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Magnetic solid-phase extraction based on magnetic multi-walled carbon nanotubes for the determination of polycyclic aromatic hydrocarbons in grilled meat samples

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ABSTRACT

A sensitive and reliable method for determination of polycyclic aromatic hydrocarbons (PAHs) in grilled meat samples was developed and validated. The method is based on magnetic solid phase extraction (MSPE) and GC-MS analysis. Magnetic carbon nanotubes (MCNTs) which have excellent adsorption capabilities, were used as adsorbent to extract PAHs, an important class of carcinogens, from meat samples. To obtain the best extraction yields, the influencing factors, including primary extraction conditions, the amount of adsorbent, adsorption and desorption time, salt addition and desorption solvent were investigated in detail. Under optimized conditions, the LODs and LOQs achieved were in the range of 0.035–0.100 and 0.075–0.200 $\mu g\,Kg^{-1}$ respectively. The calibration curves were linear ($r^2 \geq 0.988$) over the concentration ranges from 0.100 $\mu g\,Kg^{-1}$ to 250 $\mu g\,Kg^{-1}$ The relative standard deviations (RSDs) obtained by carrying out intra- and inter-day precision studies were less than 13.7% and 13.9%, respectively which confirms reproducibility of the method. In addition, the recoveries of analyzed PAHs ranged from 81.3% to 96.7% with the RSDs less than 12.7 %. Finally, the established MSPE-GC-MS method was successfully applied to determine PAHs in charcoal grilled/barbecued meat samples. benzo[a]anthracene, benzo[b]fluoranthene, Benzo[a]pyrene and chrysene were detected in beef, lamb and chicken meat samples with the mean cumulative concentration of 4.000, 3.414 and 0.931 $\mu g\,Kg^{-1}$ respectively. Taken together, the MSPE-GC-MS method developed in current study provides a new option for the determination of PAHs in grilled/barbecued meat samples.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) include several potent carcinogenic compounds consisting of two or more fused aromatic rings. PAHs are formed during incomplete combustion processes or in high-temperature pyrolysis of coal, oil and other organic materials. Therefore, PAHs can be found in complex matrices, including a variety of foodstuffs [1,2]. Food can be contaminated by environmental PAHs that are present in air, soil or water. However, most contamination of food products occurs during industrial and home food preparation methods such as heating, drying, grilling and roasting [3]. Although hundreds of individual PAHs could be formed during roasting or grilling processes, in relation to presence in food, most attention has focused on the so-called SCF-15 compounds which were considered as priority PAH pollutants by the EU Scientific Committee on Food (SCF) [4]. Since PAHs represent an important class of carcinogens, there is a great concern about their presence in food. Considering the confirmed risk of these compounds to human health, development of sensitive analytical methods for measuring their levels in environmental media and foodstuffs is of deep importance [1,2,5]. Chromatographic methods such as GC or HPLC have been frequently used for determination of PAHs in food samples [3,6–9]. The main challenges associated with analysis of these compounds are determination of extremely low concentration (at ppb level or lower) in complex mediums containing various interfering compounds. Meat is one of the main sources of food and as mentioned above, considerable amount of PAHs can be formed during cooking meat at high temperature [10–13]. Charcoal grilled/barbecued meat has a complex lipidic matrix. Therefore, to extract PAHs from this kind of food, a multi-steps sample

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preparation technique comprises of pre-extraction, extraction, clean-up and enrichment steps is necessary before instrumental analysis [14,15]. Saponification with potassium hydroxide [16], Soxhlet extraction [17,18], sonication [19,20], supercritical fluid extraction [21,22], size-exclusion chromatography [23], liquid–liquid extraction coupled to solid-phase extraction [24], microwave assisted extraction or pressurized liquid extraction (PLE) [25–27] have been used for pre-extraction of PAHs from meat matrices. After primary extraction, conventional liquid–liquid extraction (LLE) [28] and solid-phase extraction (SPE) [6,29] have been widely used for final extraction of PAHs from meat samples. However, most of the reported methods usually involve multi-steps solid-phase extraction procedure which makes them tedious, expensive and time consuming [30]. Many different adsorbents have been used in solid-phase extraction. Among them, carbon nanotubes (CNTs) have attracted great attention due to their unique properties [31–33]. The hydrophobic and π–π electron donor–acceptor interactions between CNTs and aromatic compounds make CNTs a good adsorbent candidate for PAHs [34,35]. However, due to their hydrophobic nature and nano-size, carbon nanotubes tend to aggregate and produce resistance against liquid flow when use as SPE adsorbent. This material behavior makes the SPE procedure inefficient, especially when aqueous solvents containing suspended particles are used [36]. To solve this problem, CNTs have been modified with magnetic particles by chemical process, which were further used as adsorbents of magnetic solid-phase extraction (MSPE) [37,38]. The aim of this study was to find an efficient, reliable and simpler method for determination of PAHs in meat samples, which eliminate the need for multi steps column elution process. To do this, a sample preparation process based on using MCNTs as MSPE adsorbent was developed and optimized. The applied MCNTs were hybrids of magnetite (Fe_3O_4) with multiwall carbon nanotubes (MWCNTs) which were recently developed [39]. To our knowledge, this is the first time that magnetic multiwall carbon nanotubes composites are used as MSPE adsorbent to extract PAHs from meat samples. The results indicate that magnetic solid-phase extraction based on magnetic MWCNTs coupled to gas chromatography-mass spectrometry (GC-MS) is a powerful tool for analyzing PAHs in meat samples. It is anticipated that the method may be applicable to other matrices and other similar analytes.

2. Experimental

2.1. Chemicals and standards preparation

PAH reference standards (QTM PAH-Mix, 2000 μg mL^{-1}), as well as Benzo[*a*]pyrene (solid 99%) and chrysene (solid 99%), were obtained from Supelco (Bellefonte, PA, USA). Pyrene (solid 99%) was obtained from Sigma-Aldrich (Steinheim, Germany). A standard solution containing target PAHs (1 μg mL^{-1}) was prepared in dichloromethane. Stock solutions of Benzo[*a*]pyrene, pyrene and chrysene were prepared separately at a concentration of 100 μg mL^{-1} in dichloromethane. These stock standard solutions were diluted with dichloromethane-methanol (50:50, v/v) weekly to prepare a mixed working solution with a concentration of 1 μg mL^{-1} for each compound; this working solution was applied to determine extraction performance under different conditions. Biphenyl (obtained from Merck) was used as internal standard (I. S.) and prepared in methanol at a concentration of 0.5 μg mL^{-1}. Stock and working solutions were stored at 4 °C and were used daily, diluted or directly. Multi-walled carbon nanotubes (MWCNTs, length 5.0–30 μm, diameter 30–60 nm), were obtained from Nanoshel (Panchkula, India). MWCNT-MNP composite was prepared according to the previous study [39]. All other chemicals and solvents were of analytical-reagent grade or better.

