APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Determination of Polycyclic Aromatic Hydrocarbons in Seafood by UHPLC- MS/MS

Introduction

Polycyclic aromatic hydrocarbons (PAHs) have become ubiquitous in the environment mainly due to incomplete combustion of fossil fuels. PAHs can contaminate foods during smoking, heating, and drying processes that allow combustion products to come into direct contact with food. They can also enter food supply chains through contaminated air and water, and accumulate in various food chains. Environmental pollution resulting from fossil fuel combustion, oil spillage and so on can cause contamination with PAHs, particularly in fish and fishery products. Many PAHs compounds are considered toxic because of their carcinogenic and mutagenic effects.[1] Benzo(α)pyrene (BαP) has been used as a marker for measuring the occurrence and effect of carcinogenic PAH in food. In order to protect public health, setting the maximum levels are necessary for BαP in certain foods containing fats and oils and in foods where smoking or drying processes might cause high levels of PAHs contamination. Maximum levels of PAHs are also necessary in foods where environmental pollution may cause high levels of contamination, particularly in fish and fishery products. Regulations are in place to monitor PAHs levels in foods; the EU set a stringent maximum residue limit (MRL) for BαP in muscle meat of smoked fish and smoked fishery products at 2 µg/kg.[2] In this study, seafood samples were prepared using a QueChERS extraction method followed by a dispersive solid-phase extraction clean-up step.[3-4] The samples were subsequently analyzed by coupling a UHPLC system with a triple quadrupole mass spectrometer.

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Experimental

Hardware/Software

Chromatographic separation was conducted on a PerkinElmer UHPLC System, while detection was achieved using a PerkinElmer QSight® 220 triple quadrupole mass spectrometer with a dual ionization source. All instrument control, data acquisition and data processing were performed using the Simplicity 3Q™ software package.

Method Parameters

Sample Preparation

5.0 g of homogenized sample was weighed into a QuEChERS centrifuge tube (50 mL Teflon centrifuge tube, containing 4 g MgSO4 and 1 g NaCl, part number: N9306902). 10 mL of acetonitrile was added to the tube and vortex for three minutes, and then the sample tube was centrifuged for three minutes at 6000 r/min. 5 mL of the supernatant was transferred into a clean-up tube (15 mL Teflon centrifuge tube containing 900 mg MgSO4, 150 mg PSA and 150 mg C18, part number: N9306923) and shaken vigorously for two minutes. The mixture was then centrifuged at 6000 r/min for three minutes. The sample solution was filtered through a 0.22 µm membrane filter into an auto sampler vial for LC/MS/MS analysis.

LC Conditions

The seven PAH analytes were separated using a LC-PAH column (4.6 x 250 mm, 4.6 µm). The temperature of the column oven was set at 30 °C. The mobile phases consisted of (A) containing 5 mM ammonium acetate and 0.1% formic acid in water and (B) acetonitrile. The flow rate was 1.5 mL/min and the mobile phase gradient is shown in Table 1. The injection volume was 10 µL. For Benzo[α]Pyrene (BαP) analysis, a fast LC method was developed using a C18 column (4.6 x 100 mm, 2.6 µm) and an isocratic elution for three minutes with mobile phase ratio of A and B at 95:5.

Table 1. Mobile phase gradient.

	Time (min)	A%	B%
1	0.0	50	50
2	1.0	50	50
3	3.0	1	99
4	16.0	1	99
5	17.0	50	50
6	20.0	50	50

Mass Detection Parameters

The triple quadrupole mass spectrometer source conditions are listed in Table 2. The compound dependent parameters such as collision energy (CE) and the entrance voltage (EV) were optimized for each analyte by flow injection analysis and their values are shown in Table 3, in which the retention times of all the analytes determined are also listed.

Table 2. MS source conditions.

Ion Source	ESI positive
Electrospray Voltage	5500 V
Drying Gas	90 arbitrary units
Nebulizer Gas	320 arbitrary units
Source Temperature	500 °C
HSID Temperature	320 °C
Detection Mode	MRM

Table 3. Optimized MRM parameters and analyte retention times.

