The Use of Tandem Quadrupole GC/MS/MS for the Determination of Polycyclic Aromatics Hydrocarbons (PAHs) in Food Products

Bruno Veyrand¹, Aline Brosseaud¹, Philippe Marchand¹, Fabrice Monteau¹, François Andre¹, Bruno Le Bizec¹, Timothy Jenkins², Jean-Marc Joumier³ ¹LABERCA, ENV, Atlanpôle La Chantrerie, BP 50707, NANTES Cedex 3, France. ²Waters Corporation, Manchester, UK. ³Waters EHQ, St Quentin en Yvelines FR

BACKGROUND

Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals that are formed during the incomplete burning of coal, oil, gas, wood, garbage or other organic substances, such as tobacco and charbroiled meat. There are more than 100 different compounds. PAHs generally occur as complex mixtures (for example, as part of combustion products such as soot), not as single compounds. A few PAHs are used in medicines and to make dyes, plastics, and pesticides. Others are contained in asphalt used in road construction. They can also be found in substances such as crude oil, coal, coal tar pitch, creosote, and roofing tar.

Food can be contaminated by an accumulation of PAHs in the food chain, due to their lipophilic properties and propensity to deposit in adipose tissue. Moreover, PAHs are also present due to heat processes such as smoking, grilling and smoke-drying.

Exposure to PAHs is a major concern for human health, as PAHs have been classified as carcinogenic. In the European Union, a new legislation was adopted in 2005 [1] and provided a list of 15 PAHs to monitor: Cyclopenta[c;d]pyrene, Benz[a]anthracene, Chrysene, 5-Methylchrysene, Benzo[b]fluoranthene, Benzo[j]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Indeno[1,2,3-c,d]pyrene, Dibenz[a,h]anthracene, Benzo[g,h,i]perylene, Dibenzo[a,l]pyrene, Dibenzo[a,e]pyrene, Dibenzo[a,i]pyrene, Dibenzo[a,h]pyrene.

PAHs in food

The concentration of PAHs in food is regulated by the European Union (208/2005 regulation), which determines maximum allowable limits. The objective of this application note is to demonstrate the relevance of GC/MS/MS (gas chromatography with tandem quadrupole mass spectrometry) in the detection and quantification of the broad range of PAH compounds in food products. The GC/MS/MS technology will be compared to other analytical approaches.

INSTRUMENTATION

For GC/MS experiments, a single quadrupole instrument was used. For GC/MS/MS experiments, a Waters[®] Quattro Micro GC[™] tandem quadrupole mass spectrometer was used.

Injector and transfer line temperatures were set at 280 °C and 330 °C respectively. Source temperature was set to 230 °C. GC column was a 5% phenyl substitued polydimethylsiloxane based phase with the following characteristics: 30 m x 0.25 mm id., film thickness 0.25 µm. The GC temperature parameters were set as follows 110 °C (1 min), 20 °C.min⁻¹ until 240 °C (0 min), 5 °C.min⁻¹ until 320 °C (10 min). Helium was used as carrier gas at 1 ml.min⁻¹. Electron ionization (EI) was operated at 70 eV in any case.



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RESULTS

Comparison of GC/MS and GC/MS/MS

Fragmentation of PAHs after electron ionization (EI) leads exclusively to the observation of molecular ion ($M^{+\bullet}$). The mass spectrum is generally poor in fragment ions so that identification of each compound on single MS is limited to the $M^{+\bullet}$ signal. Typical example of observed ion chromatograms for ${}^{13}C_{3}$ -pyrene (internal standard), ${}^{13}C_{6}$ -fluoranthene (internal standard), fluoranthene and pyrene in smoked tuna sample is shown on Figure 1. For some traces such as the one shown for ${}^{13}C_6$ -fluoranthene (m/z 208) and fluoranthene/pyrene (m/z 101), the signal is affected by co-extracted compounds. Moreover the signal to noise ratio of ${}^{13}C_3$ -pyrene is weak even at 0.5 µg.kg⁻¹ level. The main advantage of the MS/MS technique is the possible fragmentation of PAHs in the collision cell using high collision energies (30 to 50 V) to produce specific product ions.



Figure 1. Ion chromatograms (GC/MS, El ionization, SIM acquisition) corresponding to ${}^{13}C_3$ -pyrene (m/z: 205; internal standard), ${}^{13}C_6$ -fluoranthene (m/z 208; internal standard), fluoranthene and pyrene (both m/z 202 and 101) in a smoked tuna sample.

