 0.02 µg/kg (max. concentration 0.03 µg/kg incorporated by the addition of spices), elution sequence BbF, BkF, BjF, BeP, BaP, top native PAH, bottom ^{13}C-BaP.

Sample Measurements

Applicability for different matrices has been shown especially for those matrices known to be critical in this type of analysis. Figure 5 shows the analysis of the extract from caraway seeds with a determined concentration of benzo[a]pyrene of 0.02 µg/kg. An LOD of 0.005 µg/kg and an LOQ of 0.015 µg/kg can be estimated for the analysis of spices, when the sample weight is 1 to 1.5 g. The recovery values achieved with the described sample preparation has been between 60 and 120%.

Conclusions

The retention time of of more than 90 minutes for regular chromatography conditions was successfully reduced to approximately 25 minutes maintaining chromatographic resolution. In practice the fast GC separation combined with a high resolution GC/MS detection system has proven to be a fast and reliable quantitation of PAH at the legally required level in routine analysis.

References

[1]COMMISSION REGULATION (EC) No 208/2005 of 4 February 2005 amending Regulation (EC) No 466/2001 as regards polycyclic aromatic hydrocarbons.

[2]COMMISSION RECOMMANDATION of 4 February 2005 on the further investigation into the level of polycyclic aromatic hydrocarbons in food.

[3]Summary and Conclusion of the Joint FAO/WHO Expert Committee on Food Additives, Sixty-Fourth meeting, Rome, 8-17 February 2005, JCEFA/64/SC.

[4]Ziegenhals, K., Jira, W., High sensitive PAH method to comply with the new EU directives, Presentation at the European High Resolution GC/MS Users Meeting, Venice, Italy, March 23 -24, 2007.

[5]Ziegenhals, K., Jira, W., Bestimmung der von der EU als prioritär eingestuften polyzyklischen aromatischen Kohlenwasserstoffe (PAK) in Lebensmitteln, Kulmbach Kolloquium, Sept. 2006.

[6]Kleinhenz, S., Jira, W., Schwind, K.-H.: Dioxin and polychlorinated biphenyl analysis: Automation and improvement of clean-up established by example of spices, Molecular Nutrition & Food Research 50 (4-5) (2006) 362-367.

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