Order	Compound Name	MRM Transition Quantifier	Qualifier	RT (min)	CE (eV)	EV (V)
1	Benzo(k)Fluoranthene	253.1	250.2	8.00	-72	47
		253.1	224.2		-100	47
2	Benzo(b)Fluoranthene	253.0	250.3	8.42	-72	56
		253.0	224.1		-90	56
3	Benzo(j)Fluoranthene	253.0	250.2	9.35	-73	36
		253.0	224.2		-96	47
4	Benzo[a]Pyrene	253.1	250.2	10.65	-79	30
		253.1	224.1		-100	30
5	Dibenzo(a,h)Anthracene	279.0	276.1	11.97	-74	45
		279.0	263.1		-46	45
6	Benzo(ghi)Perylene	277.0	274.3	14.21	-85	42
		277.0	248.2		-104	42
7	Indeno(1,2,3-cd)Pyrene	277.1	274.2	15.30	-84	42
		277.1	248.2		-102	42

Results and Discussion

A LC/MS/MS method for the determination of seven PAHs was successfully developed and validated. As illustrated in Figure 1, all seven PAH compounds were detected with good peak shape and sensitivity. Using BαP as an example, the product ion mass spectra showing the fragmentations of the parent ion are shown in Figure 2. Since BαP is often used as a marker for monitoring the occurrence and effect of carcinogenic PAH in food, a fast and sensitive LC/MS/MS method was also developed for the determination of BαP within three minutes as shown in Figure 3.

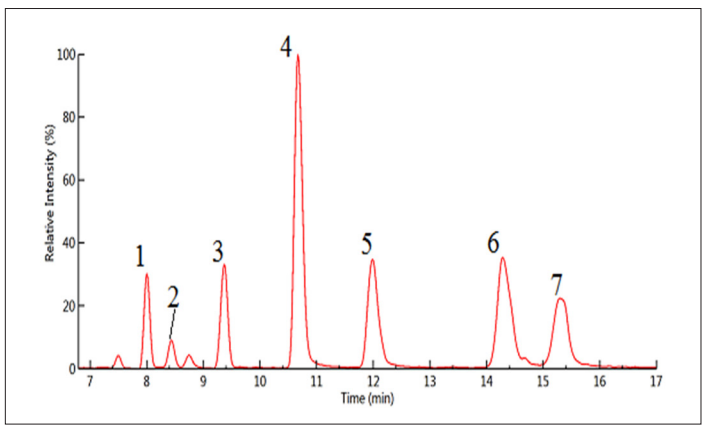

Figure 1. Typical LC/MS/MS chromatograms for the seven PAHs (10 µg/L) (compound names and orders are shown in Table 3).

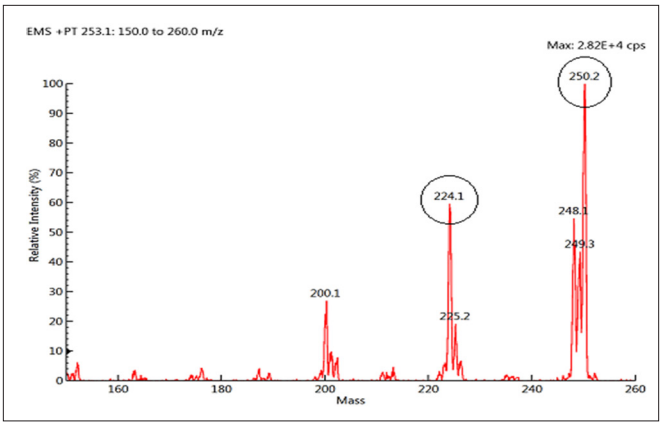

Figure 2. Product ion mass spectrum of benzo(α)pyrene (BαP).

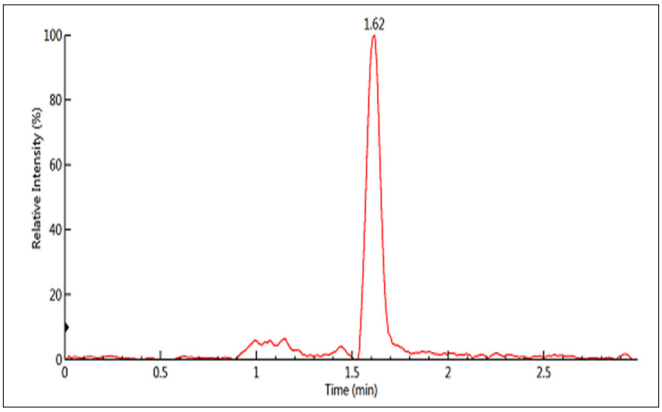

Figure 3. Typical LC–MS/MS chromatogram of benzo(α)pyrene (BαP) (at 1 µg/L).

In this study, matrix-matched calibration curves were used for quantification. As shown in Figure 4 using BαP as an example, all the matrix-matched calibration curves showed good linearity with regression coefficients (R^2) values greater than 0.994. The limit of quantification (LOQs) for all the PAHs studied were determined based on EU regulation (S/N >10 and %RSD <20%).[5] The determined LOQs for the studied PAHs ranged from 0.2 to 2 µg/kg in sample matrix as shown in Table 4. The stringent maximum residue limit (MRL) set by EU for BαP in muscle meat of smoked fish and related products is at 2 µg/kg. The method developed in this study can easily meet this requirement.