2.2. Samples

The 24 charcoal grilled meat samples (eight each of beef, lamb and chicken) were purchased from eight different local restaurants in Tehran/Iran. The restaurants were selected from different ranking levels and the samples were collected on different days. The samples were ground two times, divided into 5 g lots and stored at −70 °C in airtight containers until analysis.

2.3. Preparation of blank sample

1 Kg meat of beef were ground finely two times, divided into 100 g lots and each lot was microwave-cooked at 2450 MHz for 5 min with an output power of 900 W. The suitability of this sample as blank matrix was confirmed by preliminary studies.

2.4. Instrumental and analytical conditions

The instrument used for GC-MS analysis was an Agilent (Agilent Technologies, Palo Alto, CA, USA) 6890 plus gas chromatograph equipped with a 5973 mass selective detector quadrupole mass spectrometer. The gas chromatograph was fitted with a DB-5 ms capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness). The instrumental temperatures were as following: injector temperature 290 °C; initial oven temperature 70 °C, held for 1 min, increased to 300 °C at a rate of 10 °C min^{-1}, held for 7 min. The inlet was operated in splitless mode. The temperature of the transfer line was maintained at 300 °C. Helium (99.9999%) was used as carrier gas at 1 mL min^{-1} (constant flow). The source and quadrupole temperatures were kept at 230 and 150 °C, respectively. The electronic beam energy of the mass spectrometer was set at 70 eV. Qualification was performed by comparing the acquired mass spectra and retention times to reference spectra and retention times which were acquired by injection calibration standards under identical GC-MS conditions. The compounds were quantified using selected ion monitoring (SIM) mode. One quantitation and two qualifier ions were monitored for each compound (Table 1).

Table 1
Selected ions used for the quantification and qualification of PAHs by GC-MS (SIM mode).

Ion group	Analyte	Abbreviations	Time window ($_{min.}$)	Quantification ion (m/z)	Confirmation ions (m/z)
1	Biphenyl	Bp	8–13	154	153, 152
1	Acenaphthylene	Ace	8–13	152	153, 151
1	Acenaphthene	Ac	8–13	153	154, 152
2	Fluorene	F	13–15	166	165, 167
3	Phenanthrene	Pa	15–17	178	179, 176
3	Anthracene	A	15–17	178	179, 176
4	Fluoranthene	Fl	17–20	202	203, 101
4	Pyrene	P	17–20	202	203, 101
5	Benzo[a] anthracene	BaA	20–23	228	226, 229
5	Chrysene	Ch	20–23	228	226, 229
6	Benzo[b] fluoranthene	BbF	23–28	252	253, 126
6	Benzo[k] fluoranthene	BkF	23–28	252	253, 126
6	Benzo[a]pyrene	BaP	23–28	252	253, 126
7	Indeno[1,2,3–cd]pyrene	IP	28–31	276	277, 138
7	Dibenzo[a,h] anthracene	DhA	28–31	278	279, 139
7	Benzo[g,h,i] perylene	BgP	28–31	276	277, 138

2.5. Method optimization

500 g of the blank sample was ground again and spiked with 5 mL of mixed working solution containing Benzo[*a*]pyrene, chrysene and pyrene (0.5 μg mL^{-1}). The mixture was homogenized mechanically for 30 min and kept fridge at 4 °C for 24 h, and then used for method optimization. The method was optimized based on "one factor at a time" experiments. In this protocol, optimized conditions are determined by experiments in which all the influencing factors are kept constant except one, and the remaining one is gradually modified to find the optimum condition. After optimizing each factor, the experiment is repeated to find the optimum value for another factor while studied factors are adjusted to their determined optimum values. Finally, the overall procedure is repeated while all the factors are adjusted to their determined optimum values. The method conditions will be optimal or near-optimal if the value of determinant parameter (in this case "extraction yield") in the last experiment is the best between acquired values.

2.6. Extraction procedure

5 g of each frozen sample (blank, quality controls and tests) were pulverized by an electric lab pulverizer (Lab Grinder/Pulverizer WSG30E, WARING®, CT, USA) at high speed (19,000 rpm) for 1 min. The resulting material was placed in a glass container containing 1 mL of internal standard solution (biphenyl 0.05 μg mL^{-1} in methanol–water 50:50 v/v). The mixture was homogenized for 2 min and allowed to dry at room temperature thoroughly for 1 h.

After addition of 7.5 mL potassium hydroxide (1 M in water) and 7.5 mL methanol containing 5% acetonitrile (v/v), the sample was homogenized for 2 min then sonicated in an ultrasonic bath (Fisher Scientific FS 30 H) for 7 min at 40 °C. The mixture was then centrifuged at 8944 × *g* for 10 min and to remove the fat contents, the aqueous phase was transferred to a separatory funnel and kept at −25 °C for 1 h. Most of the lipids were precipitated as a layer on the top surface of liquid phase and as condensed lump on glassware surface. Cold extract at −25 °C was immediately filtered through a filter paper to remove the frozen lipids. Then pH decreased to 6.5 by adding hydrochloric acid (1 M). After primary extraction, the aqueous phase was transferred to another vessel. Then 10 mg of prepared MWCNT-MNP composite and 500 mg NaCl were added into the sample and the mixture was vortex-mixed vigorously for 5 min. Then an external magnet was applied to gather the magnetic adsorbent to the side of the vial. The supernatant was discarded followed by addition of 5 mL dichloromethane to elute the analytes from the adsorbent with vigorous vortex-mixing for 3 min. Then the magnetic adsorbent gathered to the side of the vial using an external magnet. This step was repeated one more time, then the desorption solvent was collected and evaporated to dryness at 30 °C under gentle stream of nitrogen. The residue was re-dissolved in methanol–acetonitrile (50:50 v/v, 50 μL) and the solution was shaken vigorously using a vortex mixer for 1 min. Finally, 1 μL of the resulting solution was injected into the GC-MS. The results of optimization studies indicated that the above procedure allowed for reproducible, quantitative extraction of PAHs from meat samples. Blank samples containing internal standard (I.S.) and quality control samples (QCs) were analyzed at the beginning, middle and at the end of the sample queue. Test samples were analyzed in duplicates and the average responses were used for quantification.

