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CALIBRATION OF GC/MS METHOD AND VALIDATION OF THE MODIFIED SAMPLE PREPARATION FOR DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS

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ABSTRACT

A modified procedure of EN 16619:2015 method for determination of four polycyclic aromatic hydrocarbons (PAH's) was proposed. A change in internal standard and in sample preparation procedure was made for two low fat food matrices (wheat flour and smoked pork leg). As a result a new calibration was obtained with very good linearity (correlation coefficient $R^2 = 0.999$). Recovery rates for spiked samples from both matrices were also calculated and were found in a good fit with the European legislation specifications. The average values of the recovery rates amounted to 99.32 % (1.83 % relative standard deviation) for wheat flour and 97.25 % (2.50 % relative standard deviation) for smoked pork leg.

Keywords: *polycyclic aromatic hydrocarbons, GC/MS, benzo[a]pyrene.*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a large group of substances with two or more fused aromatic rings. They are known as environmental and food contaminants due to their spread in the environment and our daily life [1]. They are produced through incomplete combustion or pyrolysis of organic matter or geological processes [2]. Some of them are known as very carcinogenic substances. Therefore, they are under consideration of European and International legislation [3]. It is necessary to have a fast and accurate method to identify and quantify those substances in the products of our daily life as they are present in soil and water and hence can easily penetrate the foodstuff and further the human body.

Due to the recent technology progress and instrumentation sensitivity increase, the possibility of fast and accurate measurements of food and environmental contaminants improves every day. Analytical techniques such a gas and liquid chromatography coupled with masspectrometry or fluorescence detection are largely used for determination of many pollutants, including PAHs in food and environment samples and for conformity assessment in regards with European and International legislation [4]. The combination of the power of instrumentation and variety of analytical methods makes possible the fast and the accurate determination of dangerous PAH substances in food matrices [5]. EN 16619:2015 [6] is such an analytical method. It describes the procedure of determining the content of four PAH's (Benzo[a]pyrene, Chrysene, Benzo[a]anthracene and Benzo[b]fluoranthene) in different food matrices. This method is created in response to European legislation requirements [3]. Herein, we report a modification of the calibration procedure and validation of the modified sample preparation procedure for the method pointed above. We adapt these modifications for the determination of benzo[a]pyrene. We substitute C^{13} labeled internal standard (benzo[a]pyrene $^{13}C_4$) with Chrysene D12 in

Table 1. List of substances used for the calibration step.

№	Name of CRM/RM and product number	Certified value, dimension	Expanded uncertainty of the certified value (k = 2)
1.	Benzo[a]pyrene (CRM48665)	200.0 ug/ml	± 5.8 ug/ml
2.	Chrysene-d12 (LC14491)	98.6%	-
3	9-Fluorobenzo[k]fluorantene (15738)	10.0 ug/ml	-

the calibration procedure. In addition, we successfully skip the step of size exclusion chromatography (SEC) purification in the sample preparation procedure. To check the performance of the modified method steps we realize a calibration of chromatographic system under new conditions. In addition, we spike two matrices (wheat flour and smoked pork leg) with known amount of benzo[a]pyrene and subject them to the modified sample preparation step.

EXPERIMENTAL

Reagents

Hexane, cyclohexane and toluene of GC grade purity and silica gel were used in the purification step. Hexane, toluene and silica gel were purchased from Sigma Aldrich, while cyclohexane - from Fischer Scientific. Wheat flour and pork leg matrices were purchased at random from the merchant network.

A solution of benzo[a]pyrene (Sigma Aldrich) in dichloromethane of a nominal concentration was used to calibrate a certified reference material (CRM). Chrysene D12 (Sigma Aldrich) substance was used as an internal standard, while a solution of 9-Fluorobenzo[k]fluoranthene (LGC Standards) in toluene was used for a control standard. The parameters of the above substances are listed in Table 1.

Instrumentation

The following instruments were used for the of calibration solutions and samples preparation:

- Kern ALS 250 analytical balance with accuracy to the fourth digit;

- an automatic micropipette (10 - 100 μl);

- borosilicate glass soxlet extractors coupled with reflux condensers and round bottom flasks of a volume of 250 ml;

- Buchi Rotavapor R-200 rotary evaporator provided with a vacuum system and a water bath with temperature control;

- HGC-12D system for concentration under nitrogen provided with a heating block and a sensor for temperature control;

- SPE manifold Phenomenex provided with 12 positions for samples cleaning.

