



# Determination of heterocyclic aromatic amines in airborne particulate matter (PM<sub>2.5</sub> and PM<sub>10</sub>) from different emission sources by ultra-high performance liquid chromatography-tandem mass spectrometry

Romina Canales<sup>a</sup>, Mariana Achad<sup>b</sup>, Patricia Smichowski<sup>b,c</sup>, Darío Gómez<sup>b</sup>, Mario Reta<sup>d</sup>, Soledad Cerutti<sup>a,\*</sup>

<sup>a</sup> Instituto de Química de San Luis, Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional de San Luis, Facultad de Química, Bioquímica y Farmacia Bloque III, Avda. Ejército de los Andes 950, San Luis CP: 5700, Argentina

<sup>b</sup> Comisión Nacional de Energía Atómica (CNEA), Gerencia Química, Av. Gral. Paz 1499, B1650KNA-San Martín, Pcia., Buenos Aires, Argentina

<sup>c</sup> Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Godoy Cruz 2290, C1425FQB Buenos Aires, Argentina

<sup>d</sup> Laboratorio de Investigación y Desarrollo de Métodos Analíticos (LIDMA), División Química Analítica, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 47 y 115, 1900 La Plata, Argentina

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## ABSTRACT

A novel ultrasound assisted extraction procedure was developed prior to the determination of heterocyclic aromatic amines by ultra-high performance liquid chromatography-tandem mass spectrometric detection. Airborne particulate matter PM<sub>2.5</sub> and PM<sub>10</sub> samples ( $n = 34$ ) were collected during the spring season from urban residential emission sites, as well as from biomass burning. The organic compounds were extracted from the quartz fiber filter by a quick ultrasound assisted methanol-based extraction. Linearity of the method was in the range 0.58 ng g<sup>-1</sup> to 50 ng g<sup>-1</sup> with correlation coefficients ( $R^2$ ) from 0.993 to 0.999. The limits of detection resulted to be between 0.19 ng g<sup>-1</sup> and 3.27 ng g<sup>-1</sup>. The relative standard deviations varied from 5.4 to 8.5 ( $n = 3$ ). The recoveries ranged from 83% to 109% for all of the analytes. Concentrations of the analytes in PM<sub>2.5</sub> were between 4.1 ng m<sup>-3</sup> (2.07 ng g<sup>-1</sup>) and 240.9 ng m<sup>-3</sup> (120.5 ng g<sup>-1</sup>), meanwhile in PM<sub>10</sub>, were from 23.0 ng m<sup>-3</sup> (11.4 ng g<sup>-1</sup>) to 355.1 ng m<sup>-3</sup> (176.2 ng g<sup>-1</sup>). The proposed methodology resulted simple, rapid and accurate for its application to the determination of heterocyclic aromatic amines in airborne particulate matter from different emission sources.

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## 1. Introduction

Heterocyclic aromatic amines (HAAs) are potent carcinogenic and mutagenic compounds generated in protein-rich foods during high-temperature cooking [1]. These HAAs are multi-ring aromatic compounds, which may contain imidazopyridine, imidazoquinoline, and imidazoquinoxaline or pyridoindole in their chemical structures [2].

The HAAs are likely to be ubiquitous environmental pollutants, which are produced during the combustion of several materials, such as, tobacco, wood, coal, and grass [3,4]. Moreover, the population is steadily exposed to them via diet and environment. The HAAs may induce several types of cancer including breast, colon, liver, mouth, and stomach [5]. According, the International Agency for Research on Cancer (IARC) has classified MeIQ, MeIQx, PhIP, AαC, MeAαC, Trp-P-1, and Trp-P-2 as possible human carcinogens (Class 2B) and, IQ as a probable

human carcinogenic (Class 2A) and has recommended reducing exposure to these compounds [6]. More recently, the US National Toxicology Program (NTP) has also classified IQ, MeIQ, and PhIP as reasonably anticipated human carcinogens [7].

Pollution influences everyday life, including potential harmful effects on humans and environment due to the presence of toxic substances. Thus, this area of study had received a great deal of attention in recent years [8]. Anthropogenic and biogenic sources are responsible for the emission of airborne particulate matter (APM) that may travel long distances causing pollution in remote areas [9–11]. APM is a complex mixture of materials including metals, metalloids, ions, black carbon, biogenic material and a variety of organic compounds, which constitute unambiguous chemical markers of air quality [12–15]. Hence, to gain knowledge on the presence of several organic compounds on atmospheric particles is of main interest to evaluate the relationship between APM chemical composition and their toxicity [16–18].

