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Gas chromatography-mass spectrometry (GC-MS) method for the determination of 16 European priority polycyclic aromatic hydrocarbons in smoked meat products and edible oils. — Source link

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A GC/MS method for the determination of 16 European priority polycyclic aromatic hydrocarbons in smoked meat products and edible oils

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ABSTRACT

A GC/MS method was developed for the analysis of 15 polycyclic aromatic hydrocarbons (PAHs) highlighted as carcinogenic by the Scientific Committee on Food (SCF) plus benzo[c]fluorine [recommended to be analysed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)] in fat-containing foods such as edible oils and smoked meat products,. This method includes accelerated solvent extraction (ASE) and the highly automated clean up steps gel permeation chromatography (GPC) and solid phase extraction (SPE). Using a VF-17ms GC column, a good separation of benzo[b]fluoranthene, benzo[j]fluoranthene and benzo[k]fluoranthene was achieved. Furthermore, the six methylchrysene-isomers and the PAH compounds with a molecular weight of 302 daltons in fat-containing foods attained a better chromatographic separation in comparison to a 5ms-column. The reliability of the analytical method for edible oils was demonstrated by the results from a proficiency test. Measurements with GC/HRMS and GC/MSD led to comparable results. A survey of the 16 PAHs in 22 smoked meat products showed concentrations in the range of < 0.01 µg/kg to 19 µg/kg. The median concentration for benzo[a]pyrene was below 0.15 µg/kg.

KEYWORDS: edible oils, GC/MS, HRMS, PAH, smoked meat products

INTRODUCTION

For the first time Jung and Morand reported the presence of PAH in vegetable oils and fats in 1962. Further investigations followed by Sagredos et al. 1979 and 1988, Speer and Montag 1988 and Dennis et al. 1991. Investigations of the contents of individual PAH in smoked and barbecued meat products (Simko 2002, Jira 2003) were primarily focused on benzo[a]pyrene (Kazerouni et al. 2001) or the 16 PAH compounds approved by the U.S. Environmental Protection Agency (EPA-PAH) (Mottier et al. 2000).

Since 1 April 2005 the Commission Regulation (EC) No 208/2005 of 4 February 2005 has been restricting maximum levels for benzo[a]pyrene in different food groups: Oils and fats 2 µg/kg, foods for infants and young children 1 µg/kg; smoked meat and smoked fish 5 µg/kg; unsmoked fish 2 µg/kg; crustaceans and cephalopods 5 µg/kg and bivalve molluscs 10 µg/kg. Furthermore, the European Commission (2005/108/EC) recommended that the member states should investigate not only the contents of benzo[a]pyrene in these food groups but also other polycyclic aromatic hydrocarbons (PAH) seen as carcinogenic by the Scientific Committee on Food (SCF). These are 15 PAH compounds which, due to their toxic properties, are of major concern to human health and should be monitored to make possible long-term exposure assessments (SCF, 2002). These 15 "SCF-PAH" are: benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[j]fluoranthene (BjF), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (BgP), benzo[a]pyrene (BaP), cyclopenta[cd]pyrene (CPP), dibenzo[a,h]anthracene (DhA), dibenzo[a,e]pyrene (DeP), dibenzo[a,h]pyrene (DhP), dibenzo[a,i]pyrene (DiP), dibenzo[a,l]pyrene (DlP), indeno[1,2,3-cd]pyrene (IcP), chrysene (CHR) and 5-methylchrysene (5MC). In addition, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) identified benzo[c]fluorene (BcL) as a PAH compound that should be monitored as well (JECFA 2005). These 16 EU priority PAH need to be analysed in different food groups. EFSA has established an on-line analytical database in collaboration

with European Union Member States in order to collect data for these 16 EU priority PAH (Wenzl et al. 2006).

Lately, the total contents of the 15 SCF-PAH were analysed in different Danish meat products by Duedahl-Olesen et al. 2006. After saponification, liquid liquid extraction and solid phase extraction, the chromatographic separation was carried out via a 60 m J&W DB-5 capillary column. Then in 2007, the results of 27 PAH including the 15 SCH-PAH in oils were presented by Rose et al. Again, the sample preparation included saponification, liquid liquid extraction and solid phase extraction. Also here a 60 m 5% phenyl capillary column and a low resolution mass spectrometer for separation and quantification were used.

This paper describes an analytical method without saponification for the determination of the 16 EU priority PAH compounds in the fat-containing food groups, edible oils and smoked meat products. The method includes accelerated solvent extraction (ASE) and the highly automated clean up steps gel permeation chromatography (GPC) and solid phase extraction (SPE). The determination of the PAH was carried out by applying HRGC in combination with a high resolution mass spectrometer. For separation of the individual components a 60 m 50 % phenyl capillary column was found suitable.