A gas chromatograph Shimadzu QP2010 Ultra coupled with mass spectrometer, autosampler AOC 20i+s and split/splitless injector was used for analysis of the calibration solutions and the samples spiked. A GC column Supelco SPB-5 MS with a length of 15 m, an internal diameter of 0.15 mm and film thickness of 0.10 μm was used for analytes separation. The latter were detected in SIM mode.

GC/MS analysis

GC/MS analysis was realized under the following conditions: injector temperature: 300^{o}C; injection type: splitless for 1 min; carrier gas: helium; carrier gas flow: 34.1 ml/min; column flow: 0.60 ml/min; oven temperature program: initial temperature 70^{o}C, hold 1 min; 60^{o}C/min to 180^{o}C; 4^{o}C/min to 230^{o}C, hold 10 min; 28^{o}C/min to 280^{o}C, hold 5 min; 60^{o}C/min to 340, hold 5 min; interface temperature: 300^{o}C; ion source temperature: 220^{o}C.

Procedure

Preparation of the calibration solutions

All stock and calibration solutions were prepared gravimetrically by using the substances and the solutions pointed in Table 1. EN 16619:2015 [6] procedure was followed. Nine calibration solutions were prepared. Toluene was used as a solvent. The data are provided in Table 2.

The final solutions were injected to GC/MS system in SIM mode 10 times each. The corresponding peaks were identified and a calibration curve was prepared by plotting the correlation of the concentration ratios of benzo[a]pyrene standard and Chrysene D12 internal standard against the corresponding area ratios.

Table 2. Concentration of the final calibration solutions.

Calibration solution No	Benzo[a]pyrene concentration, ng/g	Chrysene-d12 concentration, ng/g	9-Fluoro-benzo[k]fluoranthene concentration, ng/g
1	5.82	59.70	36.84
2	11.43	59.92	38.72
3	28.44	60.21	38.71
4	47.83	60.09	38.95
5	61.72	60.09	38.97
6	87.89	59.77	38.06
7	94.21	60.26	38.72
8	117.67	60.21	38.77
9	152.05	60.08	38.59

Preparation of spiked samples from wheat flour and smoked pork leg matrices and calculation of the recovery rates

At the beginning all samples were spiked with known amounts of benzo[a]pyrene. Then an amount of internal standard was added and the samples were subjected to all steps of the procedure provided by EN 16619:2015. The purification step using SEC system was skipped. The final extracts were injected to GC/MS system in SIM mode. The amount of benzo[a]pyrene was calculated based on the peaks area ratios. Samples with no spiking were also prepared in order to be monitored for any peaks due to benzo[a]pyrene or some interferences due to the matrix. The recoveries of all samples were calculated as a final step.

Mass spectral detection

Selected ion monitoring mode (SIM) for detection was used. The ions used are listed in Table 3.

The quantitation process was carried out with Q1 ion. Q2 ion served as a reference ion for confirmation in the course of the identification process.

RESULTS AND DISCUSSION

The analytical procedure of the standardized method includes four steps: 1) extraction with hexane of the analytes mixed in advance with internal standards; 2) concentration of the extract; 3) purification trough SEC chromatography and 4) SPE step with elution with cyclohexane and silica gel use as a stationary phase [6]. The final extract is concentrated, a control standard is added and the sample is diluted to the specific volume. The determination of the four PAH's is carried out with GC/MS system in SIM mode. C^{13} labeled analogues of the analytes are the internal standard used. In our study wheat flour and pork leg are chosen as model matrices because of their low fat content. Further, the C^{13} labeled internal standard is replaced by deuterated Chrysene (Chrysene D^{12}) because of its low price and greater accessibility of this substance in regard to C^{13} labeled benzo[a]pyrene. The second modification we introduce refers to the procedure of sample preparation. We skip the stage of purification of the extract from the soxlet extraction step with SEC. The SEC purification is required only for samples of a large fat content. In our case both matrices used have a low fat content.