2.7. Quantification method

Quantification of PAHs in analyzed samples was carried out using classical spiked calibration curve method. To construct the spiked calibration curve, 500 g grilled meat of beef were ground finely two times and divided into 5 g lots. Then 30 lots were used to prepare ten calibration points (100, 125, 175, 225, 500 ng Kg^{-1} and 1.0, 5.0, 25.0, 100.0 and 250.0 μg Kg^{-1}) in triplicate by adding dilutions of standard mixtures to the samples. To evaluate the method performance and validity of the results, quality control samples (QCs) were prepared at four concentration levels (0.250, 0.750, 50.0 and 150.0 μg Kg^{-1}) in triplicate. When using spiked calibration curve method, it is very important to prepare calibration and quality control samples as similar as possible to real samples. Simple addition of the PAHs into the meat matrix does not mimic the real situation in which PAHs were formed in meat during grilling or barbequing. When PAHs are simply added to the meat matrix, the spiked analytes will not bind to the matrix as well as real analytes. This difference in binding, results in significant error in quantification. Therefore, in this study we used a special method for preparing spiked samples. After spiking, the calibration and quality control samples as well as unspiked samples which were used for determining the baseline levels were placed in glass containers containing 10 mL of methanol–water (50:50) solution and homogenized for 30 min. Then the samples were left at room temperature for 2 h, after which they were kept at 4 °C for 24 h in the dark. Finally the samples were frozen at −70 °C for 48 h. After extraction under optimized conditions, the calibration curves were constructed by plotting the peak area ratios (analyte/internal standard), which were corrected based on primary levels, against nominal spiked concentrations of PAHs.

3. Results and discussion

3.1. Optimization of the extraction conditions

Analysis of PAHs in the meat samples is problematic due to their extremely low concentrations, their affinity for the fat fraction of meat and presence of many interfering compounds. Therefore, in these cases, usually a well designed sample preparation method should be applied [15,40]. In the first step of sample preparation, sample is generally converted to fine particles to maximize the surface interaction between sample matrix and extraction medium. To prepare the finest meat particle, most of previous studies used meat grinding procedures. However, due to the high lipid content of meat, the efficiency of grinding for producing fine particles is not enough and therefore the other researchers preferred to freeze-dry the meat sample and finally convert the sample to small particles with a miller. However, by using this method, they have to determine the PAHs levels in primary sample indirectly (i.e., based on the amount of weight loss during freeze-drying procedure). In this study, we used a new technique and converted the meat matrix to very fine particles by pulverizing frozen ground meat sample. By using this technique, we obtained very fine particles which guarantee the high surface interaction between sample matrix and extraction medium in the next step. For extraction of PAHs from meat samples, alkaline hydrolysis under reflux conditions for 2–6 h has been generally used [20]. Ultrasonic-assisted extraction (UAE) is a competitor and currently a popular technique which due to its advantages over other conventional techniques has increasingly been used for the primary extraction of target compounds from complex matrices [7]. UAE exhibits many substantial improvements in analytical sample preparations, as it requires much lower volume of extraction solvent, reduces extraction time and increases recovery yield [41]. Therefore, in this study, UAE was selected as primary extraction method and to optimize this step, four critical variables including: temperature of extraction medium, composition of extraction solvent, extraction time and volume of extraction solvent were investigated in details. The results demonstrated in Fig. 1(a–d).