This new method is based on the method (Jira 2004) developed at the Institute for Chemistry and Physics, Federal Research Centre for Nutrition and Food (BfEL), Kulmbach, Germany, and was adapted to the recommendation of the European Commission (2005/108/EC). The performance of the analytical method for edible oils was demonstrated in a proficiency test (IRMM 2006). Its suitability for meat products was shown by investigating suspicious samples. Smoked salts and smoke flavourings can also be analysed by using this method (Jira et al. 2006).

MATERIALS AND METHODS

Materials

The native PAH standard solutions (BcL, BaA, CHR, BbF, BkF, BjF, BaP, IcP, DhA, BgP, DlP, DeP, DiP, DhP, 1-methylchrysene (1MC), 2-methylchrysene (2MC), 3-methylchrysene (3MC), 4-methylchrysene (4MC), 5MC, 6-methylchrysene (6MC), triphenylene (TP), dibenzo[a,c]anthracene (DcA), benzo[a]fluoranthene (BaF)) were obtained from Dr. Ehrenstorfer GmbH (Augsburg Germany). The isotope labelled PAH standard solutions were purchased from Promochem (Wesel, Germany) and the fluorinated PAH standards from Biochemical Institute for Environmental Carcinogens Prof. Dr. Gernot Grimmer-Foundation (Grosshansdorf, Germany).

All solvents were obtained in picograde quality from Promochem (Wesel, Germany). The drying material (poly(acrylic acid), partial sodium salt-graft-poly(ethylene oxide)) was purchased from Sigma Aldrich (Munich, Germany), Bio Beads S-X3 (200 - 400 mesh) from Bio-Rad Laboratories (Munich, Germany) and silica gel from Merck (Darmstadt, Germany). Glass microfibre filters (18 mm i.d.) were obtained from Dionex (Idstein, Germany). The PTFE-Filters (1 µm pore size, 25 mm i.d.) and the SPE-Cartridges (12 mm i.d.) were purchased from Alltech (Unterhaching, Germany).

Sample preparation for the analysis of meat products

Accelerated solvent extraction (ASE): About 5 g homogenised meat product were levigated with the same amount of the drying material poly(acrylic acid), partial sodium salt-*graft*-poly(ethylene oxide). The resulting material was poured into 33-mL cells, which were locked with glass microfiber filters at the outlet end of the extraction cells. Afterwards, 50 µL of a PAH standard mixture containing isotope labelled (^{13}C and ^{2}H) and fluorinated PAH

compounds (5-fluorobenzo[c]fluorene, benzo[a]anthracene-$^{13}C_6$, chrysene-$^{13}C_6$, 5-methylchrysene-d_3, benzo[b]fluoranthene-$^{13}C_6$, benzo[k]fluoranthene-$^{13}C_6$, benzo[a]pyrene-$^{13}C_4$, benzo[g,h,i]perylene-$^{13}C_{12}$, dibenzo[a,h]anthracene-d_{14}, indeno(1,2,3-cd)pyrene-d_{12}, dibenzo[a,e]pyrene-$^{13}C_6$, dibenzo[a,i]pyrene-$^{13}C_{12}$ and 13-fluorodibenzo[a,l]pyrene in isooctane) were added. The extraction was performed with an ASE 200 from Dionex (Sunnyvale, USA) and carried out with n-hexane at 100°C and 100 bar at 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 (weight of filling 60 g). Samples were eluted at a flow rate of 5 mL/min applying 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 (**A**utomatic. **S**ample **P**reparation with **E**xtraction **C**olumns) (Kleinhenz et al. 2006) from Gilson (Bad Camberg, Germany). 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 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 remaining sample was carefully concentrated in a nitrogen stream to a volume of about 50 µL.

Reagent and procedural blanks were simultaneously analysed for to detect present PAH in parallel to each series of samples passing the extraction and cleanup procedures using drying material instead of real samples.

GC/MS analysis

The *GC/HRMS analysis* of PAH was performed on a HP 5890 II gas chromatograph with a split/splitless injection port. The GC was equipped with a VF-17ms capillary column (60 m x 0.25 mm i.d., 0.25 µm film thickness) purchased from Varian (Darmstadt, Germany). Helium was used as carrier gas at a constant pressure of 27 psi. The injection temperature was 300°C and the injection volume was 1 µL (splitless). The following temperature program was used: isothermal at 50°C for 1 min, at 25°C/min to 280°C, at 1°C/min to 330°C, isothermal at 330°C for 30 min.