The monitored transitions are shown for the same sample on Figure 2. The results show very significant S/N improvement. The diagnostic absence/presence of target compounds is greatly facilitated, and the quantification does not suffer from the presence of any interference. The given example is transposable to all compounds; moreover, the internal standard signals (¹³C-analyte) do not disturb the corresponding ¹²C-analyte.



Figure 2. Ion chromatograms (GC/MS/MS, EI ionization, SRM acquisition) corresponding to ${}^{13}C_3$ -pyrene (m/z: 205>203; internal standard), ${}^{13}C_6$ -fluoranthene (208>206; internal standard), fluoranthene and pyrene (both 202>152 and 202>200) in a smoked tuna sample.

Limit of detection achievable by GC/MS/MS

Figure 3 shows an example of a smoked salmon sample in which concentration of benzo[a]pyrene has been determined at 0.04 μ g/kg of wet matter. Even at this concentration level, the S/N to noise ratio is still substantial, so that the detection is made possible down to low ppt level.

Fulfillment of identification criteria

The high stability of PAHs makes them difficult to fragment even under strong El conditions so that the observation of diagnostic product ions is rather limited. A direct consequence of this is the difficulty to unambiguously identify target analytes, especially with regard to the criteria specified in the 2002/657/EC decision. MS/MS is a detection technique which enables at least 2 specific transitions for each compound to be obtained, guaranteeing reliable analyte identification.



Figure 3. Ion chromatograms (GC/MS/MS, El ionization, MRM acquisition) corresponding to ¹³C₄-benzo[a]pyrene (256>254; internal standard), benzo[a]pyrene (252>250 and 252>226) in a smoked salmon sample at 0.04 μg/kg wet matter.

Figure 4 shows the monitoring of 4 transitions for benzo[*ghi*]perylene in cockle sample at 9.9 μ g.kg⁻¹. It was found that the typical number of identification points that could be obtained for each PAH was at least 7.

Application to Certified Reference Materials

Figure 5 shows the calibration curve obtained by GC/MS/MS for the quantification of Benzo [*a*] pyrene. The calibration curve is obtained with a good correlation coefficient ($r^2=0.9999$), which allows the determination of concentration with high accuracy.

An example of the quantification is made for a 0.5 g sample of mussel tissue (Standard Reference Material 2977, National Institute of Standards & Technology). Certified concentration for phenanthrene is 35.1 ± 3.8 µg/kg (dry matter basis). The isotope dilution approach (using ${}^{13}C_{6}$ -Phenanthrene as internal standard) combined with GC/MS/MS analysis allows for high precision. In our example, the concentration was calculated at 34.8 µg/kg of dry matter (within 1% of the assigned value).







Figure 5. Calibration curve (GC-MS/MS, El ionization, MRM acquisition) for Benzo [a] pyrene (252>250) versus ¹³C₄-Benzo [a] pyrene (256>254; internal standard).



Figure 6 shows a positive result from the analysis of a smoked fish sample. The concentration of Benzo[*a*]pyrene was determined as 6.94 (± 1.73) µg.kg⁻¹ of wet matter. The maximum level in smoked fish permitted by European legislation (Regulation EC No. 208/2005) is 5 µg.kg⁻¹.



Figure 6. Ion chromatograms corresponding to ¹³C₄-benzo[a]pyrene (256>254; internal standard), benzo[a]pyrene (252>250 and 252>226) in a positive sample of smoked fish.

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CONCLUSION

This application note demonstrates that GC/MS/MS offers significant advantages for PAHs analysis in food products compared to classical GC/MS approaches. Several benefits can be achieved including;

(1) Good sensitivity (down to ppt levels) in complex matrices using MRM.

(2) Confident confirmatory analysis using multiple MRMs (up to 7 identification points per congener).

(3) Excellent quantitative accuracy for analysis of a Certified Reference Material.

With the greater sensitivity, accuracy and confidence in results afforded by the Quattro micro GC, the food safety analytical laboratory has the ability to generate more meaningful data and satisfy strict regulatory requirements.

REFERENCES

- 1. Commission Recommendation of 4 February 2005 on the further investigation into the levels of polycyclic aromatic hydrocarbons in certain foods. Off. J. Eur. Union L34 (2005) 43.
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- 3. 2002/657/EC. Commission Decision of 14th August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.
- 4. Commission Directive 2005/10/EC of 4th February 2005 laying down the sampling methods and the methods of analysis for the official control of the levels of benzo [a] pyrene in foodstuffs.



WATERS CORPORATION 34 Maple St. Milford, MA 01757 U.S.A. T: 508 478 2000 F: 508 872 1990 www.waters.com



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