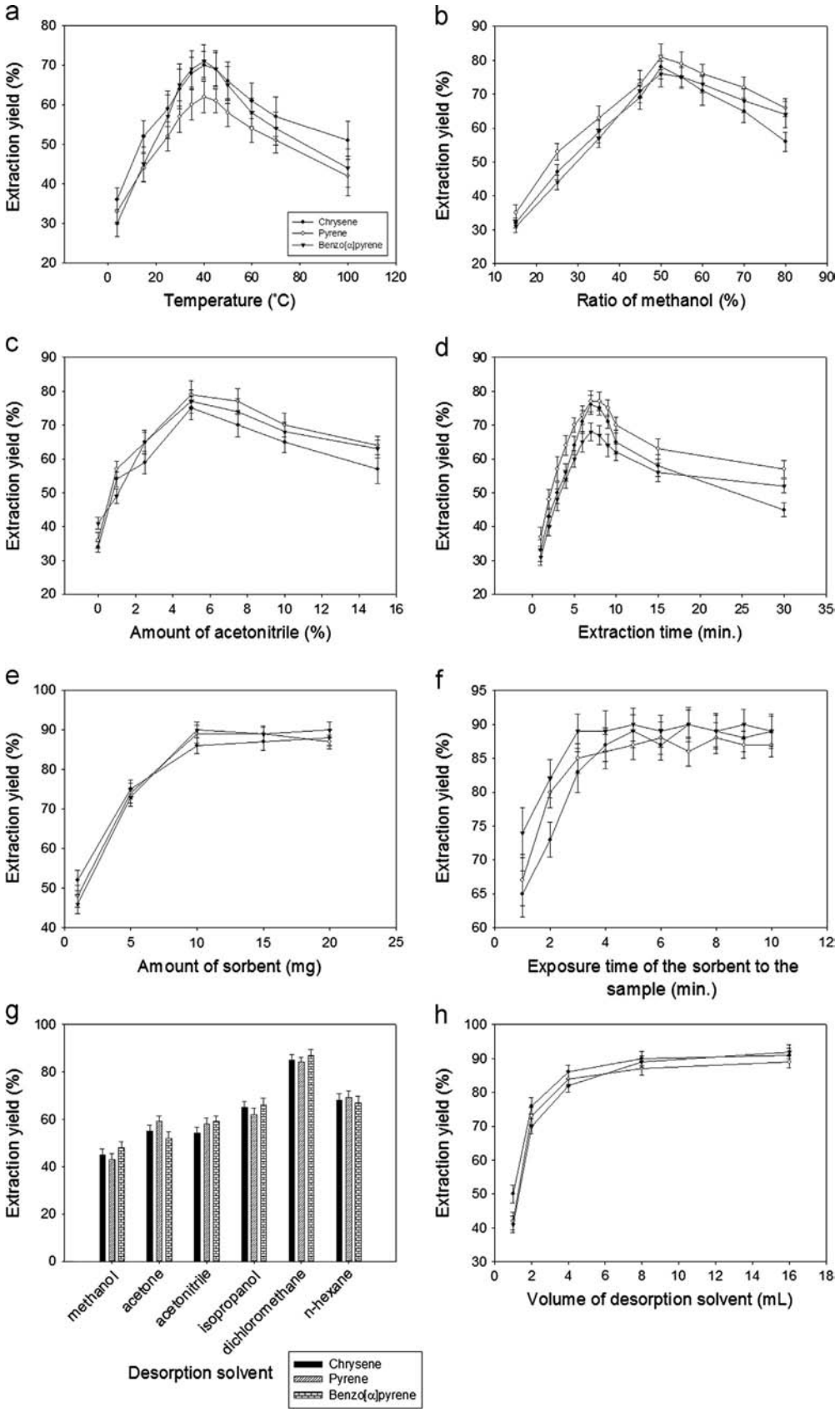

Fig. 1. The effect of different influencing factors on extraction efficiencies. (a) Effect of primary extraction medium temperature. (b) Effect of methanol content in primary extraction medium. (c) Effect of acetonitrile content in primary extraction medium. (d) Investigation of the primary extraction time. (e) Optimization of sorbent amount in second step of extraction. (f) Effect of exposure time of the sorbent to the sample. (g) Optimization of desorption solvent in the last extraction step, and (h) Investigation of the optimum volume of desorption solvent.

As the results indicated, all the extraction yields increased as temperature was increased from 4 to 40 °C. However, above 40 °C the trends reversed and significant reductions in extraction yields were observed. An explanation for this observation would be that, as the temperature increases from 4 to 40 °C, the extraction efficiency of solvent increases through different mechanisms such as increased solubility of PAHs in extraction medium as well as higher desorption of PAHs from meat matrix at higher temperature. A similar pattern was also observed for two other factors: composition of extraction solvent and extraction time. Regarding composition of extraction solvent, the best extraction yield was obtained when the ratio of methanol in the hydrolyzing solvent increased from 25% to 50%. It is also noticeable that the extraction yield was decreased at higher methanol percentage. To increase extraction yield of the PAHs, some amounts of acetonitrile were added to methanol fraction. From Fig. 1(c) it is evident that the extraction yields of PAHs are significantly increased as the content of acetonitrile increased in the range of 0–5% v/v and it is decreased beyond this range. The situation is similar to the case of methanol percentage. The reason for these observations may relate to higher solubility of PAHs in stronger solvent (i.e., higher methanol/water ratio and higher acetonitrile percentage) which inhibits its complete extraction by MCNTs in the next extraction step. The other reason could be the increased dissolution of tissue lipids in extraction medium at higher concentration of organic solvents. These dissolved lipids may occupy many adsorption sites on MCNTs, the main extraction medium in the next step. Experiments with extraction time indicated that the best extraction yields were obtainable when the extraction time was set at 7 min and the extraction yields were decreased as the time increased (Fig. 1(d)). An explanation to this observation could be that as the extraction time elapses, more lipids, proteins and other interfering compounds accumulate in extraction medium. As mentioned above, these compounds may interfere with adsorption of the analytes by MCNTs in the next step. The other studied parameter of primary extraction step was the volume of the extraction solvent. The results showed that the extraction yields reach plateau at 15 mL of extraction solvent and it remained nearly constant when the volume of solvent increased to 18, 24 or 30 mL. Therefore, 15 mL was chosen as the optimum volume of the first extraction medium. To optimize the overall extraction yield, some other critical parameters related to the next sample preparation steps (clean-up and enrichment) were also investigated. To estimate the adsorption efficiency of developed MCNTs, the capabilities of the magnetic nanoparticles (MNP) for extraction of PAHs from first extraction medium were studied and the results compared with that of obtained from MWCNT-MNP composite. The formation of MWCNT-MNP composite allowed performing an efficient extraction of the target compounds; by contrast, only unspecific adsorption of PAHs was observed when the naked Fe_3O_4 nanoparticles (MNP) were used. Additional experiments were carried out to improve the extraction efficiency of the analytes by evaluating the effect of some other parameters able to influence the extraction yield like amount of sorbent, salting out effect, the type and volume of desorption solvent and desorption and extraction time. In order to have satisfactory recoveries of target compounds, a certain amount of MWCNT-MNP sorbents is required. Addition of 1–20 mg of MWCNT-MNP was examined and as the results indicated (Fig. 1(e)), 10 mg of MWCNT-MNP was enough to extract the analytes from sample solution. Based on the results, at this level the recoveries of target analytes were all over 80%. Next, the effect of salt addition on the extraction efficiencies of PAHs was evaluated. Salting out effect has been well established in the previous works through adding different salts, mostly NaCl and Na_2SO_4 to the samples. Most authors agree on the positive effect of salt addition to the sample for improving the extraction efficiency. In this study, to investigate the effect of salt addition on the extraction efficiency, a series of experiments was performed by adding different amounts of NaCl and Na_2SO_4 (from 0 to 1 g) into the sample solution. According to the results, no significant differences were found between the salts and the highest extraction efficiencies were obtained by adding 500 mg of NaCl into the sample. Extraction time, which is the time, the MWCNT-MNP were exposed in the sample, had a significant effect on the recovery values of the extracted compounds. Fig. 1(f) shows the extraction efficiency - exposure time profiles for target analytes in the range of 1–10 min. This figure shows that for all the compounds extraction equilibrium is quickly reached within 3–5 min. Therefore, 5 min is adopted as extraction time in the following experiments. For the desorption process, three parameters need to be optimized including desorption solvent, temperature and desorption time. Six solvents including methanol, acetone, acetonitrile, isopropanol, dichloromethane and *n*-hexane were studied as desorption solvent to examine their effects on the extraction efficiencies. The results indicated that the best extraction efficiencies can be obtained with dichloromethane as desorption solvent (Fig. 1(g)). The other experiments revealed that the highest extraction yield could be achieved when the volume of desorption solvent exceeds 4 mL. As shown in Fig. 1(h), the curve rises slowly after 4 mL and reach plateau at 8 mL. This observation indicates that desorption process continues after 4 mL. Therefore, 2 × 5 mL dichloromethane were used as desorption solvent in the following experiments.