The identification of benzo[a]pyrene, Chrisene D12 and 9-Fluorobenzo[k]fluoranthene proceeds as a first step. A solution containing the three analytes is subjected to GC/MS analysis. All target compounds are identified by their retention times (t_R) and quantitation ions. The obtained data is presented in Fig. 1 and are summarized in Table 4.

Table 3. List of ions used in SIM mode.

Name	Quantifier ion Q1 [m/z]	Quantifier ion Q2 [m/z]
Benzo[a]pyrene	252	126
Chrysene D12	240	236
9-Fluorobenzo[k]fluoranthene	270	135

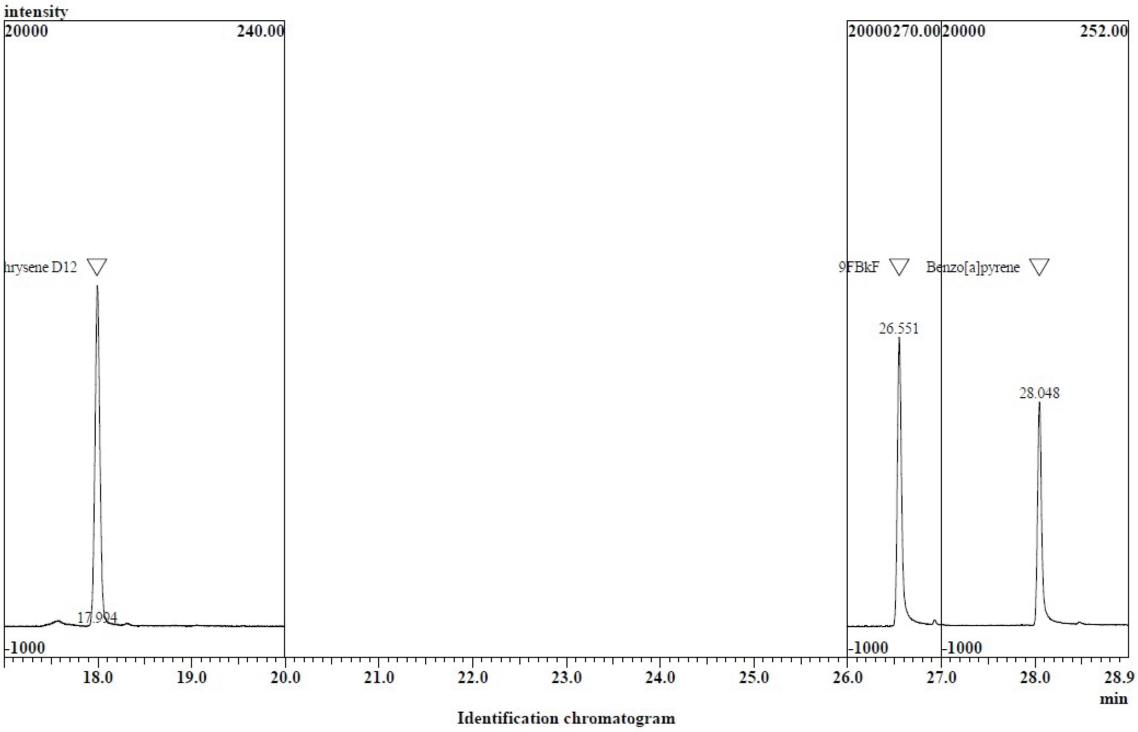

Fig. 1. GC/MS chromatogram of the three analytes in SIM mode.

Calibration of instrumentation

The second step of our study refers to instrumentation calibration using the new internal standard (Chrysene D^{12}) and exploration of the calibration function obtained. The calibration solution of a concentration ranging from 5.82 ng/g to 152.05 ng/g is injected in replicate (10 times for each concentration) to the chromatographic system. A calibration function of the peak area ratios versus the corresponding concentration ratios is obtained. It is illustrated in Fig. 2.

Fig. 2 shows clearly that the application of the new internal standard in the chosen concentration range provides a very good linearity ($R^2 = 0.999$).

Recovery rates for both matrices (wheat flour and smoked pork leg)

The final step of our study refers to the determination of the recovery rates for both matrices. Non-spiked sample extracts are initially injected and the chromatograms obtained are investigated for specificity and presence of interferences. Figs. 3 and 4 as well as 5 and 6 illustrate the comparison of chromatograms of spiked and non-spiked samples for both matrices, wheat flour and pork leg, respectively. It is evident that there are no significant interferences at benzo[a]pyrene retention time of 27 min.