The quantification of PAH by GC/HRMS was performed by using a VG Autospec (Waters, Manchester, UK) working in the EI positive ion mode using an electron energy of 35 eV. The transfer line temperature and the ion source temperature were maintained at 280°C and 250°C, respectively. The resolution of the MS was tuned to 8000 (10% valley definition). The PAH were analysed in a 4-function Selected Ion Registration (SIR) experiment.

The *GC/MSD analysis* was performed on an Agilent 7890 GC coupled with an Agilent 5975 inert mass spectrometric detector. The GC was also equipped with a VF-17ms (Varian, Darmstadt, Germany) capillary column (60 m x 0.25 mm i.d., 0.25 µm film thickness). Helium was used as carrier gas at a constant flow rate of 1 mL/min. 1 µL was injected while the injector port was held at 325°C and operated in pulsed splitless mode. Initially, the oven temperature was set to 50°C (1 min hold), then to 280°C at 30°C/min, to 340°C at 1°C/min and to 350°C at 1.5°C/min kept constant for 2 min. The mass spectrometer was operated in a selected ion monitoring (SIM) mode with an electron impact ionization of 70 eV. The

temperatures of the ion source and the quadrupole mass analyser were kept at 300°C and 150°C, respectively.

For the determination of the ratio of TP and CHR with GC/MSD a VF-Xms (Varian, Darmstadt, Germany) capillary column (60 m x 0.25 mm i.d., 0.25 µm film thickness) was used. The oven temperature was initially set at 50°C (1 minute hold) and heated to 320°C at 3.5°C/min. Helium was used as carrier gas at a constant flow rate of 1 mL/min. 1 µL was injected while the injector port was held at 325°C and operated in the pulsed splitless mode.

Sample preparation for the analysis of edible oils

Approximately 0.9 g of edible oil was dissolved in 3.5 mL cyclohexane/ethylacetate (50:50 v/v), 50 µL of the internal PAH standard were added and the mixture was chromatographed by GPC. The following clean up steps were carried out as described for meat products.

RESULTS AND DISCUSSION

Applying the GC/MS method (HRMS and MSD) developed here it is possible to achieve gas chromatographic separations of isomers which are otherwise difficult to separate. First, a sufficient chromatographic separation of CPP and CHR was achieved (see Figure 1A). Although CPP (MW = 226) has another molecular weight than BaA and CHR (MW = 228, each), a sufficient chromatographic separation of CPP and CHR is required for a reliable quantification of CPP because BaA and CHR also show fragment ions with the molecular weight MW = 226 (Nyman et al., 1993). Therefore, an unsatisfactory chromatographic separation would adulterate the quantification of CPP. In contrast, a chromatographic separation of CHR and TP on such a column is not possible. Second, with the help of this method a satisfactory chromatographic separation of the six methylchrysene-isomers is achievable (see Figure 1B). Therefore, the exact quantification of 5-methylchrysene (5MC) is possible. Third, an important chromatographic challenge is the separation of the benzofluoranthenes. The base line separation of benzo[b]fluoranthene, benzo[j]fluoranthene and benzo[k]fluoranthene is possible by means of a 60 m VF-17ms GC column (see Figure 1C). Also, benzo[a]fluoranthene does not coelute with the benzofluoranthenes. Fourth, DhA can be detected independent of dibenzo[a,c]anthracene (DcA) (see Figure 1D). Fifth, also the quantification of dibenzopyrenes (MW = 302) is easier to carry out with a GC column with 50% polyphenylsiloxane than with 5% polyphenylsiloxane because in this way a better chromatographic separation of the isomers is achievable. It is well known that there are a number of isomeric PAH compounds with MW = 302 amu (Schmidt et al. 1987, Schubert et al. 2003). In Figure 2 the chromatographic separation of PAH isomers with m/z = 302 in strongly smoked raw sausage on a VF-17ms is presented.

