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Article

FOOD CHEMICAL CONTAMINANTS

Solid-Phase Extraction Combined with Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry for the Determination of 5 Trace Nitro-Polycyclic Aromatic Hydrocarbons in Barbecued Foods

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Abstract

Background: Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) are the derivatives of polycyclic aromatic hydrocarbon which are direct mutagens and carcinogens to human. Nitro-PAHs can be produced in the process of food barbecuing/grilling. At present, there are few studies for the determination of nitro-PAHs in food.

Objective: To assess the effect of barbecued food to human health, we have established a method for the determination of 5 nitro-PAHs in barbecued foods.

Methods: The target nitro-PAHs were extracted with the mixture of methanol/acetone and then purified with an HLB SPE cartridge and finally analyzed by ultra-high-performance liquid chromatography-tandem mass spectrometry. Two pairs of target multiple reaction monitoring (MRM) ion pairs have been successfully identified for the target nitro-PAHs, and confirmed by high-resolution mass spectrometry to explore their formation mechanism.

Results: The method had linear ranges of 2.0–500 μg/L (except 1-nitronaphthalene 20–5000 μg/L) with the correlation coefficients greater than 0.995. The extraction recoveries were between 70.1% and 85.6% with the relative standard deviations less than 10.0%. The limits of detection of the method were less than 0.60 μg/L (except 1-nitronaphthalene 6.0 μg/L).

Conclusions: The method has been successfully applied to the analysis of 5 nitro-PAHs in barbecued foods. 1-nitronaphthalene, 1,8-dinitropyrene , 1-nitropyrene were detected in some charcoal grilled samples with the contents of 1.35–12.9 μg/kg. 1,8-Dinitropyrene was detected in some oil-fried samples with the contents of 2.12–5.12 μg/kg.

Highlights: This work presents an ultra-high-performance liquid chromatography-tandem mass spectrometry method for the determination of 5 nitro-PAHs in barbecued foods for the first time. The method has been successfully applied to the analysis of 5 nitro-PAHs in various barbecued foods and the nitro-PAHs were detected in some barbecued food samples. The mechanism of mass spectrometric decomposition of nitro-PAHs was investigated as well.

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Abbreviations:

nitro-PAHs, nitrated polycyclic aromatic hydrocarbons;
UHPLC-MS/MS, ultra-high-performance liquid chromatography-tandem mass spectrometry;
SPE, solid-phase extraction;
1-NN, 1-Nitronaphthalene;
3-NFL, 3-nitrofluoranthene;
1,8-DNPYR, 1,8-dinitropyrene;
9-NA, 9-nitroanthracene;
1-NPYR, 1-nitropyrene;
1-NPYR-d9, 1-nitropyrene-d9;
9-NA-d9, 9-nitroanthracene-d9,
MRM, multiple reaction monitoring;
MIM, multiple ion monitoring;
HPLC-FLD, high-performance liquid chromatography-fluorescence detection

Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) are the derivatives of polycyclic aromatic hydrocarbons (PAHs) with at least one nitro group on their aromatic rings. Like their parent PAHs, they are ubiquitous in the environment. The sources of nitro-PAHs in the atmosphere are incomplete combustion of fossil fuel or organic matter and chemical reaction of polycyclic aromatic hydrocarbons (PAHs) with nitrogen oxides in the air (1). Even nitro-PAHs contents are much lower than their parent PAHs in the different matrices, they may be more toxic than their parent PAHs (2). International Agency for Research on Cancer (IARC) has classified them as potentially potent human carcinogens (3). Nitro-PAHs have been detected and quantified in combustion residues, the emissions from industries, and the water environment (4–6). Researches showed that some food processing methods, such as barbecuing, drying, and grilling may produce nitro-PAHs as well (7–10). Nitro-PAHs in environmental samples have been analyzed by electrospray ionization mass spectrometry (11), gas chromatography-mass spectrometry (GC-MS) (12), and high-performance liquid chromatography (HPLC) (7, 8, 13–15). The detector used in GC-MS system is mainly negative ion chemical ionization-mass spectrometer (NCI-MS) (16, 17) and electron impact ionization-mass spectrometer (18). For HPLC, the detectors used include fluorescence detector (FLD) (7, 8, 19) and mass spectrometer (20–22). One of the advantages of the HPLC method compared with GC method is that the separation and detection can be carried out at room temperature, under which nitro-PAHs are more stable.

