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Application News

Gas Chromatograph Tandem Mass Spectrometer GCMS-TQ™8050 NX

Analysis of Polycyclic Aromatic Hydrocarbons (PAH) in Palm Oil by GC-MS/MS

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User Benefits

- Simple Tandem SPE sample preparation for PAH extraction and clean-up from palm oil
- GC-MS/MS with MRM acquisition mode enables the detection of PAH in palm oil with high sensitivity and selectivity

■ Introduction

Polycyclic Aromatic Hydrocarbons (PAH) are a group of organic compounds primarily formed from the incomplete combustion of organic materials[1]. PAH are carcinogenic, teratogenic and mutagenic contaminants that are toxic to human health[1]. They are highly stable and are present in environment and food[1]. It was reported that food is one of the major sources of PAH exposure, including edible oils due to their lipophilic nature and high consumption[2]. PAH may be introduced into edible oils from the environment and drying process during production[1]. In 2002, Scientific Committee on Food (SCF) identified 15 PAH that might be regarded potentially as genotoxic and carcinogenic[3]. In 2005, Joint FAO/WHO Expert Committee on Food Additives (JECFA) added an additional PAH to the list. This list is known as 15+1 EU Priority PAH. Out of this list, Commission Regulation (EU) No 835/2011 stipulated that the maximum limit of benzo[a]pyrene and the sum of PAH4 (benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene) in edible oil to be 2.0 µg/kg and 10.0 µg/kg, respectively[4]. In this study, an MRM method using Shimadzu GCMS-TQ8050 NX was established and evaluated for qualitative and quantitative determination of 15+1 PAH in palm oil.

Table 1: GC-MS/MS analytical conditions

Instrumentation	
GC-MS/MS system	GCMS-TQ8050 NX
Auto Injector	AOC-20i+s Plus
Column	SH-I-PAH (P/N: 227-36074-01) (30 m x 0.25 mm x 0.10 µm)
Gas Chromatograph	
Injection Condition	330 ℃, splitless mode
Split Ratio Program	Time: 3.00 → Split Ratio: 90 Time: 8.00 → Split Ratio: 20
Injection Volume	1 µL
Carrier Gas	Helium
Gas Flow Condition	Constant linear velocity mode Linear velocity 50.0 cm/s Purge flow 3.0 mL/min
High Pressure Injection	2 min at 250 kPa
Column Temperature Program	110 ℃ (1min) → 30 ℃/min to 240 ℃ (2 min) → 3 ℃/min to 270 ℃ (2 min) → 8 ℃/min to 340 ℃ (8 min)
Mass Spectrometer	
Ion Source Temperature	230 ℃
Interface Temperature	300 ℃
Solvent Cut Time	3 min
Acquisition Mode	MRM

■ Experimental

Instrumental and Analytical Conditions

A triple quadrupole GC-MS/MS system, GCMS-TQ8050 NX (Shimadzu Corporation, Japan) was employed in this work. The details of the system and analytical conditions for the GC-MS/MS method are shown in Table 1. Each of the 15+1 PAH was monitored via one quantitative MRM transition and two qualitative MRM transitions. The details of MRM transitions, collision energy (CE) values and internal standard (IS) grouping are tabulated in Table 2.

Table 2: MRM transitions, collision energies of PAH and internal standards.