For the qualitative detection of the different PAH compounds using GC/MSD, in addition to the retention time and the molecular ion (M+), a third criterion is needed to identify a substance with a high selectivity because of the low resolution of the MSD. A second ion in addition to the quantifier ion is called qualifier ion and was used for the purpose of verification (Simon et al. 2006). The ratio between the quantifier ion and the qualifier ion had to match a specific ratio (see Table I) to show a positive result. The ratios need to be determined by measuring an external PAH standard solution. The quantification of the different PAH compounds is based on the comparison of the peak area of the most abundant ion (M^+) of the native PAH compound with the peak area of the corresponding deuterated, ^{13}C-labelled or fluorinated internal standards. As not all of the 16 PAH compounds are commercially available as isotope labelled standard compounds, it was necessary to use response factors for the quantification of PAH with no corresponding internal standards (cyclopenta[c,d]pyrene, benzo[j]fluoranthene and dibenzo[a,h]pyrene). These response factors were determined by the analysis of a PAH standard mixture containing the 16 PAH compounds. The range of linearity of both GC/MS-systems was determined by an automated statistical test and ranged from 1 or 5 to 1000 pg/µl. GC/HRMS and GC/MSD show different limits of detection (LOD) and limits of quantification (LOQ). The LOD, which is defined as the lowest concentration of an analyte detectable in a sample (signal-to-noise ratio = 3:1) and the LOQ, which is defined as the lowest content of the analyte that can be measured with reasonable statistical certainty (2007/333/EC) (signal-to-noise ratio = 9:1) for the different PAH compounds in smoked meat products, are shown in Table II. Two groups are defined: 1) PAH with a lower molecular weight which can be detected more sensitively than 2) PAH with a high molecular weight. The two different groups are:

1. BcL, BaA, CHR, CPP, 5MC, BbF, BkF, BjF, BaP, BgP

2. IcP, DhA, DlP, DeP, DiP, DhP

Since a gas-chromatographic separation of the two isomers TP and CHR could not be obtained with a VF-17ms capillary column, a VF-Xms capillary column had to be used. With a low heating rate of 3.5°C/min not only a separation of TP and CHR but also a sufficient separation of BaA, CPP, TP and CHR was achieved (see Figure 3).

PAH in edible oils

The reliability of the described analytical method was demonstrated by participation in a proficiency test (IRMM 2006). Three edible oils spiked with PAH, sunflower oil, olive oil, maize oil and one native sunflower oil, were analysed in duplicate. The results of GC/HRMS were submitted to IRMM. Subsequently, the same extracts were measured by GC/MSD. These results were not submitted to the IRMM. Both results determined were compared with the real spiked concentrations (Table III). In the case of the native sunflower oil the results were compared to the median of the PAH concentration of the different participants of the collaborative trial.

The results determined by both GC/MS-systems were in a good agreement. The recoveries for different PAH compounds ranged from 75% to 95%. The repeatability was better in the case of MSD. The averaged relative standard deviations (RSD) of all PAH compounds were 17% for MSD and 22% for HRMS.

PAH in smoked meat products

In order to test the suitability of the described GC/HRMS method developed at the BfEL Kulmbach, 22 smoked raw meat products (mainly raw smoked hams) which looked like very strongly smoked products were collected from different producers at the end of 2005.

The resulting benzo[a]pyrene contents of these products were in the median below 0.15 µg/kg and therefore classified as being relatively low. With the exception of one sample, which showed a benzo[a]pyrene content of 18 µg/kg (Table IV), all of the samples were below the new maximum level of 5 µg/kg. Including the sample with a BaP content of 18 µg/kg four samples were above the old maximum level in Germany of 1 µg/kg. The sample with a benzo[a]pyrene content of 18 µg/kg was a smoked belly of pork with a strongly fading, black surface.

For the clarification of the question, in which quantitative ratio the present leading substance benzo[a]pyrene is compared to the other investigated PAH compounds, the contents determined for the 15 priority PAH compounds and the JECFA-PAH benzo[c]fluorene were added to calculate the percentage contributions of the single PAH compounds to the total content of the 16 PAH compounds. The result of these calculations is presented in Figure 4. Hereby, the results were differentiated between PAH with a higher (marked dark) and a lower (marked bright) carcinogenic potential. The differentiation between these two groups resulted from the toxic equivalent factors (TEF) in the literature (Müller et al. 1995). In this way a higher carcinogenic potential was assumed for PAH with a TEF $\geq$ 0.89 and a lower carcinogenic potential for TEF $\leq$ 0.11. The results clearly show that within the group of PAH compounds with a higher carcinogenic potential benzo[a]pyrene (BaP) represents the largest contribution to the total content of the 16 investigated PAH compounds. The contribution of BaP in these samples was 8% of the total PAH content (median) and varied from approximately 6% to 12%. The contributions of the other PAH compounds with a higher carcinogenic potential (dibenzo[a,h]anthracene (DhA), dibenzo[a,e]pyrene (DeP),

dibenzo[a,h]pyrene (DhP) and dibenzo[a,l]pyrene (DlP)) were significantly lower and were in the median range of about 1% and lower. Consequently, benzo[a]pyrene was the dominating compound within the group of PAH with a higher carcinogenic potential and therefore, seems to be justified to be used as the leading substance in smoked meat products. The percentage contributions of the other PAH with a low carcinogenic potential show relatively high variations.