The sources of nitro-PAHs in barbecued foods are mainly from contamination by nitro-PAH due to incomplete combustion of coal fuel and the reaction of food-containing nitrogen or the nitrogen dioxides in the air and the PAHs produced during the grilling/barbecuing process. But up to now, only a few studies on the analysis of nitro-PAHs in barbecued/grilled foods have been reported (7, 8, 10). For example, Schlemtz et al. (7) reported an online platinum and rhodium catalyst column reduction–high-performance liquid chromatography for the analysis of seven nitro-PAHs in grilled and smoked foods. They have detected 1-nitronaphthalene, 1-nitrofluorene, and 1-nitropyrene in some smoked and grilled foods with the contents less than 19.6 μg/kg. Deng et al. (8) used SPE extraction and Fe/H+-induced nitro-reduction and UHPLC-FLD analysis of 1-nitronaphthalene, 2-nitrofluorene, and 1-nitropyrene in meat products with the detection limits of 0.59, 0.51, and 0.31 μg/kg, respectively. These methods required the conversion of nitro-PAHs to amino-PAHs, whose conversion efficiency directly affects the recovery rate. And the main problem of fluorescence detection is weak qualitative ability because of interference. Lung and Liu (23) used HLB SPE cartridge to extract nine nitro-PAHs in aerosol samples and detect them using UHPLC-APPI-MS/MS. Pre-treatment of samples is the key step in the analysis of trace nitro-PAHs in food samples because of the complexity of the food matrices, especially the grease existing in barbecued foods. In this study, we extracted ultrasonically the trace nitro-PAHs in various barbecued foods with the mixture of methanol/acetone (75:25, v/v) and then purified the sample solution using HLB SPE cartridge and finally analyzed the nitro-PAHs by ultra-high-performance liquid chromatography–tandem mass spectrometry. Two pairs of target MRMs ion pairs have been successfully identified for the target nitro-PAHs and confirmed by high-resolution mass spectrometry to explore their formation mechanism. The established method has been applied to the detection of nitro-PAHs in barbecued/grilled food samples. The mechanism of nitro-PAHs formed by different processing methods was investigated and satisfactory results have been obtained. Table 1 shows the chemical structures and the important physico-chemical properties of the five nitro-PAHs in this study.

Experimental

Samples

Twenty barbecued samples including meat, vegetables and seafood were collected from a local barbecue food shop. For each sample, ca. 500 g was collected and kept into a sterile food bag. During transportation, the samples were kept out of light and stored at 4°C refrigerator. All the samples were pretreated and analyzed within 24 h.

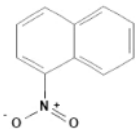
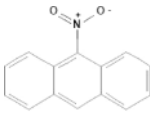
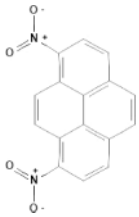
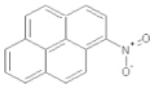
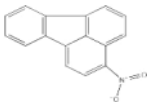
Apparatus

(a) *LC-MS system*.—The qualitive and quantitative analysis were carried out by using a Waters I-Class ultra-performance liquid chromatograph equipped with a 6500+ MS/MS trap system (SCIEX, USA) and the quantification was done in MRM mode.
(b) *LC-column*.—BEH C18 column (2.1 × 50mm, 1.7 μm, Waters Corporation, USA).
(c) *Mass spectrometric detection*.—SCIEX QTRAP 6500 plus mass spectrometer (AB SCIEX Corp., USA) equipped with an APCI ion source.
(d) *LC-QTOF system*.—The identification of MS fragmentation was performed on 5600 QTOF system (SCIEX, USA).

Reagents

(a) *Standard*.—1-Nitronaphthalene (1-NN), 3-nitrofluoranthene (3-NFL), 1,8-dinitropyrene (1,8-DNPYR), 1-nitropyrene-d9 (1-NPYR-d9), 9-nitroanthracene-d9 (9-NA-d9), 9-nitroanthracene (9-NA), 1-nitropyrene (1-NPYR) were purchased from AccuStandard Chemical (USA).
(b) *Solvent*.—PLC-grade methanol, methylene chloride, and acetone were all purchased from Merck, USA.
(c) *SPE column*.—HLB (6 mL, 500 mg), MIP-PAHs (6 mL, 500 mg), and Carbon-GCB solid-phase extraction cartridges (6 mL, 500 mg) were purchased from Anpel company (Shanghai, China). Cleanert PEP (6 mL, 500 mg) and MCX (6 mL, 500 mg) solid-phase extraction cartridges were purchased from Agela Technologies (Shanghai, China).

Table 1. The information of the five nitro-PAHs in this study

Nitro-PAH, Abbreviation	CAS No.	Chemical structure	Molecular formula	Molecular weight, g/mol	Boiling Point, °C	Melting point, °C	Water solubility, mg/L, 25°C	log Kow	IARC group
(1) Nitronaphthalene (1-NN)	86-57-7		$C_{10}H_7NO_2$	173.17	304.0	61	9.18	3.19	3
9-Nitroanthracene (9-NA)	602-60-8		$C_{14}H_9NO_2$	223.23	275.0	146	0.114	4.78	3
1,8-Dinitropyrene (1,8-DNPYR)	42397-65-9		$C_{16}H_8N_2O_4$	292.25	515.2	204.76	5.40E− 02	4.57	2B
1-Nitropyrene (1-NPRY)	5522-43-0		$C_{16}H_9NO_2$	247.26	445.5	155	1.18E − 02	5.06	2A
3-Nitrofluoranthene (3-NFL)	892-21-7		$C_{16}H_9NO_2$	247.26	445.5	170.56	1.18E − 02	4.75	3

The data were from https://pubchem.ncbi.nlm.nih.gov/.