In the next step of this study the final extracts of five spiked samples from each matrix are injected to GC/MS system and the corresponding amounts of benzo[a]pyrene are calculated. These results are compared with the initial values. The recovery rates are also calculated. They are summarized in Tables 5 and 6.

The stated range of the recovery rates for a method analyzing benzo[a]pyrene in food matrices is between 50 % and 120 % according to EC regulation No 333/2007 [7]. The results obtained by the modified method show compliance with those in the mentioned regulation.

Table 4. Identification data.

Name	t_R [min]	Quantifier ion 1 [m/z]	Quantifier ion 2 [m/z]
Benzo[a]pyrene	28,048	252	126
Chrysene D12	17,994	240	236
9-Fluorobenzo[k]fluoranthene	26,551	270	135

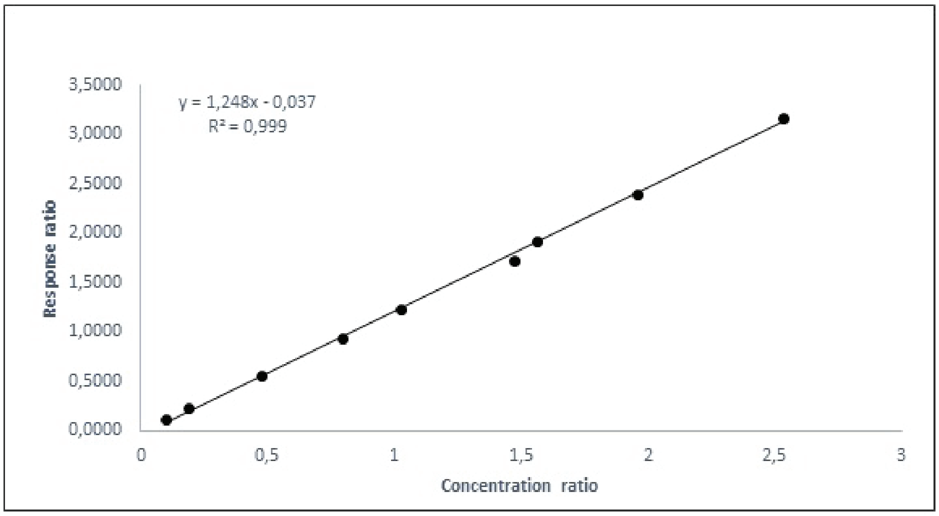

Fig. 2. Calibration correlation function for benzo[a]pyrene.