Compound name	IS Grp	Target transition	CE (V)	Reference transition	CE (V)
Benzo[c]fluorene	1	216.10>215.10	22	216.10>189.10	30
Benz[a]anthracene	1	228.10>226.10	28	228.10>202.10	26
Chrysene-D12 (IS)	1	240.20>236.20	28	240.20>238.20	26
Cyclopenta[c,d]pyrene	1	226.10>224.10	38	226.10>200.10	30
Chrysene	1	228.10>226.10	28	228.10>202.10	26
5-Methylchrysene	1	242.10>239.10	32	242.10>215.10	22
Benzo[b]fluoranthene	2	252.10>250.10	28	252.10>226.10	30
Benzo[j]fluoranthene	2	252.10>250.10	30	252.10>226.10	30
Benzo[k]fluoranthene	2	252.10>250.10	30	252.10>226.10	30
Benzo[a]pyrene	2	252.10>250.10	30	252.10>226.10	24
Perylene-D12 (IS)	2	264.20>260.20	47	264.20>262.20	44
Indeno[1,2,3-cd]pyrene	2	276.10>274.10	34	276.10>250.10	30
Dibenz[a,h]anthracene	2	278.10>276.10	30	278.10>252.10	30
Benzo[g,h,i]perylene	2	276.10>274.10	32	276.10>275.10	28
Dibenzo[a,l]pyrene	2	302.10>300.10	36	302.10>298.10	60
Dibenzo[a,e]pyrene	2	302.10>300.10	36	302.10>276.10	28
Dibenzo[a,i]pyrene	2	302.10>300.10	36	302.10>276.10	28
Dibenzo[a,h]pyrene	2	302.10>300.10	36	302.10>276.10	28

Standards and Sample Preparation

Palm oil was purchased from Indonesia. EU 15+1 PAH standard mixture (100 mg/L) was obtained from Restek Corporation, USA while chrysene-D12 and perylene-D12 internal standards were obtained from Cambridge Isotope Laboratories Inc., USA.

Matrix blank was prepared according to Xu et al. with some modification[1], using a tandem solid-phase extraction (SPE) comprising of SupelMIP® SPE-PAHS (50 mg/3 mL) and Supelclean™ ENVI-Carb™ (500 mg/ 6 mL) SPE, purchased from Merck KGaA. First, 0.5 mL of the palm oil (the weight was recorded for calculation) was vortex-mixed with 0.5 mL of cyclohexane. Prior to the extraction, SupelMIP® SPE-PAHS and Supelclean ENVI-Carb SPE were conditioned separately with 3 mL and 6 mL of cyclohexane, respectively. Next, the SupelMIP SPE-PAHS (top) was coupled to Supelclean ENVI-Carb SPE (bottom) and loaded with the mixture of palm oil and cyclohexane. Flow rate of sample loading was controlled at below 0.1 mL/min. Washing was performed with 3 x 1 mL of cyclohexane. The position of cartridges was then switched, followed by elution with 8 mL of 5% toluene in ethyl acetate. Afterwards, the eluate was evaporated using TurboVap® (Biotage Sweden) to a final volume of 1 mL of matrix blank.

The PAH standard mixture was diluted to 10 mg/L in toluene as working standard. A 100 µg/L internal standard working solution, consisting of chrysene-D12 and perylene-d12, in toluene was prepared. Matrix-matched internal standard calibration solutions were then prepared accordingly in the matrix blank. The PAH concentrations prepared were 0.2, 0.5, 1, 2, 5, and 10 µg/L (which covers 0.447 – 22.346 µg/kg, subject to the weight of palm oil used). The internal standard concentrations of 5 µg/L chrysene-D12 and 5 µg/L perylene-D12 were in each of the calibration solutions.

To determine the recovery, the matrix blank which was spiked with PAH standard mixture and internal standards before extraction was analyzed.

■ PAH Analysis Results Using GC-MS/MS

Detection and Separation

The EU 15+1 PAH were separated in the GC-MS/MS using SH-I-PAH column. The mass chromatograms of all compounds are displayed in Figure 1, while mass chromatograms of chrysene-D12 and perylene-D12, which are the internal standards, are shown in Figure 2.