(d) *Water*.—The ultrapure water used in this study was produced from a Milli-Q water system (Millipore, Merck).

Preparation of Standard Solutions

All the standard stock solutions were prepared in methanol with a concentration of 1.00 mg/L (except 1-nitronaphthalene 10.00 mg/L), respectively.

The standard series concentrations of 2.00 μg/L to 500 μg/L (except 1-nitronaphthalene 20.0 μg/L to 5000 μg/L) with the internal standard at 10.0 μg/L were prepared with standard stock solution using methanol as diluent. All standard solutions were stored in a 4°C refrigerator.

Chromatographic Conditions

(a) *Mobile phase*.—Methanol (A) and 0.1% formic acid aqueous solution (B).

(b) *Gradient elution program*.—0–0.5 min, 20% B; 0.5–3.5 min, 20–80% B; 3.5–5.6 min, 80% B; 5.6–6.5 min, 80%–95% B; 6.5–7.2 min, 95% B; 7.2–7.5, 95%–20% B; 7.5–10, 20% B.

(c) *Column temperature*.—30°C.

(d) *Flow rate*.—0.35 mL/min.

(e) *Equilibration time*.—1.0 min.

Mass Spectrometric Detection Conditions

(a) Curtain gas (CUR). –40 psi.

(b) Nebulizer current. –1μA.

(c) Auxiliary gas (GS1) pressure. –40 psi.

(d) Source temperature. –550°C.

(e) The declustering potential (DP) and collision energy (CE) were optimized to match MRMs for analytes.

(f) The first pairs of MRM ion pairs were used for quantification and the others were used for qualitation.

The MRM parameters are shown in Table 2.

Table 2. MS parameters for the nitro-PAHs

Nitro-PAHs	Q1 Mass, Da	Q3 Mass, Da	Time, msec	DP, Volts	CE, Volts
1-Nitronaphthalene	173	46 173	100	−40	−5
9-Niroanthracene	223	46 193	40	−20	−16
9-Nitroanthracene-d9	232	46 202	40	−25	−16
1,8-Dinitropyrene	292	26 223 221 646	40	−40	−23
1-Nitropyrene-d9	256	46 226	40	−30	−22
1-Nitropyrene	247	46 217	40	−30	−22
3-Nitrofluoranthene	247	46 217	40	−30	−22

Figure 1. The barbecuing and grilling process of meat, etc., frying on a heated iron plate with vegetable oil (A) and the shrimp, etc., grilling with charcoal (B).

Sample extraction procedure

The samples were divided into two groups according to their processing method, i.e., fried with vegetable oil (Figure 1A) and grilled with charcoal (Figure 1B). Five hundred grams of samples were chopped and reduced to ca. 100 g by quartering method and then the remaining sample was ground to paste with a food mixer. Five grams of homogeneous sample were weighed into a 50-mL centrifuge tube, and 20 μL internal standard solution (100 μg/L) was added to make its final concentration at 10 μg/L. Then 20 mL methanol/acetone (75:25, v/v) was added, followed by supersonic extraction for 30 min at room temperature. After centrifugation for 5 min at 10 000 rpm, all of the supernatant was used for further SPE purification. The HLB cartridges were first activated with 5 mL dichloromethane and 5 mL methanol. The supernatant was transferred into the cartridge at an outflow rate of about a drop per second by controlling the vacuum, followed by addition of 10 mL of methanol for clean-up, and finally the nitro-PAHs absorbed on the cartridge were eluted with 10 mL dichloromethane. The eluate was collected and evaporated to dryness under gentle nitrogen flow and finally the residue was dissolved in 200 μL methanol. After centrifugation at 13 000 rpm for 10 min, 10 μL of the solution was injected into the UHPLC-MS/MS system for analysis.

Qualitative and quantitative analysis

According to the ratio of the peak area of a certain nitro-PAH to the internal standard peak area, the concentration (μg/L) of the nitro-PAH in the sample solution was calculated by the internal standard curve. By using the following formula, the content (μg/kg) of nitro-PAH in the barbecue food sample was calculated.

$$W = \frac{0.20C}{m}$$

where W is the content of a nitro-PAH in barbecued food (μg/kg); C is the concentration of a nitro-PAH in the sample solution calculated based on internal standard curve (μg/L); and m is the sample weight (g) and 0.20 is the sample solution volume (mL).