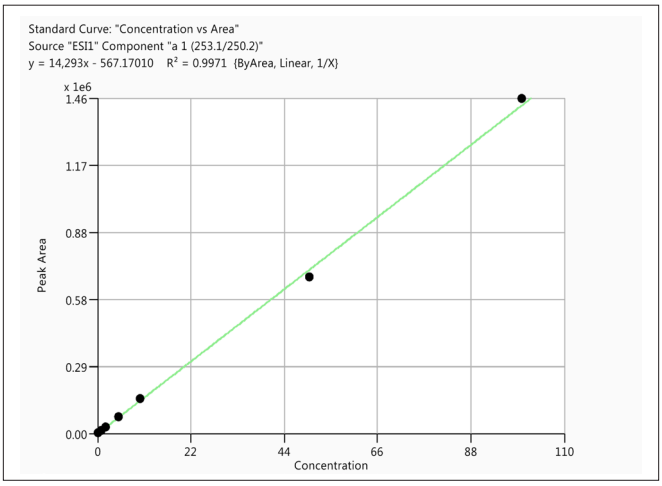

Figure 4. Calibration curves (ranging from 0.1 to100 µg/L) for BαP.

Table 4. Matrix matched calibration curve results and LOQs of the method.

Compound	Linear Range (µg/L)	Regression Coefficients (R^2)	LOQ (µg/kg)
Benzo(k)Fluoranthene	0.5 - 100	0.999	1
Benzo(b)Fluoranthene	1 - 100	0.994	2
Benzo(j)Fluoranthene	0.5 - 100	0.998	1
Benzo[a]Pyrene	0.1 - 100	0.997	0.2
Dibenzo(a,h)Anthracene	0.5 - 100	0.998	1
Benzo(ghi)Perylene	0.5 - 100	0.996	1
Indeno(1,2,3-cd)Pyrene	0.5 - 100	0.997	1

The recoveries of the analytes were evaluated by spiking analytes to the samples at concentrations of 5 and 50 µg/kg, respectively. As shown in Table 5, the recoveries for all the analytes were within the acceptable range (70 to 120%),[5] with mean values ranging from 71.2% to 103.2% with RSD <11%.

Table 5. Recovery results for the PAHs studied.

Compound Name	Spiked Level (5 µg/kg)		Spiked Level (50 µg/kg)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Benzo(k)Fluoranthene	78.3	6.8	76.3	6.1
Benzo(b)Fluoranthene	71.2	10.3	77.6	9.6
Benzo(j)Fluoranthene	80.1	5.9	86.6	6.5
Benzo[a]Pyrene	98.7	3.6	103.2	4.2
Dibenzo(a,h)Anthracene	75.9	7.1	83.5	7.8
Benzo(ghi)Perylene	87.5	8.8	88.7	6.7
Indeno(1,2,3-cd)Pyrene	83.9	7.9	92.6	5.9

Conclusions

An LC/MS/MS method for the determination of polycyclic aromatic hydrocarbons (PAHs) in seafood has been developed by coupling a UHPLC system to a QSight 220 triple-quad mass spectrometer. This method can be applied to the determination of PAHs in seafood samples with good accuracy and precision. The QuEChERS extraction method provides a simple and efficient routine sample preparation procedure for PAHs in seafood samples. The method has the advantages of high sensitivity and selectivity for detecting PAHs by coupling UHPLC to tandem mass spectrometry. Two mass transition ion pairs were employed for each analyte in the MRM mode to enhance the confidence for compound identification according to the EC guidelines.[5] In addition, a rapid LC/MS/MS method has been developed in this study for the analysis of targeted compound benzo[α]pyrene (BαP) within three minutes. These methods can be easily applied by analytical testing laboratories for routine analysis of a large number of samples.

References

1. Xue W, Warshawsky D. Toxicology and Applied Pharmacology, 2005, 206(1), 73-93.
2. European Union Commission Regulation No. 1881/2006, Off. J. Eur. Comm. 2006, L364, 5-24.
3. Ramalhosa M J, Paiga P, Morais S, et al. Journal of Separation Science, 2009, 32(20):3529-3538.
4. Guo M, Wu H, et al. Environmental Chemistry,2013,32(6):1025-1031.
5. European Commission, SANCO. 2015. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed, SANTE/11945/2015 https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_11945.pdf.

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