



## Application Note GCMS-05

# Quantification of PAHs below regulatory limits in food using the EVOQ GC-TQ

### Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are a large group of molecules containing two or more aromatic rings which are produced by natural and anthropogenic processes. Among these sources, pyrogenic (such as incomplete burning of coal, oil, gas, wood, garbage, and other organic substances) and petrogenic inputs are the two main sources of PAHs. These chemicals are considered as food and environment contaminants.

Non smokers are mainly exposed to PAHs through food and air. PAHs are significantly present in food due to heat processes such as smoking, grilling and smoke drying. Food can also be naturally contaminated by an accumulation of PAHs in the food chain, due to their lipophilic properties and their tendency to accumulate in adipose tissue.

Exposure to PAHs is a major concern for human health, since most PAHs are classified as carcinogenic.

PAHs generally occur in complex mixtures which may consist in hundreds of compounds. Among these compounds, fifteen were identified in 2005 by the European Commission as a priority.

### Authors

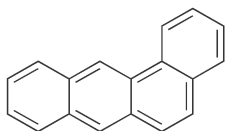
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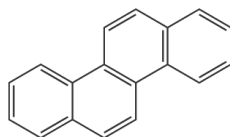
Keywords	Instrumentation and Software
Polycyclic Aromatic Hydrocarbons	EVOQ GC-TQ
PAHs	
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### Chemical structure of the 4 studied PAHs

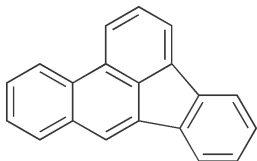
**Benzo[a]anthracene**



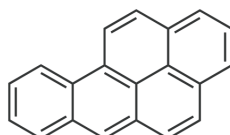
**Chrysene**



**Benzo[b]fluoranthene**



**Benzo[a]pyrene**



Regulation 1881/2006/EC has been amended (European Union (EU), 2011), for application starting on September 2012, to introduce new maximum levels for the 4 PAHs of interest whilst maintaining a separate maximum level for benzo[a]pyrene, thus ensuring a more efficient associated risk assessment. Quantification of these 4 PAHs results in the "PAH4 value" corresponding to the sum of concentration for benzo[a]pyrene, chrysene, benzo[b]fluoranthene and benz[a]anthracene.

In September 2014, these levels were lowered from 5 to 2 µg/kg for benzo[a]pyrene and from 30 to 12 µg/kg for PAH4.

A method has been developed for 20 PAHs, including the fifteen compounds of interest in the EU plus benzo[c]fluorene, and four other PAHs listed in US EPA. Quantification was performed using isotope dilution with <sup>13</sup>C-labeled compounds, and recovery rates were evaluated with fluorinated PAHs external standards.

The use of the EVOQ GC-TQ system enables the efficient analysis of PAHs at low levels, and in particular the four PAHs as follows: benzo[a]pyrene, chrysene, benzo[b]fluoranthene and benz[a]anthracene. Structures of these four compounds are illustrated above.

### Instrument

Gas chromatography (436 GC, Bruker) coupled to a triple quadrupole mass spectrometer (EVOQ GC-TQ™, Bruker).

### Material and reagents

All stock solutions of <sup>12</sup>C, <sup>13</sup>C-labelled and fluorinated PAHs were purchased from LGC Standards (Molsheim, France) and Chiron (Trondheim, Norway). For calibration purposes, mix solutions were prepared in toluene.

The calibration solutions of <sup>12</sup>C PAHs were at the following concentrations: 0, 5, 10, 25, 50, 250, 500 and 2000 pg/µL. Internal standards (<sup>13</sup>C) and fluorinated external standards were at 25 pg/µL. All solvents used were "Picograde" quality, from LGC Standards.

### Sample preparation

Ten grams of food sample were freeze-dried. The dry residue obtained was weighed in order to determine its water content. Extraction was performed with a hexane / acetone (50 / 50, v/v) mixture using pressurized liquid extraction system (ASE): the ASE cell was filled up with 1g of celite and 15g of pre-washed florisil. One gram of the dry residue sample was introduced into the cell and spiked with <sup>13</sup>C-labelled internal standard.

The extract was purified onto a SPE cartridge (Envi Chrom-P): after conditioning, the sample was loaded. The cartridge was washed with a mixture of cyclohexane/ ethanol (70/30, v/v) and compounds were eluted using 12 mL of cyclohexane/ethyl acetate (40/60, v/v).

Fluorinated PAHs were used as external standards. Two microliters of the final extract (in toluene) were analyzed by GC-MS/MS.