Results and Discussion

Optimization of the Extraction Conditions

Selection of extraction solvent

To effectively extract the nitro-PAHs from the samples, we compared the extraction efficiencies of several extraction solvents with different polarity, including methanol, acetone and n-hexane. One milliliter of 100 μg/L mixed standard solution and 10 μg/L internal standard were spiked in the 5 mL blank matrix which was prepared with 5 g raw minced and homogenized pork with no nitro-PAHs detected and extracted with 5 mL methanol/acetone (75:25, v/v). After extraction with the solvents, the extracts were concentrated and purified with HLB cartridges. After eluted with 10 mL dichloromethane, the eluate was collected and evaporated to dryness under gentle nitrogen flow and finally the residue was dissolved in 1.00 mL methanol. Figure 2A shows that the extraction efficiencies of methanol and methanol/acetone (75:25, v/v) were both the highest. It was found that n-hexane could dissolve the oil in the food sample resulting in matrix interference and lower recovery. Considering that the sample matrix contained fat, methanol/acetone (75:25, v/v) was selected as the extraction solvent.

Selection of SPE cartridge

According to the polarity of the nitro-PAHs, we compared Cleanert PEP, HLB, MIP-PAHs, Carbon-GCB and MCX for their extraction efficiencies (7–9, 22, 23). One milliliter of the mixed standard solution (100 μg/L spiked with 10 μg/L internal standard solution) was loaded onto the activated SPE cartridge, eluted with 10 mL dichloromethane, blown to near dryness under gentle nitrogen flow, and the residue was re-dissolved with

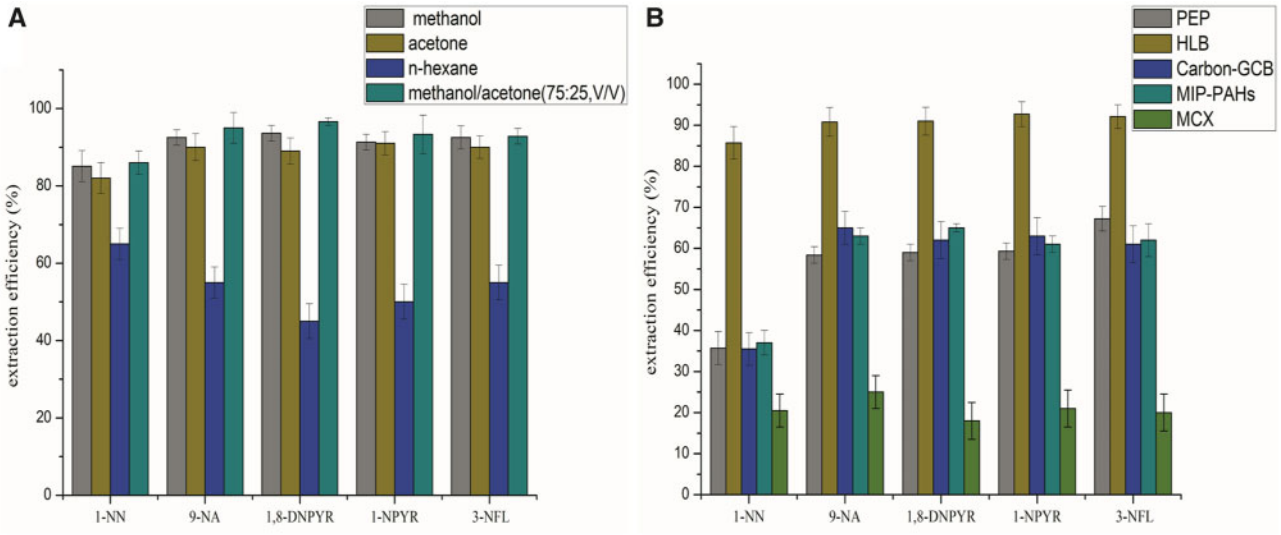


Figure 2. The effect of extraction solvents (A) and SPE cartridges (B) on extraction efficiencies.

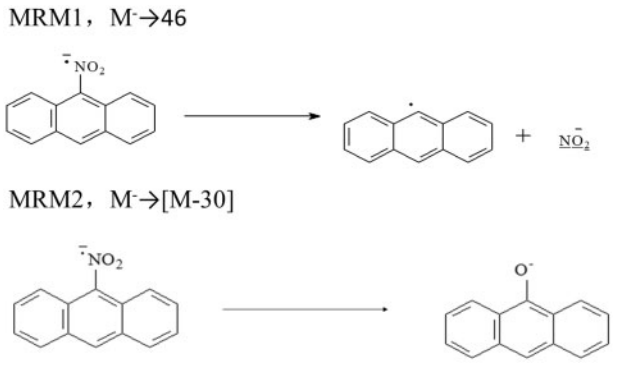


Figure 3. The MRM fragment pattern of 9-aminoanthracene.