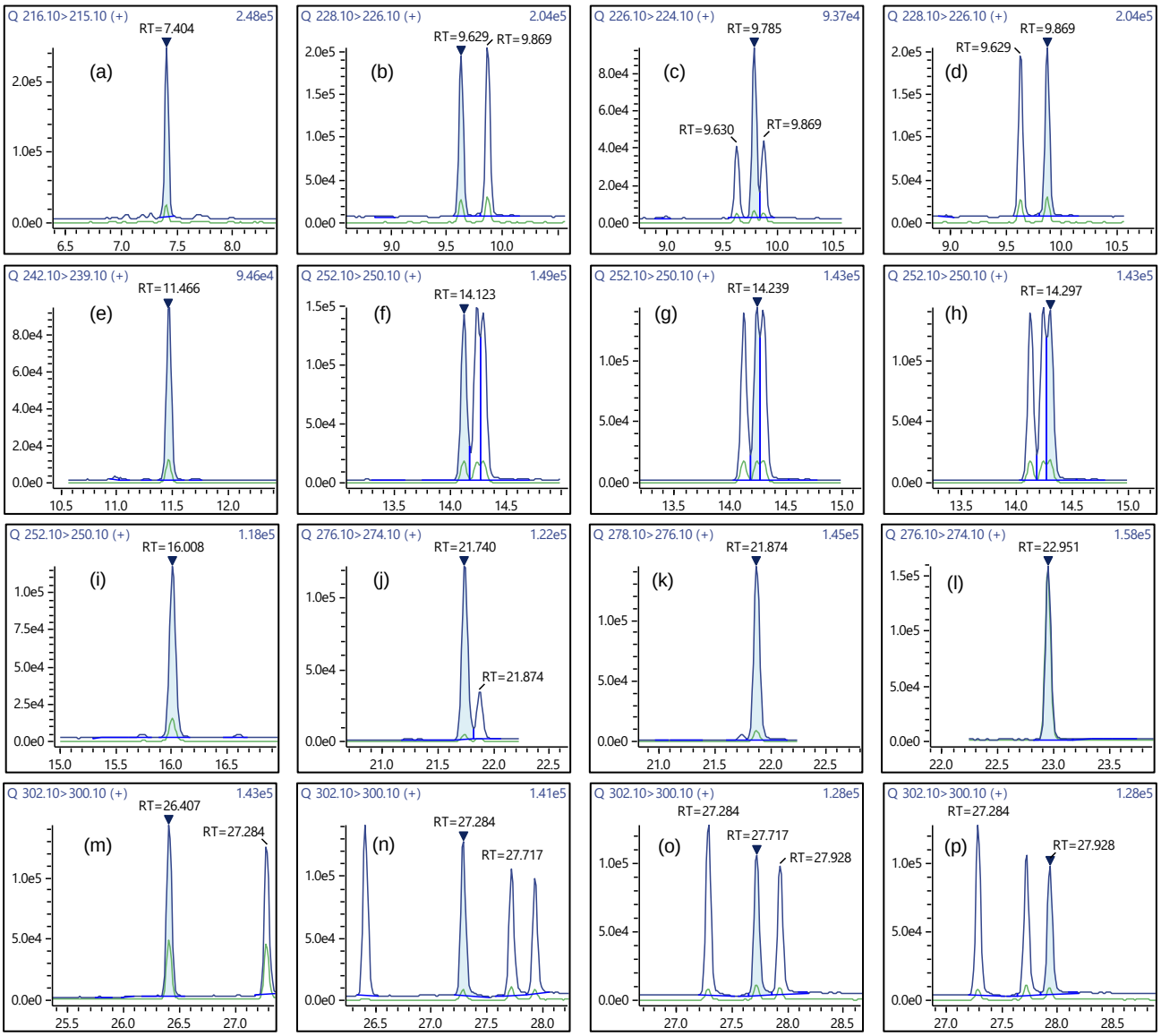

Figure 1: PAH mass chromatograms of: **(a)** benzo[c]fluorene, **(b)** benz[a]anthracene, **(c)** cyclopenta[cd]pyrene, **(d)** chrysene, **(e)** 5-methylchrysene, **(f)** benzo[b]fluoranthene, **(g)** benzo[j]fluoranthene, **(h)** benzo[k]fluoranthene, **(i)** benzo[a]pyrene, **(j)** indeno[1,2,3-cd]pyrene, **(k)** dibenz[a,h]anthracene, **(l)** benzo[ghi]perylene, **(m)** dibenzo[a,l]pyrene, **(n)** dibenzo[a,e]pyrene, **(o)** dibenzo[a,i]pyrene, **(p)** dibenzo[a,h]pyrene at 5 µg/L. Each of the PAH target peak is shaded.