After developing the method for the separation of TP and CHR the ratios of these isomers were analysed in 25 samples from different meat products. Some of these samples were the same samples that looked like very strongly smoked products because of their black surface. But some of the samples that looked very strongly smoked had very low concentrations, which could not be analysed by using the GC/MSD. For these measurements the MSD was not sensitive enough. Therefore, samples of bacon were analysed additionally.

The results showed that the ratio of TP/CHR varied also within the ham and bacon samples (Table V). The maximum value was reached for wild boar ham.

CONCLUSIONS

With the help of both GC/MS methods (HRMS and MSD) a reliable determination of the 16 European priority PAH compounds in smoked meat products and edible oils is possible. Except for chrysene the 16 EFSA-PAH can be detected separately from each other and from interfering isomers not classified as priority. This method also allows an analysis with low limits of quantification (LOQs). Such a sensitive analytical method is necessary for the determination of PAH in smoked meat products with low PAH contents. Otherwise, a quantification of PAH in meat samples with lower concentration is very difficult. (Reinik et al. 2007).

The contents of the 16 European priority PAH compounds in the analysed suspicious samples of smoked meat products point out that BaP can be used as the leading substance in smoked meat products. However, the suitability of BaP as a marker for carcinogenic PAH compounds in smoked meat products has to be confirmed by the investigation of representative samples. In particular, next to a representative study it is important to get more information about the content of DlP in meat products. DlP has been in the spotlight of scientific interest recently because toxicological investigations indicated that DlP probably shows a hundredfold higher toxicity in comparison to BaP (McClure and Scheny 1995, Müller et al. 1995). In consideration of this potentially extremely high toxicity DlP should not to be underestimated – although the contents in the investigated smoked meat products were very low. The GC chromatograms show that a 50% phenyl capillary column is superior to a 5% phenyl capillary column (Jira 2004, Rose et al. 2007), especially because of a better chromatographic separation of the high number of PAH compounds with MW = 302 (Schubert et al. 2003).

Toxicological investigations indicated that CHR has a much stronger carcinogenic potential than TP (Müller et al. 1995). CHR und TP elute on polar columns at the same retention time. Because of their identical molecular mass they could only be quantified in sum. Most studies consider only the content of CHR. For the determination of the TP/CHR-ratio a separate

method was developed. Applying this method, differing ratios of TP/CHR within different samples were analysed and as a result the content of TP is not to be neglected. Because of these results, the previously description “CHR” should be replaced by “TP and CHR”, to avoid an over-evaluation of the carcinogenic potential of the sample.

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Figure 1. Structures, abbreviations and molecular weights of the 16 priority PAH

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Figure 5. Linear range HRMS (1 – 1000 pg/µL)

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Table II. LOD und LOQ in meat products

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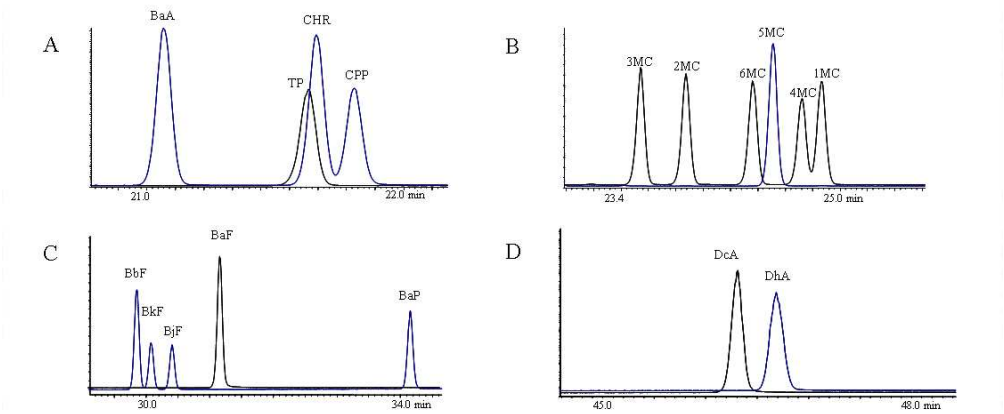