1.00 mL methanol, and finally the solution was injected for analysis. The extraction efficiencies of different SPE cartridges were compared.

As the most common used SPE cartridge for acidic, neutral and alkaline compounds, HLB cartridge is packed with macroporous co-polymers polymerized by lipophilic diethylbenzene and hydrophilic n-vinyl pyrrolidone monomers in a certain proportion. Different from traditional silica gel columns, HLB polymers have hydrophilic and hydrophobic groups on the surface, with good water infiltration, and are very stable in the pH range of 1 to 14. The surface of PEP (polar enhanced polymer) has both hydrophilic and hydrophobic groups, so it has a more balanced adsorption effect on all kinds of polar and non-polar compounds. MCX column is based on the cationic exchange mixing mechanism of water permeable polymer matrix. It is stable in the medium with pH 0~14.0, and has a large binding capacity. MIP-PAHs cartridge is designed for adsorption of PAHs by using molecular imprinting polymers. Although the specific surface area of Carbon-GCB cartridge is smaller than that of silica gel, its adsorption capacity is two times of silica gel. Due to the positive six-membered ring structure on its surface, it can extract many polar substances, and has more stable properties than C18 cartridge, so it is suitable for the extraction and purification of many kinds of organic analytes. From Table 1, we can see that the five nitro-PAHs in this study have different polarity, for example, the log Kow of 1-nitronaphthalene is 3.19, meaning that it has some hydrophilicity, while the log Kow of 1-nitropyrene is 5.06, which means it is almost hydrophobic. Therefore, the HLB cartridge is more suitable for the extraction of the five nitro-PAHs in this study with different polarities. The results (Figure 2B) also indicated that the extraction efficiencies of HLB cartridge were much higher than other SPE cartridges, so we chose HLB SPE cartridge for the extraction of the nitro-PAHs.

Optimization of mass spectrometric parameters

The analysis of nitro-PAHs in airborne particulates or diesel soot etc. have been reported by many authors by using LC-MS with ESI ionization sources (9) or APCI (13, 14, 24, 25). Nitro-PAHs with low polarity showed stronger MS signal by using APCI than ESI according to the most literature, and mainly get radical anions $[M\cdot]^-$ in negative mode (only 2-nitrofluorene with partially aromatic ring structure forms deprotonated molecules $[M\text{-}H]^-$). In the MS/MS spectrum of nitro-PAH by CID, $[M\text{-}30]^-$ peak was usually observed with high intensity which can be interpreted as a neutral loss of NO leading to [M-NO]-, or as the reduction of nitro-PAH to the correspondent amine $[M\text{-}2O+2H]^-$. The results from high resolution MS support the fragment pattern of neutral loss NO which means a new bond formation (the remaining oxygen of nitro group attaches to the ring of PAH). Other researchers used $m/z = 46$ (NO_2^-) as the main product ion of MRM mode (14).

Based on the references, we used APCI negative ionization mode for nitro-PAHs detection. The high resolution MS/MS spectra of nitro-PAHs and their deuterium internal standards showed the unique fragmentation with two typical product ions: $m/z = 46$ (NO_2^-) and [M-30]([M-NO]$^-$), while $[M\text{-}2(NO)]^-$ and $[M\text{-}NO\text{-}NO_2]^-$ were found in the spectrum of 1,8-dinitropyrene. Our results also supported neutral loss of NO instead of reduction. $m/z = 46$ (NO_2^-) and [M-30] ([M-NO]$^-$), were used as product ions in MRM mode, and their source and analytical parameters such as declustering potential, collision energy were optimized by infusion of standard solution into the MS/MS spectrometer. For nitro-naphthalene, only one product ion NO_2^-, $173 > 173$ (multiple ion monitoring mode), was chosen as quanlitative MRM.

Use of 9-nitro anthracene as an example to show the fragment pattern of nitro-PAHs (Figure 3). If [M-30] is formed by a