### GC-MS/MS Conditions

The sample and calibrations solutions were injected with the following GC and MS/MS parameters. For ionization, filament current was set to 80 µA. Argon was used for SRM as collision gas at a pressure of 2.0 mTorr.

For acquisition, windows of 2 minutes width were centered at the retention time for each compound, and scan times were set by the software between 60 and 100 ms, adjusted for peak width (in sec) and desired number of data points per peak.

### Chromatographic conditions

Column	Agilent J&W Select PAH 30m x 0.25 mm x 0.15 µm
Gas	Helium, 1.30 mL/min constant flow
Injector	280°C, Splitless
Injection volume	2 µL

### Oven temperature program

Rate (°C/min)	Temperature(°C)	Hold (min)
-	110	1
60	220	0
5	270	0
3	295	0
20	330	10
2	340	0

## MS Conditions

Ionisation mode	Electron Impact, 70eV
Source temperature	250°C
Transfer line temperature	310°C
Acquisition mode	SRM
Quadrupoles Resolutions	Standard on Q1 (i.e. 2.0 amu width) Unit on Q3 (i.e. 0.7 amu width)

## SRM Transitions monitored for each target analyte

Compound	Transition 1 (CE, V)	Transition 2 (CE, V)
Benz[a]-anthracene	228.1>226.1 30 V	228.1>202.1 20 V
<sup>13</sup> C <sub>6</sub> -Benz[a]-anthracene	234.1>232.1 30 V	-
Chrysene	228.1>226.1 30 V	228.1>202.1 20 V
<sup>13</sup> C <sub>6</sub> -Chrysene	234.1>232.1 30 V	-
Benzo[b]-fluoranthene	252.1>250.1 30 V	252.1>226.1 30 V
<sup>13</sup> C <sub>6</sub> -Benzo[b]-fluoranthene	258.1>256.1 30V	-
Benzo[a]pyrene	252.1>250.1 30 V	252.1>226.1 30 V
<sup>13</sup> C <sub>4</sub> -Benzo[a]-pyrene	256.1>254.1 30 V	-

In every sequence, 7 points of calibration, blanks, and quality control sample were injected to ensure the best level of performance of the system. Prior to SRM analysis, SRM parameters were optimized.

Following criteria described in Commission Decision 2002/657/EC, two SRM transitions were chosen for each analyte, providing unambiguous identification of compounds.

## Results

The following chromatogram illustrates the instrumental response with 5 pg/μL of each compound (figure 1). For this chromatogram, S/N ratio of different peaks varies from 40 to 55 (peak to peak). Separation of benzo[b]fluoranthene from the other isomers of benzofluoranthene (l) and (k), in green) was acceptable. Calibration curves exhibited R<sup>2</sup> better than 0.9999 for all compounds, as shown in figures 2 and 3.

In order to assess the performance level of the system, several samples consisting in different kinds of matrices were analyzed. Among matrices submitted to regulation, smoked products like meat, fish or crustaceans were investigated.

Figure 4 shows a chromatogram obtained for a smoked meat product that contained very low levels of PAHs.

Peak intensities were approximately those obtained for the lowest calibration point (at 5 pg/μL).

For this smoked meat sample, the determined concentrations were:

- Benzo[a]pyrene: 0.06 μg/kg.
- PAH4 value: 0.52 μg/kg.

These values show how low the system could quantify PAHs. For benzo[a]pyrene and PAH4 value, the system could easily quantify PAHs levels from 20 to 30 times lower than the maximum residue limit of the September 2014 regulation.