Table 2
Calibration curve parameters for the determination of target PAHs in grilled meat samples.

Target compound	Linear range ($\mu g\ Kg^{-1}$)	Limit of detection (LOD) ($\mu g\ Kg^{-1}$)	Limit of quantification (LOQ) ($\mu g\ Kg^{-1}$)	Coefficient of estimation (r^2)
Acenaphthylene (Ace)	0.125–250	0.050	0.100	0.993
Acenaphthene (Ac)	0.125–250	0.050	0.100	0.995
Fluorene (F)	0.100–250	0.035	0.075	0.994
Phenanthrene (Pa)	0.100–250	0.035	0.075	0.992
Anthracene (A)	0.100–250	0.035	0.075	0.996
Fluoranthene (Fl)	0.125–250	0.050	0.100	0.995
Pyrene (P)	0.125–250	0.050	0.100	0.993
Benzo[a]anthracene (BaA)	0.175–250	0.075	0.150	0.996
Chrysene (Ch)	0.175–250	0.075	0.150	0.994
Benzo[b]fluoranthene (BbF)	0.175–250	0.075	0.150	0.989
Benzo[k]fluoranthene (BkF)	0.175–250	0.075	0.150	0.992
Benzo[a]pyrene (BaP)	0.225–250	0.100	0.200	0.991
Indeno[1,2,3–cd]pyrene (IP)	0.225–250	0.100	0.200	0.988
Dibenzo[a,h]anthracene (DhA)	0.225–250	0.100	0.200	0.988
Benzo[g,h,i]perylene (BgP)	0.225–250	0.100	0.200	0.988

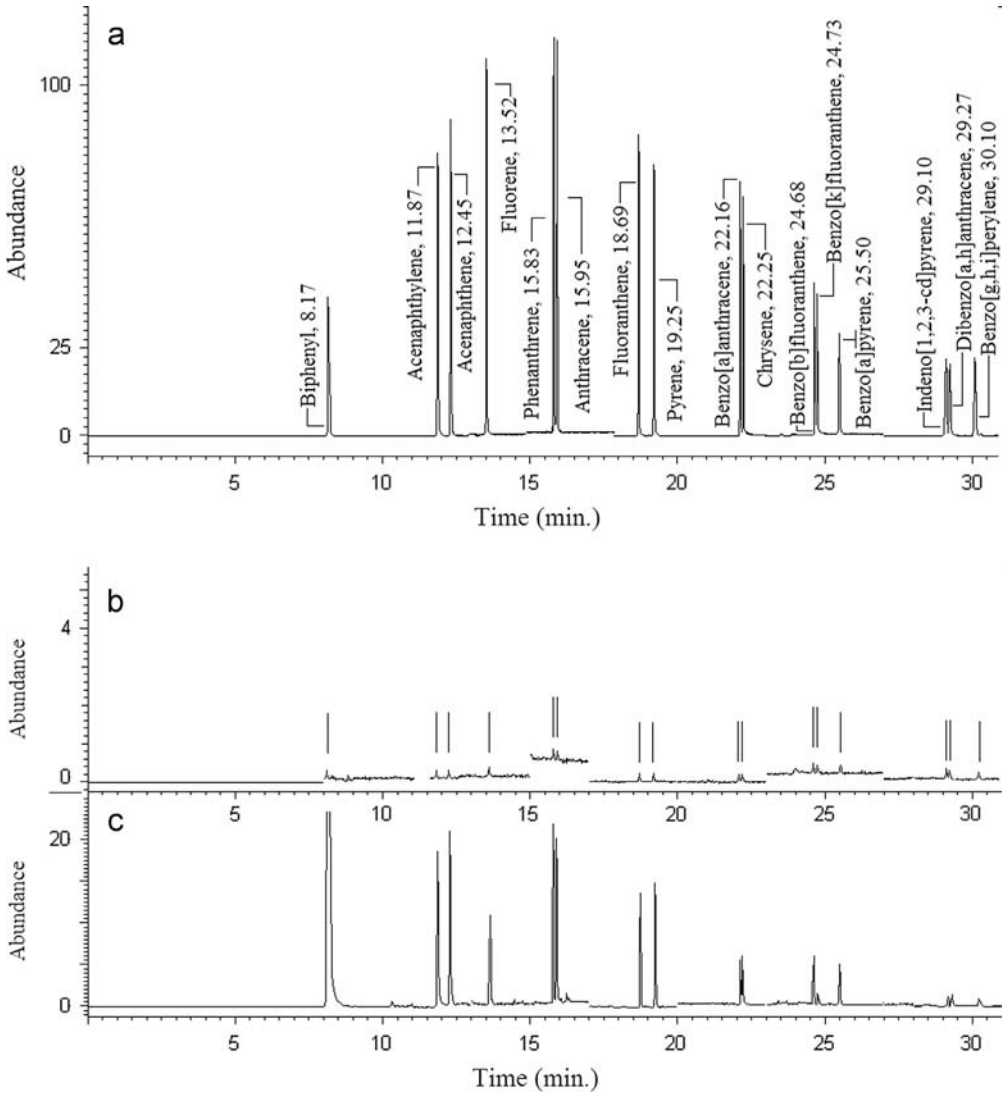

Fig. 2. Representative MSPE-GC-MS chromatogram (SIM mode) of (a) A blank sample, spiked with the analytes at 10 μg Kg^{-1}. (b) A blank sample spiked with the analytes at their LOD levels (c) one of the analyzed sample. Quantitation results (μg Kg^{-1}): Ace: 2.23, AC: 2.65, F: 1.18, Pa: 1.64, A: 1.58, Fl: 1.63, P: 2.11, BaA: 0.92, Ch: 0.85, BbF: 1.45, BkF: 0.32, BaP: 2.12, IP: 0.43, DhA: 0.49, BgP: 0.33.

Table 3
Comparison of different analytical methods for the determination of PAHs in grilled meat samples.