Chromatograms of the priority PAH and some isomers on a VF-17ms
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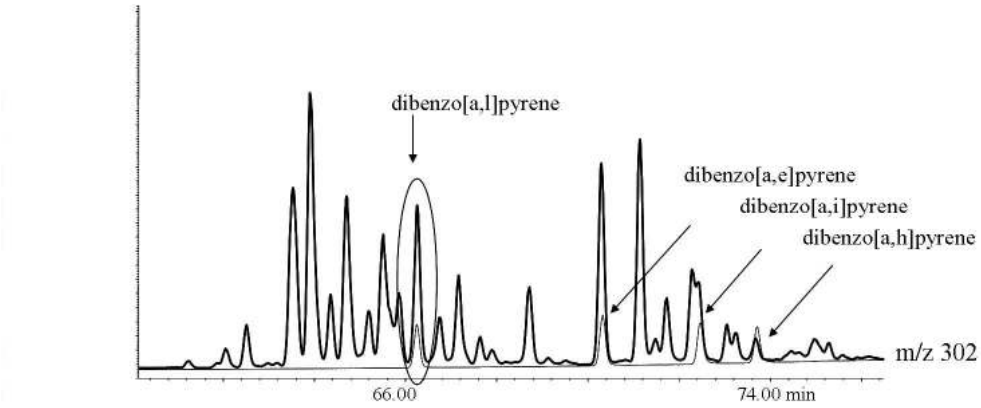

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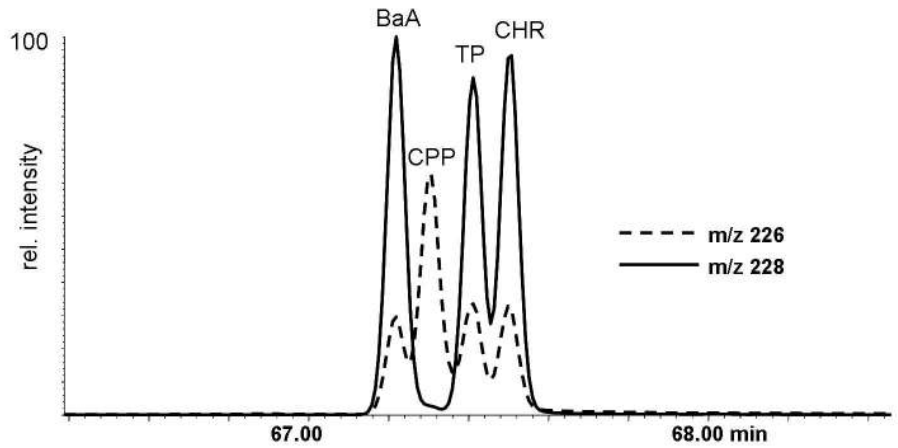
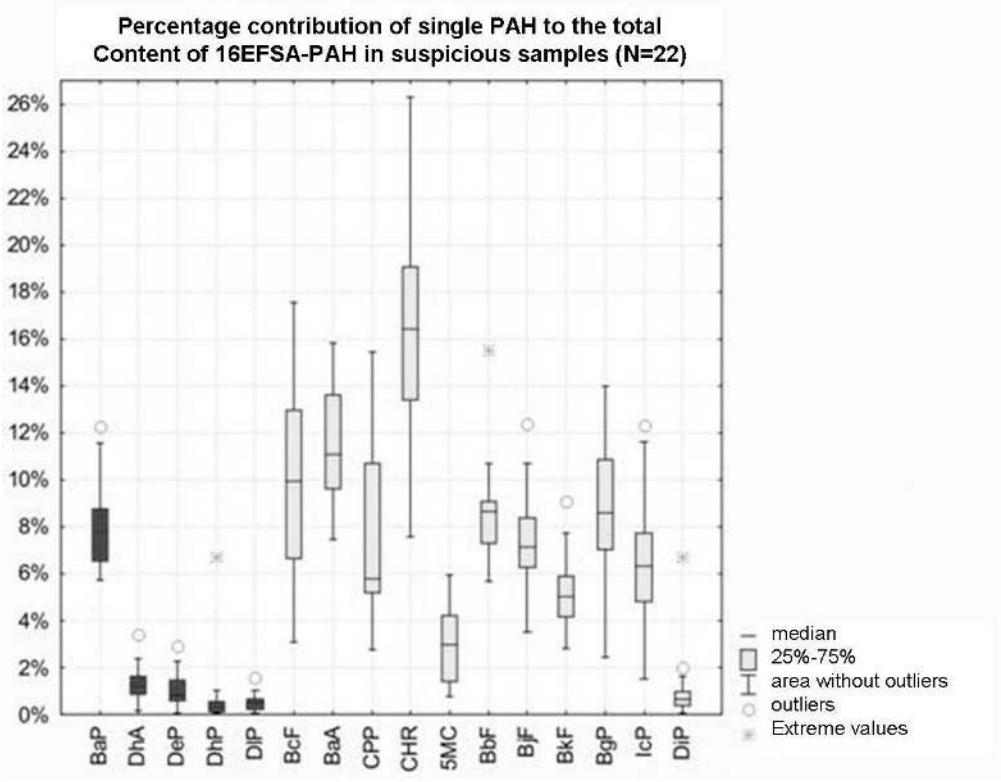
252x120mm (150 x 150 DPI)