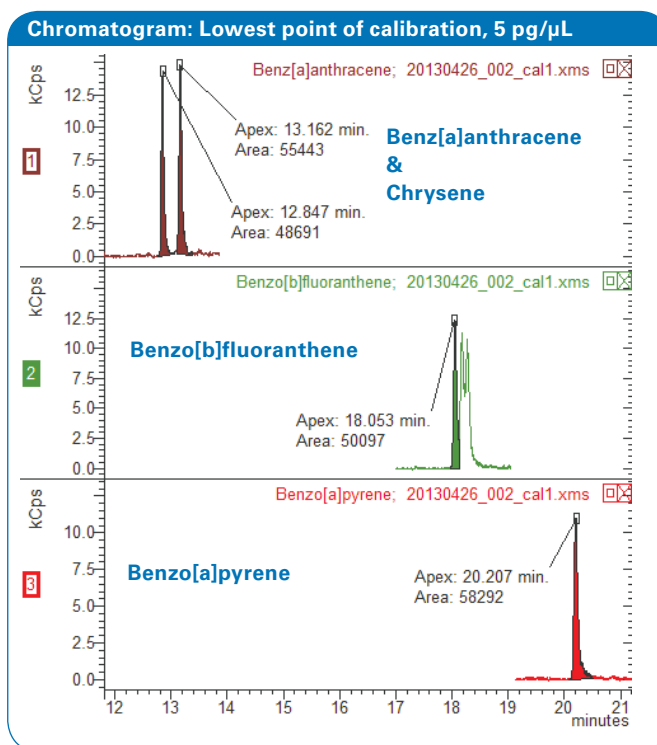


Figure 1: Typical diagnostic ion chromatograms (quantifier SRM transition) obtained for standard of benz[a]anthracene, chrysene, benzo[b]fluoranthene, and benzo[a]pyrene.

PAH	R <sup>2</sup>
Benz[a]anthracene	0.999996
Chrysene	0.999995
Benzo[b]fluoranthene	0.999980
Benzo[a]pyrene	0.999991

Figure 2: Linearity obtained from each target analyte for standard solutions using isotope dilution.

### Calibration curve for Melamine

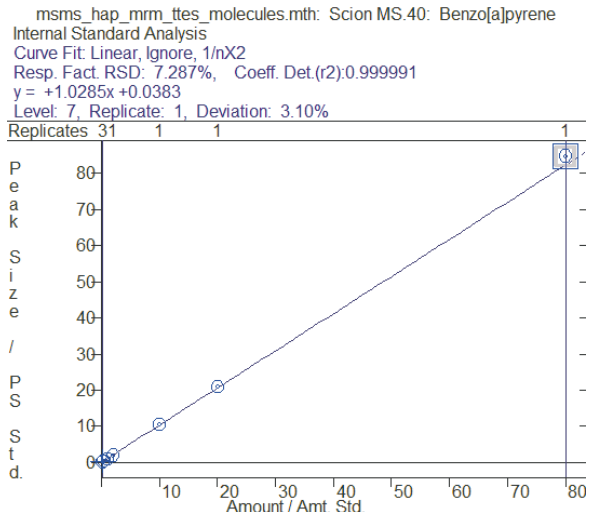


Figure 3: Example of calibration curve obtained for benzo[a]pyrene.

### Chromatogram: Lowest point of calibration, 5 pg/μL

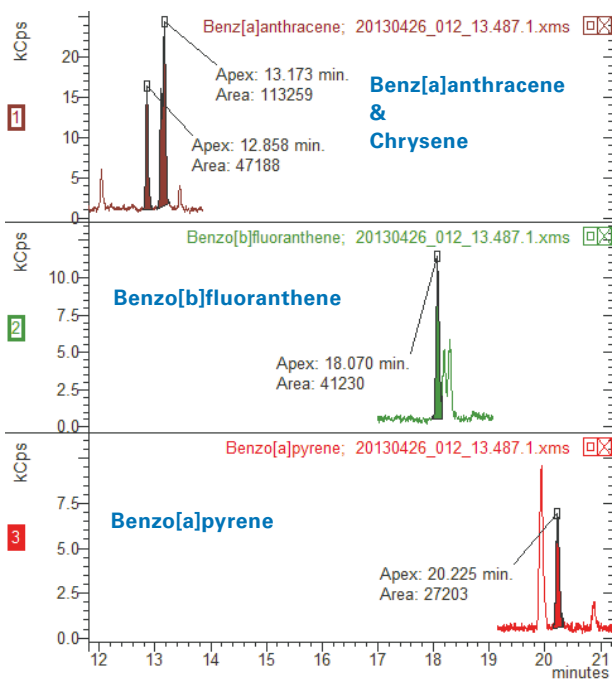


Figure 4: Typical diagnostic ion chromatograms (quantifier SRM transitions) obtained for a smoked meat sample. benzo[a] anthracene and chrysene (brown, two most intense peaks), benzo[b] fluoranthene (green, first peak), and benzo[a]pyrene (red, second peak in intensity).

### Conclusion

The excellent sensitivity of the instrument, combined with specificity of the SRM acquisition, provided efficient quantification of 4 regulated PAHs at very low-levels. In fact, detection limits were more than 20 times lower than the maximum residue limits proposed in the September 2014 regulation. These capabilities enable the Bruker EVOQ GC-TQ system to easily quantify low-level PAHs in complex food matrices.

### Acknowledgements

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- COMMISSION REGULATION (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs.
- EU COMMISSION DECISION 2002/657/EC N° L221/8 of 12 August 2002, Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

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