Sample	Method of Sample preparation	Method of instrumental analysis	Recovery range (%)	Recovery values variation (RSD%)	LOD or LOQ (μg Kg^{-1})	Ref.
Pork meat	alkaline hydrolysis, solid phase extraction and adsorption column chromatography	HPLC-fluorescence	Approximately: 50–60	Not reported	Not reported	3
Different grilled meats	lyophilisation, Ultrasonic-assisted extraction, solid phase extraction	HPLC-fluorescence	16–82	2–58	LOQ: 0.02-0.10	7
Fish meat	Microwave-assisted extractions	HPLC-fluorescence	65–97	0.4–8.5	LOQ: 0.15-27.16	8
Different grilled meats	Cold NaOH treatment, Two steps solid-phase extraction	HPLC-fluorescence	68.3–118	15.9–21.3	LOQ: 0.04-0.10	10
Meat	Acid hydrolysis, liquid-liquid extraction, gel chromatography	HPLC-fluorescence, GC-MS	85–101	Not reported	LOQ: 0.3-1.0	13
Different oils and foods	preparative size-exclusion chromatography without saponification	HPLC-fluorescence	Not reported	Not reported	LOD For all PAHs: 0.1	23
Salmon meat	freeze-drying, liquid-liquid extraction, solid-phase extraction	GC–MS-MS, GC–MS	43–99	2–15	LOQ: 0.1-0.5	24
Smoked meat	lyophilisation, microwave assisted extraction, solid-phase extraction,	HPLC-fluorescence	77–103	1.4–102.3	LOQ: 0.2-0.6	27
Beef, lamb, chicken meats	Ultrasonic-assisted extraction, Magnetic solid-phase extraction	GC-MS	81.3–96.7	4.3–12.7	LOQ: 0.075-0.200	This study

Experiments with different desorption times from 0.5 to 6 min were carried out, and based on the results 3 min was sufficient for complete desorption of all the analytes. Since the experiments did not show any significant differences between different desorption temperatures in the range of 20–50 °C, room temperature (25 °C) was selected as the optimum condition.

3.2. Quantitative analysis

The calibration curves parameters listed in Table 2 were obtained under optimized condition. Linearity of the calibration curves was determined in the range of 0.100–250 μg Kg^{-1}. Coefficient of correlation ranged from 0.987 to 0.996. The LODs were

Table 4
Estimated recoveries, accuracies and precisions for determination of PAHs at different concentrations ($n=6$).

QC Samples	Nominal concentration (μg Kg^{-1})	(intra-day)				(inter-day)				Estimated recoveries (%)	Precision (RSD%) of calculated recovery
		Calculated concentration (μg Kg^{-1})	Standard deviation	Precision (RSD%)	Accuracy (%)	Calculated concentration (μg Kg^{-1})	Standard deviation	Precision (RSD%)	Accuracy (%)		
Ace	0.250	0.245	0.030	12.2	98.1	0.244	0.031	12.7	97.8	87.4	11.7
	0.750	0.744	0.083	11.1	99.3	0.758	0.089	11.7	101.0	89.7	9.4
	50.000	52.703	2.846	5.4	105.4	52.066	3.228	6.2	104.1	92.7	7.5
	150.000	145.197	6.098	4.2	96.8	143.809	5.465	3.8	95.9	93.5	5.4
Ac	0.250	0.240	0.029	12.1	96.0	0.245	0.031	12.6	98.1	89.4	10.2
	0.750	0.734	0.087	11.8	97.8	0.737	0.089	12.1	98.3	91.2	8.4
	50.000	49.443	3.115	6.3	98.9	48.693	3.019	6.2	97.4	93.5	6.6
	150.000	147.920	6.508	4.4	98.6	146.018	6.133	4.2	97.3	94.7	4.5
F	0.250	0.242	0.031	12.7	96.7	0.238	0.030	12.8	95.2	89.5	11.4
	0.750	0.773	0.094	12.2	103.1	0.705	0.087	12.4	94.0	92.3	8.4
	50.000	50.501	3.283	6.5	101.0	49.501	2.673	5.4	99.0	93.5	6.7
	150.000	147.022	6.322	4.3	98.0	146.337	7.463	5.1	97.6	95.3	4.5
Pa	0.250	0.246	0.029	11.8	98.2	0.246	0.031	12.6	98.3	90.2	10.2
	0.750	0.755	0.087	11.5	100.7	0.731	0.085	11.7	97.4	91.4	9.3
	50.000	50.421	2.723	5.4	100.8	47.221	3.164	6.7	94.4	93.5	5.6
	150.000	147.925	7.840	5.3	98.6	147.450	8.405	5.7	98.3	94.6	5.7
A	0.250	0.236	0.029	12.3	94.2	0.233	0.029	12.6	93.2	90.3	11.4
	0.750	0.765	0.086	11.2	102.0	0.764	0.090	11.8	101.9	91.7	8.5
	50.000	48.833	3.321	6.8	97.7	50.634	3.646	7.2	101.3	93.5	5.6
	150.000	146.327	6.585	4.5	97.6	145.527	6.112	4.2	97.0	95.7	5.3
Fl	0.250	0.251	0.031	12.4	100.2	0.243	0.031	12.8	97.3	91.6	11.7
	0.750	0.740	0.087	11.7	98.7	0.739	0.085	11.5	98.6	93.6	9.2
	50.000	47.521	2.994	6.3	95.0	48.596	3.256	6.7	97.2	95.4	6.7
	150.000	147.781	6.798	4.6	98.5	147.539	7.672	5.2	98.4	96.7	4.4
P	0.250	0.254	0.031	12.4	101.4	0.247	0.031	12.7	87.4	90.8	11.4
	0.750	0.764	0.090	11.7	101.9	0.726	0.088	12.1	96.9	92.6	8.6
	50.000	48.879	2.737	5.6	97.8	49.191	3.247	6.6	98.4	94.6	5.7
	150.000	148.030	6.365	4.3	98.7	146.008	8.468	5.8	97.3	95.8	4.3
BaA	0.250	0.258	0.033	12.6	103.2	0.241	0.031	12.8	87.6	90.4	10.2
	0.750	0.749	0.091	12.1	99.9	0.725	0.086	11.9	94.9	92.4	7.4
	50.000	49.914	3.344	6.7	99.8	47.929	3.259	6.8	95.9	93.6	4.8
	150.000	145.271	8.426	5.8	96.8	143.380	7.456	5.2	95.6	95.6	5.1
Ch	0.250	0.255	0.033	12.8	102.2	0.243	0.031	12.6	88.8	91.5	10.4
	0.750	0.730	0.085	11.6	97.3	0.739	0.090	12.2	96.0	92.7	7.3
	50.000	48.957	3.476	7.1	97.9	47.557	3.662	7.7	95.1	93.8	6.5
	150.000	146.175	7.893	5.4	97.4	145.474	9.165	6.3	97.0	95.5	4.7
BbF	0.250	0.260	0.033	12.8	104.1	0.237	0.031	12.9	86.8	90.4	11.3
	0.750	0.732	0.087	11.9	97.6	0.738	0.092	12.4	99.4	92.5	8.7
	50.000	48.870	3.079	6.3	97.7	47.999	3.168	6.6	96.0	93.6	6.5
	150.000	148.072	7.848	5.3	98.7	147.645	6.939	4.7	98.4	95.7	5.4
BkF	0.250	0.269	0.034	12.8	107.6	0.247	0.032	12.9	89.8	91.3	10.6
	0.750	0.700	0.087	12.4	93.3	0.695	0.086	12.4	94.6	92.6	8.3
	50.000	48.578	3.303	6.8	97.2	48.178	3.613	7.5	96.4	93.7	6.4
	150.000	145.580	7.716	5.3	97.1	145.180	8.130	5.6	96.8	95.8	5.3
BaP	0.250	0.241	0.033	13.6	96.5	0.253	0.035	13.8	91.0	85.3	11.3
	0.750	0.774	0.094	12.2	103.3	0.694	0.091	13.1	95.0	86.4	9.2
	50.000	49.027	3.628	7.4	98.1	48.425	3.487	7.2	96.9	88.5	7.5
	150.000	146.203	8.918	6.1	97.5	147.623	7.824	5.3	98.4	88.7	6.7
IP	0.250	0.249	0.034	13.7	99.4	0.248	0.034	13.9	99.2	81.5	12.5
	0.750	0.753	0.094	12.5	100.4	0.766	0.098	12.8	102.1	82.5	10.2
	50.000	48.021	3.265	6.8	96.0	47.778	3.679	7.7	95.6	83.8	6.4
	150.000	148.061	5.034	3.4	98.7	147.831	6.800	4.6	98.6	85.2	5.7
DhA	0.250	0.268	0.036	13.5	107.1	0.240	0.033	13.7	95.9	81.3	12.7
	0.750	0.722	0.092	12.7	96.3	0.721	0.088	12.2	96.1	82.2	10.2
	50.000	49.353	3.405	6.9	98.7	48.858	3.078	6.3	97.7	83.7	7.4
	150.000	148.081	7.996	5.4	98.7	148.952	9.533	6.4	99.3	84.6	5.4
BgP	0.250	0.225	0.030	13.3	90.0	0.229	0.030	12.9	91.8	81.7	10.2
	0.750	0.738	0.093	12.6	98.4	0.734	0.092	12.5	97.9	83.4	8.1
	50.000	49.548	3.171	6.4	99.1	49.750	3.731	7.5	99.5	84.5	6.3
	150.000	148.340	7.862	5.3	98.9	148.841	8.037	5.4	99.2	84.7	4.3