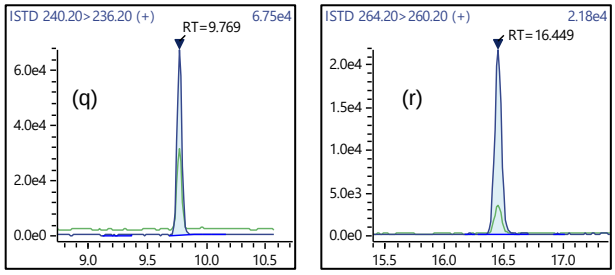

Figure 2: Mass chromatograms of internal standards **(q)** chrysene-D12 and **(r)** perylene-D12 at 5 µg/L.

Calibration Range & Linearity

Matrix-matched internal standard calibration curves were set up for the 15+1 PAH using calibration solutions prepared. The calibration range of each PAH is shown in Table 3. Calibration curves of all PAH demonstrated excellent linearity with R^2 of at least 0.9979 (Table 3). The calibration curves of PAH4 (benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene) regulated by EU are shown in Figure 3.

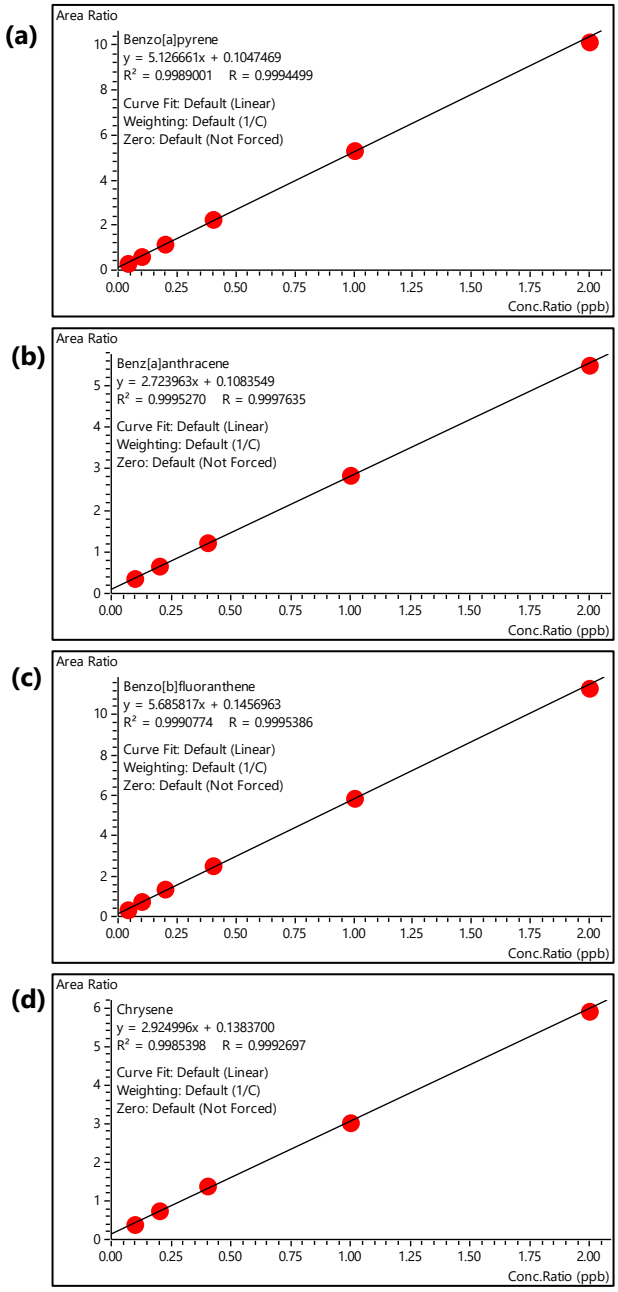

Figure 3: Internal standard calibration curves of **(a)** benzo[a]pyrene, **(b)** benz[a]anthracene, **(c)** benzo[b]fluoranthene, and **(d)** chrysene.