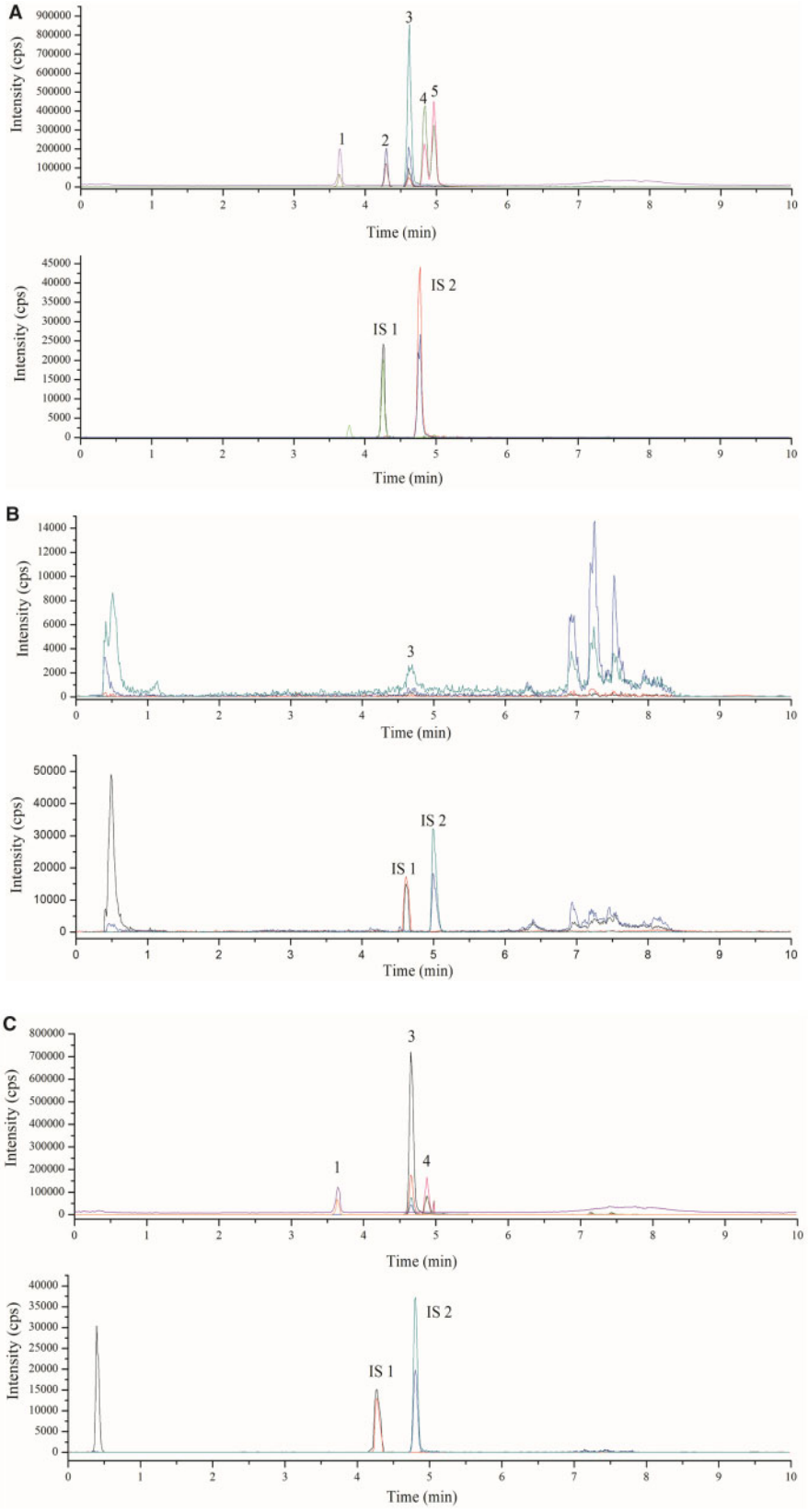


Figure 4. The MRM chromatograms of the mixed standard solution (A) at 200 μg/L (1-nitronaphthalene, 2000 μg/L), a fried squid sample (B) and a charcoal grilled fish sample (C). Peak identifications: 1.1-nitronaphthalene; 2.9-nitroanthracene; 3.1,8-dinitropyrene; 4.1-nitropyrene; 5.3-nitrofluoranthene; IS1. 9-nitroanthracene-d9; IS2. 1-nitropyrene-d9.

Table 3. The linear range, regression equations, correlation coefficients, LODs, and LOQs of the method

Peak No.	Nitro-PAHs	RT, min	Linear ranges, μg/L	Regression equations	Correlation coefficie,nts r	LODs, μg/L	LOQs, μg/L
1	1-Nitronaphthalene	3.65	20.0–5000	y =0.1056x + 0.0685	0.9980	6.00	20.0
2	9-Niroanthracene	4.30	2.00–500	y =0.4079x + 0.1177	0.9996	0.60	2.00
3	1,8-Dinitropyrene	4.70	2.00–500	y =1.9427x − 0.5712	0.9998	0.60	2.00
4	1-Nitropyrene	4.85	2.00–500	y = 0.4758x − 0.00418	0.9997	0.60	2.00
5	3-Nitrofluoranthene	4.98	2.00–500	y = 1.1461x +0.00815	0.9995	0.60	2.00

Table 4. The accuracy and precision of the method (n = 6)

Peak No.	Nitro-PAHs	Added, μg/L	Found, μg/L	Recovery, %	RSD, %
1	1-Nitronaphthalene	200	140.2	73.6 ± 3.5	8.67
		2000	1512	75.6 ± 5.1	7.96
2	9-Niroanthracene	20.0	16.3	81.5 ± 1.2	6.32
		200	166.2	83.1 ± 1.9	6.21
3	1,8-Dinitropyrene	20.0	16.5	82.6 ± 2.9	5.32
		200	166.4	83.2 ± 1.8	5.65
4	1-Nitropyrene	20.0	17.0	83.1 ± 2.1	6.34
		200	170.4	82.2 ± 3.1	7.96
5	3-Nitrofluoranthene	20.0	17.0	83.1 ± 2.5	8.26
		200	170.6	82.3 ± 2.5	5.35