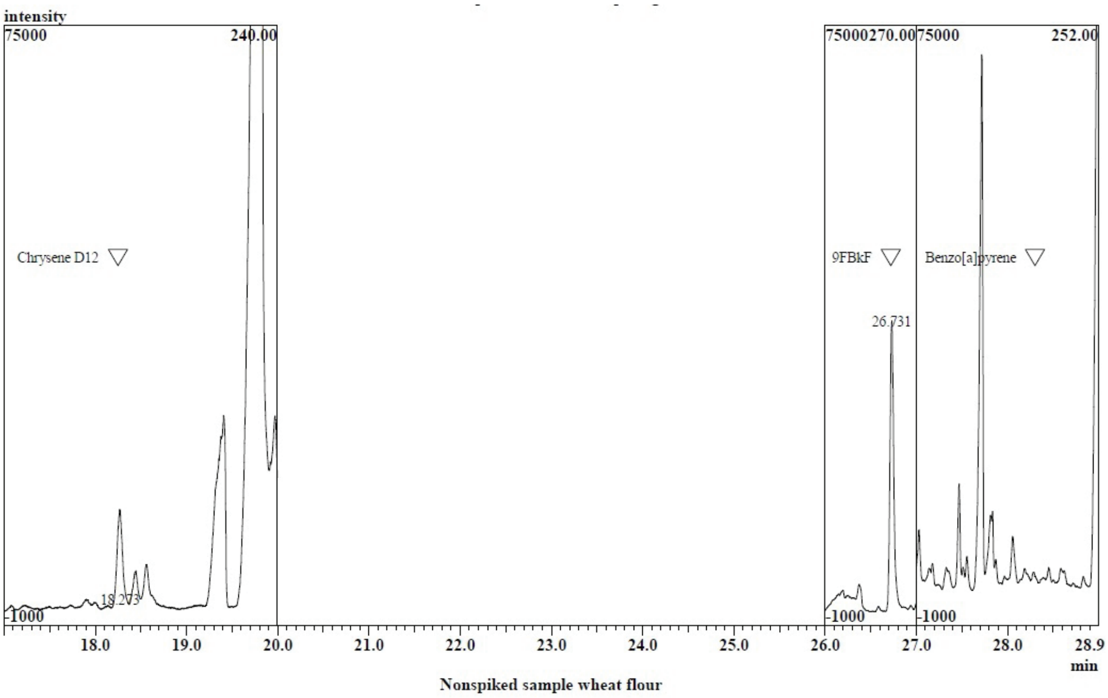

Fig. 3. A chromatogram of non-spiked sample for wheat flour matrix.

CONCLUSIONS

A modified procedure for calibration and sample preparation for two matrices was successful verified. A calibration with very good correlation coefficient (0.999) was obtained. It was proven that the skip of the purification step with size exclusion chromatography had no significant effect on the obtained results for both studied matrices, wheat flour and pork leg, characterized by a low fat content. The obtained recovery rates were within the range stated by the European legislation. The created modified method could be successfully used for analysis of benzo[a]pyrene in low fat content matrices.

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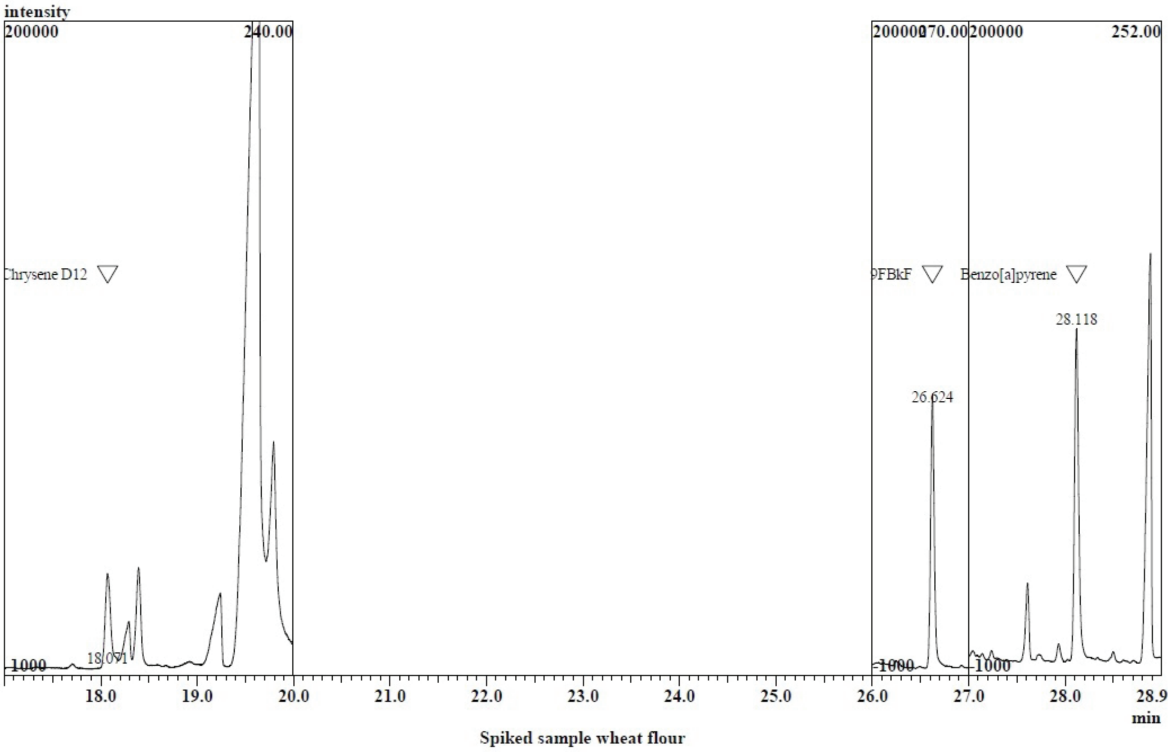

Fig. 4. A chromatogram of spiked sample for wheat flour matrix.