Chromatogram of the separation of triphenylene (TP) and chrysene (CHR)
267x131mm (150 x 150 DPI)



Percentage contribution of single PAH to the total content of the 16 EFSA-PAH in smoked meat products (N=22)
214x167mm (150 x 150 DPI)

Table I. Ratio qualifier to quantifier ion of different PAH compounds

	Qualifier/Quantifier Ion	
BcL	*108/216*	10.0
BaA	*114/228*	11.0
CHR	*113/228*	11.5
CPP	*113/226*	16.7
5MC	*215/242*	11.9
BbF	*126/252*	12.5
BkF	*125/252*	9.5
BjF	*125/252*	12.6
BaP	*126/252*	13.2
IcP	*138/276*	21.7
DhA	*139/278*	13.8
BgP	*138/276*	18.7
DlP	*150/302*	28.8
DeP	*150/302*	15.0
DiP	*150/302*	9.7
DhP	*150/302*	10.3

Table II. LOD und LOQ in meat products

µg/kg	meat products			
	5 g sample weight			
	MSD		HRMS	
	LOD	LOQ	LOD	LOQ
group 1	0.01	0.03	0.003	0.009
group 2	0.02	0.06	0.01	0.03

Table III: Contents of PAH determined with both GC/MS-systems in three spiked and one native oil

	spiked sunflower oil			spiked olive oil			spiked maize oil			native sunflower oil		
	HRMS	MSD	median conc.*	HRMS	MSD	median conc.*	HRMS	MSD	median conc.*	HRMS	MSD	median conc.*
benzo[a]anthracene	0.9	0.8	0.9	2.7	2.7	2.9	7.9	8.1	7.8	5.6	5.5	5.7
benzo[a]pyrene	1.0	0.8	0.9	2.5	2.2	2.3	7.6	7.1	7.5	4.8	4.3	4.7
benzo[b]fluoranthene	6.3	6.3	6.6	1.2	1.1	1.1	2.1	1.9	1.9	5.0	4.6	4.6
benzo[c]fluorene	1.6	1.4	1.5	6.7	5.9	6.9	0.7	0.6	0.7	1.0	0.8	1.5
benzo[ghi]perylene	8.6	9.2	9.5	1.6	1.3	1.5	2.9	2.8	2.8	3.7	3.2	3.6
benzo[j]fluoranthene	2.8	2.7	2.7	10.2	10.6	10.0	1.4	1.2	1.1	2.7	2.6	2.6
benzo[k]fluoranthene	1.0	0.7	0.8	2.3	2.2	2.3	7.3	7.3	7.4	2.6	2.4	2.4
chrysene	1.1	1.0	1.0	10.1	10.0	9.9	2.3	2.5	2.3	8.1	7.6	7.5
cyclopenta[cd]pyrene	7.1	8.2	6.3	1.1	1.0	1.1	2.1	2.1	1.8	2.5	2.4	2.6
dibenzo[a,e]pyrene	2.0	1.6	1.7	1.1	0.7	0.8	6.3	6.2	6.8	1.0	0.7	0.8
dibenzo[a,h]anthracene	1.3	1.0	1.0	3.1	2.8	2.9	8.9	9.3	11.0	1.1	0.8	0.8
dibenzo[a,h]pyrene	2.1	1.7	1.8	1.1	0.7	0.8	7.0	7.1	6.8	0.6	0.1	-
dibenzo[a,i]pyrene	1.0	0.6	0.6	7.2	7.2	6.8	2.1	1.8	1.9	0.9	1.0	0.3
dibenzo[a,l]pyrene	7.3	7.0	7.5	1.9	2.0	2.1	1.1	0.7	1.0	0.5	0.2	0.6
Indeno[1,2,3-cd]pyrene	1.9	1.6	1.8	6.2	6.1	6.9	2.8	2.3	0.8	3.0	2.8	3.2
5-methylchrysene	1.0	0.7	0.9	0.4	0.3	0.8	3.6	2.5	3.5	-	-	-

* recovery corrected median concentration of all participants.

