WEJ Contaminants

Simple, Fast, Innovative and Automated Determination of 27 Polycyclic Aromatic Hydrocarbons (PAHs) in Oils and Fats by LC-LC-GC-MS

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are due to their mutagenicity, carcinogenicity and toxicity important food contaminants. In routine, the extraction of PAHs in oils and fats is either performed by saponification or by caffeine-complexation in combination with a solid-phase extraction clean-up and analysis by GC-MS [1]. This method is complex and time-intensive. Therefore, the aim was to simplify the sample preparation and to decrease chromatographic interferences. Thus, a fast and robust method by LC-LC-GC-MS was tested [2]. Prior to analysis by LC-LC-GC-MS no further extraction or clean-up is needed and the extract can be injected directly.

Method

Sample Preparation

The sample preparation consists of weighing of 300 mg sample into a 2 mL autosampler vial, the addition of internal standard and vortexing for 10 seconds.

LC-LC-GC-MS

Normal-Phase LC based on silica gel is a well-known technique for the analysis of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH). Because PAHs belong to MOAH, this approach was tested. However, the analysis is interfered by co-eluting olefinic hydrocarbons. Epoxidation for the removal of these interferences is not applicable due to possible loss of PAHs. Therefore, a combination of two LC columns is used. The first LC dimension (silica gel) is used for the separation of matrix interferences from the fraction containing PAHs/polyunsaturated compounds. Using the second LC dimension (tetrachlorophthalimidopropyl-modified silica gel), the separation of PAHs and polyunsaturated compounds is achieved (**figure 1**).

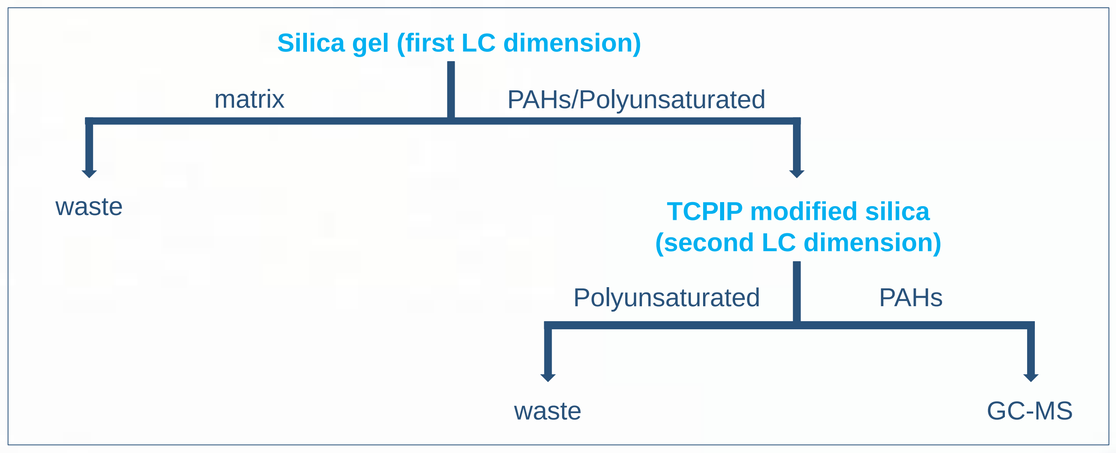

Figure 1 Scheme of LC-LC-GC-MS analysis

LC-System:	1260 Infinity II HPLC, Agilent Technologies
First column:	Allure Si HPLC column (250 mm × 2.1 mm, 5 µm, 60 Å), Restek
Second column:	Chromspher Pi (80 mm × 3.0 mm, 5 µm, 120 Å), Agilent Technologies
Eluent A:	Hexane
Eluent B:	Dichloromethane
Injection Volume:	67 µL
Interface:	Chronect LC-GC, Axel Semrau
GC-System:	7890B, Agilent Technologies
MS-System:	5977B MSD, Agilent Technologies
GC-Column:	PAH Select (15 m x 0,15 mm, 0,10 µm), Agilent Technologies

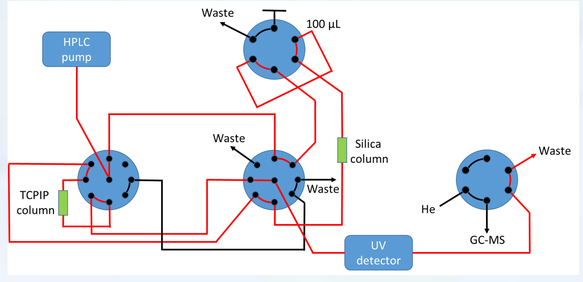

Figure 2 Scheme of LC-LC-GC-MS valve configuration [2]

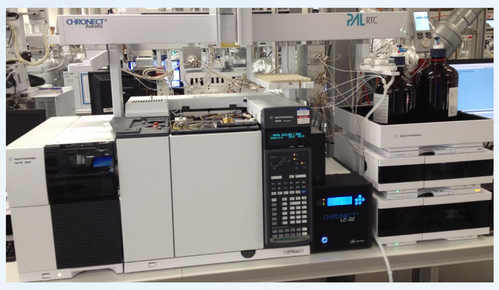

Figure 3 LC-LC-GC-MS system by Axel Semrau

Results

More than 200 different oils and fats including sunflower oil, coconut oil, olive oil, sesame oil, rapeseed oil, walnut oil, soy oil, cacao butter and fish oil were analyzed by the routine method and using the LC-LC-GC-MS method. A mixture of sunflower oil, soy oil and pumpkin seed oil was spiked with PAHs and prepared using the routine method (**figure 4**) and LC-LC-GC-MS (**figure 5**). Matrix interferences were enormously reduced by LC-LC-GC-MS due to the efficient clean-up of the two-dimensional heart-cut LC. Therefore, all samples analyzed by LC-LC-GC-MS delivered evaluable chromatograms with less matrix interferences compared to the routine method.