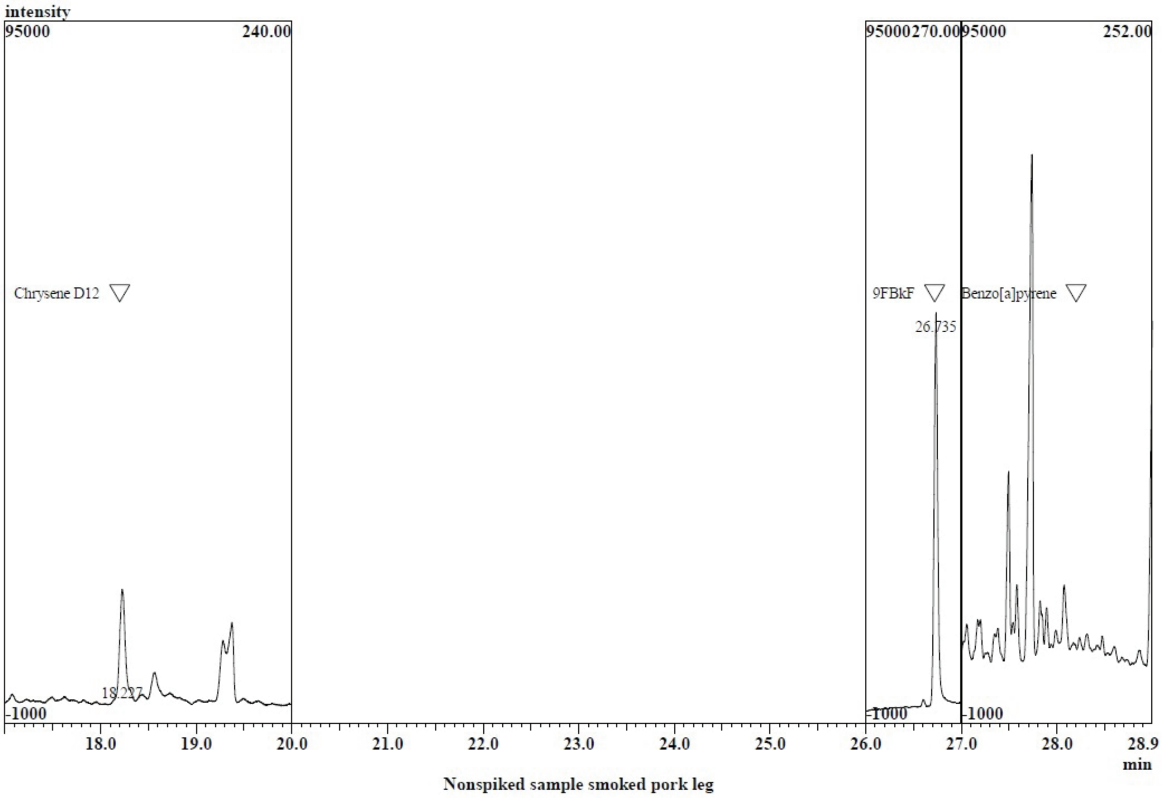

Fig. 5. A chromatogram of non-spiked sample for smoked pork leg matrix.