Table IV. Contents of PAH in smoked meat products

µg/kg	BcL	BaA	CPP	CHR + TP	5MC	BbF	BjF	BkF	BaP	BgP	DhA	IcP	DeP	DhP	DiP	DlP
smoked ham	1.59	2.02	1.42	2.22	0.38	0.87	0.94	0.66	1.03	0.61	0.20	0.62	0.10	<0.03	0.05	0.07
smoked ham	0.19	0.32	0.41	0.36	0.05	0.20	0.22	0.15	0.23	0.21	0.06	0.20	0.04	<0.01	<0.03	<0.03
smoked ham	0.05	0.09	0.05	0.14	0.03	0.09	0.07	0.05	0.08	0.10	<0.03	0.08	<0.03	<0.01	<0.01	<0.03
smoked ham	0.79	0.84	0.91	0.78	0.06	0.38	0.42	0.29	0.50	0.39	0.08	0.35	0.05	<0.01	0.04	0.03
smoked ham	0.14	0.14	0.11	0.20	0.02	0.12	0.10	0.07	0.12	0.15	0.03	0.11	<0.03	<0.01	0.03	<0.03
smoked ham	0.36	0.93	0.36	3.55	0.39	0.80	0.64	0.29	0.54	0.79	0.17	0.47	<0.01	<0.01	<0.01	<0.01
smoked ham	0.12	0.49	0.48	0.65	0.03	0.41	0.38	0.25	0.40	0.37	<0.03	0.27	0.03	<0.01	<0.03	<0.01
smoked ham	0.05	0.08	0.05	0.12	0.03	0.09	0.07	0.05	0.08	0.11	<0.03	0.08	<0.01	<0.01	<0.01	<0.01
smoked ham	0.18	0.15	0.16	0.20	0.01	0.10	0.10	0.06	0.09	0.16	<0.01	0.10	<0.01	<0.01	<0.01	<0.01
smoked ham	0.95	0.79	0.56	4.29	0.2	0.59	1.29	0.43	0.76	0.26	0.03	0.16	<0.01	<0.01	<0.01	<0.01
smoked ham	0.12	0.12	0.03	0.15	0.04	0.07	0.05	0.03	0.06	0.06	<0.03	0.05	<0.01	<0.01	<0.01	<0.01
smoked ham	4.60	4.70	2.93	4.99	0.96	5.00	1.14	2.49	2.31	1.34	0.17	1.25	0.20	<0.03	0.12	<0.03
smoked ham	2.25	4.47	4.04	4.27	0.34	2.66	3.14	2.25	3.89	2.60	0.27	2.57	0.48	0.06	0.32	0.05
smoked ham	0.08	0.12	0.08	0.15	0.03	0.13	0.13	0.10	0.18	0.20	<0.03	0.18	0.04	<0.03	<0.03	<0.01
smoked ham	0.06	0.06	0.03	0.09	0.02	0.05	0.04	0.03	0.04	0.08	<0.03	0.04	<0.03	<0.01	<0.03	<0.01
smoked ham	0.09	0.10	0.04	0.19	0.03	0.06	0.04	0.03	0.04	0.05	<0.03	0.03	<0.03	<0.01	<0.01	<0.01
smoked ham	0.01	0.03	0.02	0.05	0.02	0.03	0.02	0.01	0.02	0.04	<0.01	<0.03	0.005	<0.01	<0.01	<0.01
smoked ham	0.09	0.07	0.02	0.11	0.03	0.04	0.02	0.02	0.03	0.04	0.004	0.02	<0.03	<0.03	<0.03	<0.03
smoked ham	0.13	0.15	0.04	0.18	0.04	0.08	0.06	0.04	0.08	0.07	<0.03	0.05	<0.03	<0.03	<0.03	<0.03
smoked ham	0.06	0.06	0.03	0.09	0.02	0.04	0.03	0.02	0.03	0.05	<0.03	0.03	<0.03	<0.03	<0.03	<0.03
belly pork ham	13.88	13.48	13.04	13.69	2.54	16.04	19.38	16.46	17.63	19.29	6.21	21.03	3.09	0.27	1.93	2.88
smoked wild boar ham	0.86	0.82	0.23	3.22	0.21	0.61	0.51	0.24	0.49	0.52	0.13	0.41	0.09	<0.03	0.07	0.06

Table V. Ratios of triphenylene (TP) to chrysene (CHR) in smoked meat products

meat product	ratio TP/CHR	meat product	ratio TP/CHR
raw ham_1	0.39	bacon_8	0.37
raw ham_2	0.11	bacon_9	0.49
raw ham_3	0.51	bacon_10	0.28
raw ham_4	0.24	bacon_11	0.25
raw ham_5	0.32	bacon_12	0.46
belly of pork	0.12	bacon_13	0.36
bacon_1	0.37	wild boar ham	2.70
bacon_2	0.34	smoked ham_1	0.46
bacon_3	0.25	smoked ham_2	0.20
bacon_4	0.53	smoked ham_3	0.18
bacon_5	0.54	smoked ham_4	0.17
bacon_6	0.47	smoked ham_5	0.35
bacon_7	0.28		