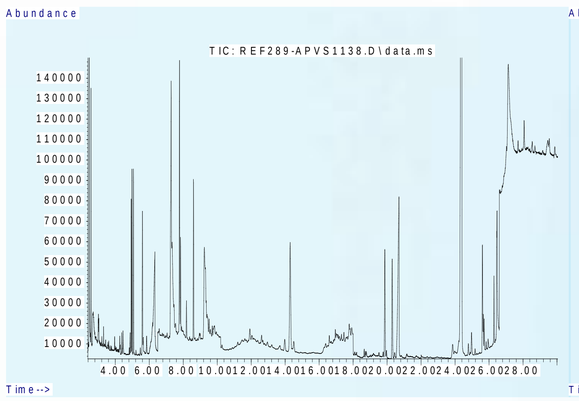

Figure 4 GC-MS TIC chromatogram of routine method of a mixture of sunflower oil, soy oil and pumpkin seed oil

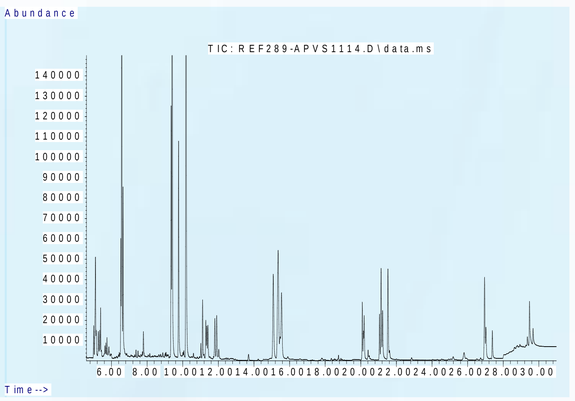

Figure 5 LC-LC-GC-MS TIC chromatogram of a mixture of sunflower oil, soy oil and pumpkin seed oil

The results of the analyzed fats and oils were comparable to the routine method. In addition, for the verification of the trueness several proficiency test samples were analyzed by LC-LC-GC-MS. Results of the Ducares proficiency test “PAK15-2” in animal fat were within the limits for both methods indicating the trueness of the LC-LC-GC-MS method (**table 1**).

Table 1 Results of Ducares proficiency test “PAK15-2” in animal fat

Analyt	Assigned value [ppb]	Limits [ppb]	Routine method [ppb]	LC-LC-GC-MS [ppb]
Anthracene	3,1	0,79 - 5,4	2,4	3,4
Benzo[a]anthracene	5,6	4,6 - 6,6	6,0	5,2
Benzo[b]fluoranthene	14,7	10,1-19,4	17,0	13,7
Benzo[k]fluoranthene	13,5	11,4-15,7	15,0	13,2
Benzo[a]pyrene	16,2	6,5-25,8	17,0	15,4
Chrysene	5,4	3,5-7,4	5,3	5,3
Dibenzo[a,h]anthracene	9,6	3,4-15,8	9,4	8,8
Fluoranthene	32,4	27,0-37,9	35,0	32,0
Indeno[1,2,3-cd]pyrene	37,5	25,2-49,7	45,0	33,3
Phenanthrene	20,9	6,2-35,6	19,0	19,4
Pyrene	10,5	3,8-17,2	10,0	10,4

Furthermore, verification data including intra-day precision, linearity, trueness, recovery, limit of quantification (LOQ) and robustness were sufficient and in compliance with the European Regulation 836/2011 (**table 2**). Therefore, the method is suitable for the determination of PAHs in fats and oils.

Table 2 Validation parameter of LC-LC-GC-MS

Parameter	Value
Intra-Day Precision	0,1-7,8%
Linearity	$R^2 > 0,995$
Recovery	66-96%
LOQ	0,03-0,07 ppb [2]

Conclusion

Using this method the sample throughput can be enhanced by decreasing the consumable costs and solvent consumption and increasing the chromatographic quality. In addition, the blank values of volatile PAHs such as phenanthrene, pyrene and fluoranthene are reduced dramatically due to the decreased use of consumables and solvents. Furthermore, the LC-LC-GC-MS-system is suitable for improving the LOQ without an elaborated sample preparation and clean-up.

The results of the analyzed samples were comparable to the routine method and the obtained verification results were in full compliance with the EU Regulation 836/2011. However, the configuration of the instrument is very complex. Therefore, experience in LC-GC-technique is necessary to achieve good robustness of the system. Overall, the analysis by LC-LC-GC-MS combines a fast and easy sample preparation for the analysis of PAHs in oils and fats with an automated sample clean-up and analysis without obvious matrix interferences.

Literature

[1] C.M. Schulz, H. Fritz, A. Ruthenschrör, *Food Addit. Contam. A*, **2014**, *31* (10), 1723-1735. [2] M. Nestola, R. Friedrich, P. Bluhme, T.C. Schmidt, *Anal. Chem.*, **2015**, *87* (12), 6195-6203

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