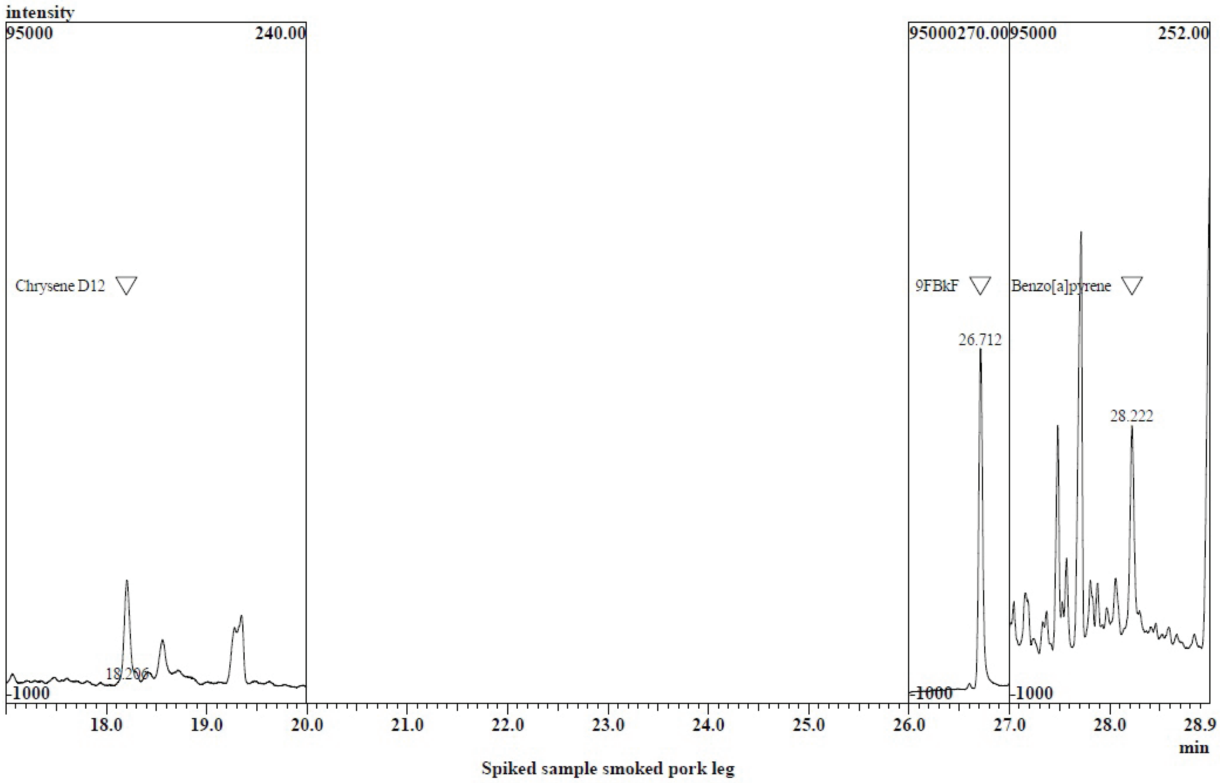

Fig. 6. A chromatogram of spiked sample for smoked pork leg matrix.

Table 5. Recovery rates data for benzo[a]pyrene in spiked sample (wheat flour matrix).

Sample No	Spiked amount of benzo[a]pyrene, ng/g	Recovery amount of benzo[a]pyrene, ng/g	Recovery rate, %
1	132.80	131.31	98.87
2	132.80	129.79	97.73
3	132.80	134.63	101.38
4	132.80	134.22	101.07
5	132.80	129.55	97.55

Table 6. Recovery rates data for benzo[a]pyrene in spiked sample (smoked pork leg matrix).

Sample No	Spiked amount of benzo[a]pyrene, ng/g	Recovery amount of benzo[a]pyrene, ng/g	Recovery rate, %
1	70.12	71.00	101.25
2	70.12	67.32	96.01
3	70.12	68.58	97.80
4	70.12	66.72	95.15
5	70.12	67.34	96.03

REFERENCES

1. H. Alomirah, S. Al-Zenki, S. Al-Hooti, S. Zaghloul, W. Sawaya, N. Ahmed, K. Kannan, Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (PAHs) from grilled and smoked foods, Food Control, 22, 2011, 2028-2035.
2. G. Purcaro, S. Moret, L.S. Conte, Overview on polycyclic aromatic hydrocarbons: Occurrence, legislation and innovative determination in foods, Department of Food Science, University of Udine, via Sondrio 2A,Udine 33100, Italy.
3. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
4. G. Purcaro, S. Moret, L.S. Conte, Overview on polycyclic aromatic hydrocarbons: Occurrence, legislation and innovative determination in foods, Review, Talanta, 105, 2013, 292-305.
5. S. Moret, L. S. Conte, Polycyclic aromatic hydrocarbons in edible fats and oils: occurrence and analytical methods, Review, J. Chromatography A, 882, 2000, 245-253.
6. EN 16619:2015. Food analysis - Determination of benzo[a]pyrene, benz[a]anthracene, chrysene and benzo[b]fluoranthene in foodstuffs by gas chromatography-mass spectrometry (GC-MS).
7. Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo[a]pyrene in foodstuffs.