defined as five times the standard deviation of baseline noise ($n=6$) and determined by spiking serially diluted analyte standards into blank meat samples (Fig. 2(a, b)). According to the ICH (International Conference on Harmonization of Technical Requirements for Bioanalytical Methods) guideline for analytical method validation, limit of quantification (LOQ) for each analyte was determined as the lowest concentration on the calibration curve with a precision of less than 20% coefficient of variation (CV%) and an accuracy of 80–120% [42]. The results of validation experiments showed that the LODs and LOQs for the target analytes ranged from 0.035–0.100 μg Kg^{-1} and 0.075–0.200 μg Kg^{-1}, respectively (Table 2).

A brief comparison of different analytical methods for determination of PAHs was demonstrated in Table 3. For most of target PAHs, the method developed in the current study gave lower LOQ and higher recovery value than previous methods, which indicate the superior performance of the developed MSPE-GC-MS method. Furthermore, the total analysis time (including sample preparation and instrumental analysis) of the developed method is about 3 h which is considerably shorter than previous reports.

The precision of the method was evaluated in terms of intermediate precision (or interday precision) through calculating the analyte concentrations in quality control samples, prepared at four levels (each six replicates) on three consecutive days. Interday precision values for the analytes were always less than 13.9% (Table 4). Expression of the repeatability (or intraday precision) is based on the RSDs% of determined responses of six replicates of quality control (QC) samples, which were prepared at four levels and reported in Table 4. The estimated recoveries at four different concentration levels are also listed in Table 4. To determine the recovery, mean peak area of each analyte at each concentration level was determined for a blank meat sample spiked with the analyte ($n=6$). The determined value was compared with the mean value obtained from spiking the same amount of the analyte in the final extracts of a blank meat sample. All these results indicate the feasibility and reliability of the developed method for determining PAHs in grilled meat samples. The selectivity of the method was confirmed by analyzing twelve different meat samples from each of beef, chicken and lamb. There was no interfering peak in the region of the analytes and internal standard.

Table 5
Estimated concentrations of PAHs in analyzed samples (n=3).