Table 3: Calibration range and R^2 value for 15+1 PAH

Compound name	Calibration range (µg/L)	R^2
Benzo[c]fluorene	1.0 – 10	0.9994
Benz[a]anthracene	0.5 – 10	0.9995
Cyclopenta[c,d]pyrene	0.5 – 10	0.9997
Chrysene	0.5 – 10	0.9985
5-Methylchrysene	0.2 – 10	0.9993
Benzo[b]fluoranthene	0.2 – 10	0.9991
Benzo[j]fluoranthene	0.2 – 10	0.9987
Benzo[k]fluoranthene	0.2 – 10	0.9985
Benzo[a]pyrene	0.2 – 10	0.9989
Indeno[1,2,3-cd]pyrene	0.2 – 10	0.9988
Dibenz[a,h]anthracene	0.2 – 10	0.9990
Benzo[g,h,i]perylene	0.2 – 10	0.9991
Dibenzo[a,l]pyrene	0.2 – 10	0.9986
Dibenzo[a,e]pyrene	0.2 – 10	0.9981
Dibenzo[a,i]pyrene	0.2 – 10	0.9979
Dibenzo[a,h]pyrene	0.5 – 10	0.9988

Quantitation and Recovery

Quantitation of palm oil sample was done by analyzing palm oil matrix blank that was spiked with internal standards. None of 15+1 PAH was detected in this palm oil sample. For recovery calculation, the matrix blank was spiked with 5 µg/L (equivalent to 11.173 µg/kg in palm oil) of both Internal standards and PAH standards before the tandem SPE sample preparation. Then, concentrations were quantified using the internal standard calibration curves. The recovery of PAH4 were between 76.15%-88.11% which is within the criteria determined by EU Regulation No 836/2011 (recovery should be between 50%-120%)[5]. The quantitation and recovery results of palm oil spiked with 15+1 PAH are presented in Table 4.

It is worth noting that triphenylene, which shares the same MRM target transition as chrysene, could be an interference during quantification of chrysene. Confirmation method with a different column is recommended to verify the quantitation when chrysene concentration falls above the regulated upper limit.

Table 4: Summary of quantitation of 15+1 PAH in PAH-spiked palm oil sample and their recovery

Compound name	Concentration (µg/kg)	Recovery (%)
Benzo[c]fluorene	6.530	58.44
Benz[a]anthracene	8.508	76.15
Cyclopenta[c,d]pyrene	10.326	92.42
Chrysene	9.846	88.11
5-Methylchrysene	9.125	81.67
Benzo[b]fluoranthene	8.985	80.42
Benzo[j]fluoranthene	8.262	73.95
Benzo[k]fluoranthene	9.489	84.93
Benzo[a]pyrene	9.353	83.71
Indeno[1,2,3-cd]pyrene	9.448	84.56
Dibenz[a,h]anthracene	9.493	84.96
Benzo[g,h,i]perylene	9.450	84.57
Dibenzo[a,l]pyrene	9.010	80.64
Dibenzo[a,e]pyrene	9.643	86.30
Dibenzo[a,i]pyrene	9.856	88.21
Dibenzo[a,h]pyrene	9.259	82.86

Limit of Detection (LOD)

The following formula was used to determine the calculated LOD (Cal. LOD) of the PAH based on the lowest level in the calibration:

$$Cal.\ LOD = \frac{Conc.of\ the\ Lowest\ Calib\ Std}{Signal - to - Noise\ Value} \times 3$$

The LODs of all 15+1 PAH in palm oil sample were determined to be in the range of 0.038 - 0.327 µg/kg (Table 5). The LODs of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene (PAH4) regulated by EU were in the range of 0.071 - 0.157 µg/kg, fulfilling the criteria of ≤ 0.30 µg/kg stated in Commission Regulation (EU) No 836/2011[5]. This demonstrates the capability of this MRM method in detecting low concentrations of PAH in palm oil.

Limit of Quantitation (LOQ)

Calculated LOQ (Cal. LOQ) was determined to be 3.33 times of the Cal. LOD. The LOQs of all 15+1 PAH in the palm oil were calculated to be in the range of 0.127 – 1.090 µg/kg (Table 5). The LOQs of PAH4 regulated by EU were in the range of 0.236 – 0.522 µg/kg which fulfils the criteria of ≤0.9 µg/kg as stated in Commission Regulation (EU) No 836/2011[5]. This demonstrates the capability of this MRM method in quantifying PAH in palm oil within the regulated limit.