Table 5. The Nitro-PAHs contents (μg/kg) detected in various barbecued foods

No.	Sample	Processing method	1-Nitronaphthalene	1,8-Dinitropyrene	9-Niroanthracene	1-Nitropyrene	3-Nitrofluoranthene
1	Squid	Fried	<LOQ	5.12	N.D.	N.D.	N.D.
		Grilled	10.3	12.9	N.D.	5.63	N.D.
2	Chicken	Fried	<LOQ	2.13	N.D.	N.D.	N.D.
		Grilled	3.62	7.62	N.D.	3.19	N.D.
3	Mutton	Fried	<LOQ	2.26	N.D.	N.D.	N.D.
		Grilled	1.98	3.52	N.D.	2.13	N.D.
4	Beef	Fried	<LOQ	2.19	N.D.	N.D.	N.D.
		Grilled	2.35	3.65	N.D.	1.92	N.D.
5	Pork	Fried	<LOQ	2.15	N.D.	N.D.	N.D.
		Grilled	4.32	3.62	N.D.	3.52	N.D.
6	Shrimp	Fried	<LOQ	3.19	N.D.	N.D.	N.D.
		Grilled	<LOQ	5.13	N.D.	N.D.	N.D.
7	Scallops	Fried	<LOQ	3.23	N.D	N.D.	N.D.
		Grilled	N.D.	N.D.	N.D.	3.13	N.D.
8	Salmon	Fried	<LOQ	2.12	N.D.	N.D.	N.D.
		Grilled	N.D.	3.15	N.D.	2.13	N.D.
9	Hairtail	Fried	<LOQ	<LOQ	N.D.	N.D.	N.D.
		Grilled	2.12	<LOQ	N.D.	1.92	N.D.
10	Perch	Fried	<LOQ	<LOQ	N.D.	N.D.	N.D.
		Grilled	1.98	<LOQ	N.D.	2.16	N.D.
11	Corn	Fried	<LOQ	<LOQ	N.D.	N.D.	N.D.
		Grilled	1.35	<LOQ	N.D.	1.91	N.D.
12	Potatoes	Fried	<LOQ	<LOQ	N.D.	N.D.	N.D.
		Grilled	1.65	<LOQ	N.D.	1.82	N.D.
13	Eggplant	Fried	<LOQ	<LOQ	N.D.	N.D.	N.D.
		Grilled	1.71	<LOQ	N.D.	2.12	N.D.
14	Onions	Fried	<LOQ	<LOQ	N.D.	N.D	N.D.
		Grilled	2.15	<LOQ	N.D.	1.96	N.D.
15	Garlic	Fried	<LOQ	<LOQ	N.D.	N.D.	N.D.
		Grilled	1.92	<LOQ	N.D.	N.D.	N.D.

(continued)

Table 5.. (continued)

No.	Sample	Processing method	1-Nitronaphthalene	1,8-Dinitropyrene	9-Niroanthracene	1-Nitropyrene	3-Nitrofluoranthene
16	Mushrooms	Fried	<LOQ	<LOQ	N.D.	N.D.	N.D.
		Grilled	1.52	<LOQ	N.D.	N.D.	N.D.
17	Bread	Fried	<LOQ	<LOQ	N.D.	N.D.	N.D.
		Grilled	1.96	<LOQ	N.D.	N.D.	N.D.
18	Tofu	Fried	<LOQ	<LOQ	N.D.	N.D.	N.D.
		Grilled	2.33	<LOQ	N.D.	1.98	N.D.
19	Sausage	Fried	<LOQ	<LOQ	N.D.	N.D.	N.D.
		Grilled	3.96	<LOQ	N.D.	2.52	N.D.
20	Taro	Fried	<LOQ	<LOQ	N.D.	N.D.	N.D.
		Grilled	3.12	<LOQ	N.D.	2.19	N.D.

N.D. = Not detected; LOQ = Limit of quantification (S/N = 10).