Sample	Target compound	No. of positive samples (%)	Mean concentration (μg Kg^{-1}) ± 95% confidence interval (CI)	Minimum concentration (μg Kg^{-1})	Maximum concentration (μg Kg^{-1})	Median of concentration (μg Kg^{-1})	Q3 (μg Kg^{-1})	Content of PAH4[a] (μg Kg^{-1})
Beef meat	Ace	50	0.854 ± 0.931	< LOQ	2.756	0.338	1.993	4.000
	Ac	62.5	1.511 ± 1.386	< LOQ	4.786	1.295	2.459	
	F	87.5	1.584 ± 0.913	< LOQ	3.426	1.646	222%	
	Pa	62.5	1.886 ± 1.572	< LOQ	4.676	1.846	3.813	
	A	87.5	1.578 ± 0.999	< LOQ	3.244	1.057	2.986	
	Fl	75	1.819 ± 1.374	< LOQ	4.289	1.676	3.478	
	P	75	2.24 ± 1.933	< LOQ	6.839	1.684	3.763	
	BaA	87.5	0.91 ± 0.585	< LOQ	2.345	0.78	1.208	
	Ch	75	1.55 ± 1.339	< LOQ	3.777	1.247	3.068	
	BbF	62.5	0.615 ± 0.778	< LOQ	2.778	0.316	0.815	
	BkF	62.5	0.131 ± 0.128	< LOQ	0.451	0.122	0.204	
	BaP	75	0.925 ± 0.754	< LOQ	2.672	0.722	1.452	
	IP	62.5	0.535 ± 0.547	< LOQ	1.771	0.246	1.026	
	DhA	62.5	0.284 ± 0.236	< LOQ	0.673	0.276	0.555	
	BgP	62.5	0.377 ± 0.316	< LOQ	1.034	0.368	0.651	
Lamb meet	Ace	75	0.837 ± 0.707	< LOQ	2.134	0.677	1.746	3.414
	Ac	62.5	0.868 ± 0.836	< LOQ	2.347	0.556	2.044	
	F	75	0.562 ± 0.479	< LOQ	1.577	0.405	1.092	
	Pa	62.5	1.339 ± 1.247	< LOQ	4.223	1.181	2.377	
	A	87.5	1.311 ± 0.762	< LOQ	2.537	1.222	2.154	
	Fl	62.5	1.174 ± 1.007	< LOQ	3.256	1.002	2.106	
	P	75	1.888 ± 1.558	< LOQ	5.556	1.448	3.084	
	BaA	75	0.725 ± 0.507	< LOQ	1.734	0.647	1.176	
	Ch	87.5	1.309 ± 0.829	< LOQ	3.236	1.059	1.908	
	BbF	100	0.871 ± 0.708	0.118	2.453	0.52	1.519	
	BkF	62.5	0.139 ± 0.108	< LOQ	0.324	0.147	0.252	
	BaP	75	0.509 ± 0.471	< LOQ	1.431	0.294	1.116	
	IP	62.5	0.359 ± 0.349	< LOQ	1.034	0.218	0.812	
	DhA	62.5	0.209 ± 0.192	< LOQ	0.568	0.13	0.444	
	BgP	62.5	0.117 ± 0.104	< LOQ	0.368	0.124	0.164	
Chicken meat	Ace	62.5	0.325 ± 0.252	< LOQ	0.683	0.347	0.634	0.931
	Ac	62.5	0.503 ± 0.667	< LOQ	2.345	0.175	73%	
	F	62.5	0.432 ± 0.420	< LOQ	1.363	0.28	0.821	
	Pa	62.5	0.685 ± 0.664	< LOQ	1.897	0.338	1.508	
	A	62.5	0.399 ± 0.470	< LOQ	1.674	0.235	0.527	
	Fl	62.5	0.569 ± 0.550	< LOQ	1.673	0.291	1.169	
	P	75	0.849 ± 0.744	< LOQ	2.334	0.609	1.657	
	BaA	62.5	0.347 ± 0.410	< LOQ	1.431	0.201	0.569	
	Ch	62.5	0.281 ± 0.230	< LOQ	0.664	0.239	0.552	
	BbF	50	0.193 ± 0.194	< LOQ	0.588	0.116	0.404	
	BkF	50	0.067 ± 0.067	< LOQ	0.211	0.052	0.116	
	BaP	62.5	0.111 ± 0.084	< LOQ	0.245	0.13	0.201	
	IP	50	0.080 ± 0.080	< LOQ	0.245	0.0535	0.161	
	DhA	37.5	0.098 ± 0.125	< LOQ	0.375	0	0.236	
	BgP	50	0.058 ± 0.053	< LOQ	0.129	0.054	0.117	

[a] Summed concentration of benzo[a]anthracene, benzo[b]fluoranthene, Benzo[a]pyrene and chrysene.

3.3. Application to real samples

As mentioned in the introduction section, PAHs represent an important class of carcinogens and their presence in food has been intensively studied. However, due to insufficient method sensitivity, some of the PAHs which are expected to be found in food, have been not determined in most of the previous studies and there are few reports about simultaneous determination of all these fifteen PAHs in a single analytical run. Therefore, to show the application of the developed method, some real samples were collected and analyzed. Determined concentrations of target PAHs in these samples were listed in Table 5 and one of the acquired chromatograms was shown in Fig. 2(c). From Table 5, it is apparent that all of target PAHs were detected in most of the beef and lamb samples. In addition, the mean concentration of PAHs were higher in both beef and lamb than chicken samples ($p < 0.05$). For BaP in smoked meat a maximum concentration limit of 5 $\mu g\ kg^{-1}$ was announced by European commission [43] and from September 2012 an additional maximum concentration limit of 30 $\mu g\ kg^{-1}$ for PAH4 (summed content of benzo[a]anthracene, benzo[b]fluoranthene, Benzo[a]pyrene and chrysene) was recommended [44]. As indicated in Table 5, the highest value of PAH4 was observed in beef samples (average 4 $\mu g\ kg^{-1}$) compared with lamb (average 3.414 $\mu g\ kg^{-1}$) and chicken (average 0.931 $\mu g\ kg^{-1}$) samples. However, the determined PAH4 values are still much lower than recommended maximum concentration limit of 30 $\mu g\ kg^{-1}$. Furthermore, the mean concentrations of BaP in all of the samples were lower than the established maximum concentration limit of 5 $\mu g\ kg^{-1}$ for smoked meat. The acquired values are almost comparable with those reported in previous studies [2,5,11,45]. The listed values in Table 5 reveal that chicken meat samples contained the lowest concentration of PAHs. This result is also in accordance with the commonly accepted theory which states that production of PAHs could be the result of fat dripping down on the charcoal and since the fat content of chicken meat is lower than beef meat, therefore less PAHs will be formed during chicken meat grilling [5]. The results of real sample analysis confirm the applicability of the developed method for analysis of PAHs in meat samples.

4. Conclusion

In this work, a sensitive and reliable method for determination of PAHs in meat samples was developed. The method is based on using magnetic carbon nanotubes (MCNTs) as extraction medium. By using MCNTs the method was simplified and the time-consuming procedures such as column passing and filtration steps were eliminated. Furthermore, the capabilities of MCNTs and spiked calibration curve technique, let us to improve the sensitivity and reliability of the developed method. Under optimized condition the acquired LODs and LOQs, as well as within and between run variations were better than most of pervious works. In addition to analysis quality control samples, the method performance was also evaluated by analysis real samples. The results were in accordance to previous works and findings. The results of this study revealed that magnetic solid-phase extraction based on magnetic multi-walled carbon nanotubes is a useful tool for determination of PAHs in grilled meat samples.

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