Repeatability

Analysis method precision was determined by analyzing 8 consecutive runs of the low concentration (0.5 µg/L) and mid concentration (5 µg/L) of calibration levels in matrix. The %RSD of peak area ratio of all PAH with respect to their internal standard were <8% and <5% for low and mid concentration levels, respectively (Table 5). To stretch it further, the 5 µg/L calibration solution in matrix was analyzed for another 72 times within 48 hours duration. The %RSD of peak area ratio of all PAH (n=72) with respect to their internal standard were <5%, except for dibenzo[a,i]pyrene, which was <8% (Table 5). These results indicate that the optimized MRM method has high stability and analysis precision.

Table 5: Summary of LOQ, LOD and repeatability of low and mid concentration levels

Compound name	Cal. LOD (µg/kg) in oil sample	Cal. LOQ (µg/kg) in oil sample	Area ratio %RSD of 0.5 µg/L solution (n=8)	Area ratio %RSD of 5 µg/L solution (n=8)	Area ratio %RSD of 5 µg/L solution (n=72)
Benzo[c]fluorene	0.327	1.090	6.2	1.5	2.2
Benz[a]anthracene	0.176	0.588	5.3	1.6	2.4
Cyclopenta[c,d]pyrene	0.157	0.522	3.1	1.5	4.2
Chrysene	0.175	0.583	5.9	2.0	3.2
5-Methylchrysene	0.112	0.373	2.8	1.6	2.4
Benzo[b]fluoranthene	0.088	0.293	3.0	1.8	2.7
Benzo[j]fluoranthene	0.077	0.256	5.2	4.1	4.3
Benzo[k]fluoranthene	0.077	0.255	5.1	3.9	4.8
Benzo[a]pyrene	0.071	0.236	4.0	2.1	2.8
Indeno[1,2,3-cd]pyrene	0.049	0.163	1.4	2.2	2.3
Dibenz[a,h]anthracene	0.045	0.150	1.9	1.7	2.6
Benzo[g,h,i]perylene	0.038	0.127	2.7	2.2	2.5
Dibenzo[a,l]pyrene	0.107	0.358	7.3	3.4	4.4
Dibenzo[a,e]pyrene	0.127	0.423	7.2	2.4	3.7
Dibenzo[a,i]pyrene	0.114	0.378	5.3	3.0	7.9
Dibenzo[a,h]pyrene	0.137	0.458	5.3	3.0	4.3

■ Conclusion

This application news illustrates the development of an MRM method using Shimadzu GCMS-TQ8050 NX for the analysis of polycyclic aromatic hydrocarbons (PAH) in palm oil with tandem SPE sample preparation. The results show that this method allows the detection and quantification of 15+1 PAH in palm oil at low concentration, down to 0.071 µg/kg LOD and 0.127 µg/kg LOQ, respectively. Excellent linearity of internal standard calibration curve with R^2 values of at least 0.9979 was obtained. On top of the high sensitivity and selectivity, the optimized MRM method also showed good repeatability of <5% and <8% at mid and low concentration levels (n=8) of the calibration levels, respectively.

■ References

1. Xu, T. et al, Simultaneous Determination of 24 Polycyclic Aromatic Hydrocarbons in Edible Oil by Tandem Solid-Phase Extraction and Gas Chromatography Coupled/Tandem Mass Spectrometry, Journal of AOAC INTERNATIONAL 98(2), 529-537.

2. Zacs, D. et al, Multi-walled carbon nanotubes as effective sorbents for rapid analysis of polycyclic aromatic hydrocarbons in edible oils using dispersive solid-phase extraction (d-SPE) and gas chromatography-tandem mass spectrometry (GC-MS/MS), Food Analytical Methods (11), 2508–2517

3. Polycyclic Aromatic Hydrocarbons in Food, Scientific Opinion of the Panel on Contaminants in the Food Chain (Question N° EFSA-Q-2007-136), The EFSA Journal (2008) 724, 1-114.

4. Commission Regulation (EU) No 835/2011

5. Commission Regulation (EU) No 836/2011

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