Table 6. Comparison of the proposed method with the reported methods

Analytes	Sample	Sample Treatment	Analytical Method	Linear range	LODs	RSD, %	Recovery,%	References
1-NN, 2-NN, 2-NFL, 9-NA, 3-NFL, 1-NPYR and 6-NBaP	Food	SPE (biobeads SX-3)	On-line reduction HPLC-FLD	0.5–500 pg	0.5–150 pg	1.2 (1-NPYR)	ca.80	(7)
1-NN, 2-NFL and 1-NPYR	Meat	SPE (silica Sep-Pak)	Precolumn reduction UPLC-FLD	40.0–4600 μg/L	0.31–0.59 μg/kg	7.5–12.8	73.1–91.0	(8)
1-NN, 3-NFL, 1,8-DNP, 9-NA, 1-NPYR	Food	SPE(HLB)	LC-MS/MS	2.00–500 μg/L (except 1-nitronaphthalene 20.0–5000 μg/L)	0.6μg/L–6.0μg/L	<10	70.1–85.6	This work

reduction of 9-nitroanthracene to 9-aminoanthracene whose formula is $C_{14}H_{11}N$ with accurate molecular weight 193.0886. This hypothesis is not consistent with the MS/MS spectrum result of 9-nitro anthracene acquired by LC-TOF, so [M-30] peak should be interpreted as a neutral loss of NO. 1,8-Dinitropyrene fragment 3 : [M-2(NO)]-, theoretical m/z = 232.05188, meet the result in MS/MS spectrum (232.0508); fragment 4 : [M-NO-NO2]-, theoretical m/z = 216.05697, also meet the result in MS/MS spectrum (216.0550).

Method Performance

The linear ranges, correlation coefficients, LODs, and LOQs

We choose 9-nitroanthracene-d9 and 1-nitropyrene-d9 as the internal standards at 10.0 μg/L. The calibration curves were plotted based on the ratio of the peak areas to the internal standard peak areas as a function of the analyte concentration of the standards (μg/L). Under the optimal conditions, the linearities of the method were investigated in the range from 2.00 to 500 μg/L (1-nitronaphthalene 20.0 to 5000 μg/L) for all the nitro-PAHs, and the correlation coefficients (r) obtained ranged from 0.995 to 0.999. The limits of detection (LODs), based on a signal-to-noise ratio (S/N) of 3, were less than 0.60 μg/L (except 1-nitronaphthalene 6.0 μg/kg). Limits of quantification (LOQs), based on a signal-to-noise ratio (S/N) of 10, were less than 2.00 μg/L (but 1-nitronaphthalene was 20.0 μg/L). Figure 4A is the MRM chromatogram of the mixed standard solution at 200 μg/L. Table 3 shows the regression equations, correlation coefficients, LODs, and LOQs of the method.

The accuracy and precision

A set of 6 parallel blank fried pork samples, each weighing 5 g, were spiked with the mixed standard solution to make its final content of 0.80 μg/kg (except 1-nitronaphthalene 8.0 μg/kg), and another set of 6 parallel parallel blank pork samples with no nitro-PAHs detected, each weighing 5 g too, were spiked with the mixed standard solution to make its final content of 8.0 μg/kg (except 1-nitronaphthalene 80.0 μg/kg). In addition, 20 μL internal standard solution (100 μg/L) was added to each sample. All the samples were extracted, purified and analyzed by the proposed method. The accuracy and the precision of the method were evaluated by calculating the recoveries and the relative standard deviations (RSDs) of the six parallel detection results of each level. Table 4 shows that the recoveries of the method ranged from 70.1 to 85.6% with the relative standard deviations (RSDs) less than 10.0%.

Method application

To validate the feasibility of the method, 20 fried/grilled samples were analyzed by the established method. The samples included livestock and poultry meat, seafood, vegetables, etc. Each sample was prepared in two different ways: fried with oil and grilled with charcoal. By comparing the measured results (Table 5), we can see that nitro-PAHs were more easily detected in grilled foods by charcoal. 1-nitronaphthalene, 1,8-dinitropyrene, and1-nitropyrene were detected in some charcoal grilled samples with the contents from 1.35 to 12.9 μg/kg. 1, 8-Dinitropyrene was detected in some oil-fried samples with the contents of 2.12 to 5.12 μg/kg. Figure 4B shows the MRM

chromatogram of a fried squid sample. Figure 4C shows the MRM chromatogram of a charcoal grilled fish.

We compared the proposed method with the reported methods for the analysis of nitro-PAHs in food or meat in terms of linear ranges, limits of detection, recoveries, and relative standard deviations (Table 6). Our proposed method has similar sensitivities to those reported methods, but it is more simple, fast because it doesn't need reduction procedure.

Conclusions

Analysis of nitro-PAHs in barbecued foods has rarely been reported before. We established a quick, sensitive, and accurate method for analysis of five trace nitro-PAHs in barbecued food samples based on the HLB SPE extraction and ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) with internal standard calibration. Compared with the traditional HPLC-FLD method, the proposed method has stronger qualitative capability. The method has been validated and applied to the analysis of 20 barbecued food samples prepared by two different ways and found that the samples grilled with charcoal have much higher contents of 1-nitronaphthalene, 1,8-dinitropyrene, and 1-nitropyrene, which indicates that these foods have potential risk to the health of consumers.

Conflict of interest statement

The authors declare they have no conflict of interest.

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