The adsorption of HAAs into surfaces of airborne fine particles with a greater relative surface area is a possible pathway for the distribution of these compounds in the different environmental compartments. Among the key non-regulated components, HAAs deserve special

\* Corresponding author at: Instituto de Química de San Luis (INQUISAL-CONICET), Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Ejército de los Andes 950, C.P. 5700 San Luis, Argentina.

E-mail address: [ecerutti@unsl.edu.ar](mailto:ecerutti@unsl.edu.ar) (S. Cerutti).

attention as possible chemical markers of biomass burning. Therefore, it is required to develop and to validate sensitive and specific methodologies.

A technique considered as an effective approach for the extraction of organic compounds from solid samples is UAE, which was introduced by Manabe et al., for the determination of amino- $\alpha$ -carbolines (A $\alpha$ C, MeA $\alpha$ C) and amino- $\gamma$ -carbolines (Trp-P-1, Trp-P-2) in diesel exhaust particles [3]. This extraction procedure is based on the formation of high-frequency ultrasonic waves, which are capable of causing cavitation due to the expansion and contraction cycles suffered by the material. Consequently, both extraction and mass transfer are favored by the penetration of the solvent. The UAE is an inexpensive technique, which can offer high reproducibility in short time, simplicity of operation, and low consumption of organic solvents [19,20]. The efficiency of UAE is affected by several experimental variables, such as solvent composition, pH and solvent-solid ratio, sonication time, and temperature [21]. Statistical procedures for optimization of the different experimental factors influencing the extraction efficiency by a variety of experimental design techniques have been encouraged. These approaches result to be faster, more economical and effective, and allow more than one variable to be optimized simultaneously. Additionally, a better understanding of the system's behavior and how the input variables (factors), and their interactions, affect the response can be obtained [22]. Taking the later into account, the optimization of the extraction parameters was carried out by applying an experimental design.

The aim of this study was to propose an alternative, simple, and fast approach based on UAE extraction followed by UHPLC-(+)ESI-MS/MS quantification of specific HAAs in APM from urban areas and biomass burning emission. The influence of diverse variables affecting the analytical efficiency of both extraction and separation/detection techniques was evaluated and optimized over the HAAs percentage recoveries. The overall performance of the methodology was assessed and was applied to the analysis of ultra-trace levels of eight HAAs in the selected samples. This work constitutes the first report about the monitoring of HAAs in APM samples from different emission sources of Argentina.

## 2. Material and methods

### 2.1. Chemicals and reagents

Analytical standards of 2-amino-3-methylimidazo-[4,5-f]-quinolone (IQ), 2-amino-3,4-dimethylimidazo-[4,5-f]-quinolone (MeIQ), 2-

amino-3,4,8-trimethylimidazo-[4,5-f]-quinoxaline (4,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo-[4,5-b]-pyridine (PhIP), 3-Amino-1,4-dimethyl-5H-pirido-[4,3-b]-indole (Trp-P-1), 3-amino-1-methyl-5H-pirido-[4,3-b]-indole (Trp-P-2), 2-amino-9H-pyrido-[2,3-b]-indole (A $\alpha$ C) and 2-amino-3-methyl-9H-pyrido-[2,3-b]-indole (MeA $\alpha$ C) were obtained from Toronto Research Chemicals (North York, ON, Canada). Structures of the HAAs studied are shown in Fig. 1.

Acetonitrile (ACN), methanol (MeOH) and water Optima® LC-MS grade were purchased from Fisher Scientific (Fair Lawn, New Jersey, USA). Formic acid was obtained from Fisher Scientific (Loughborough, UK). Working standard solutions in MeOH/H<sub>2</sub>O (1:3) were prepared daily by stepwise dilution from 5.0 mg L<sup>-1</sup> stock standard solutions of each compound. Quantification was achieved by preparing spiked water samples with proper amounts of the analytes. The solutions were maintained at 4 °C, protected from light and kept in amber flasks.

### 2.2. Instrumentation

Mass spectrometry analyses were performed on a Quattro Premier™ XE Micromass MS Technologies triple quadrupole mass spectrometer with a Z-Spray™ electrospray ionization source (Waters, Milford, USA).

An Acquity™ Ultra High Performance LC system (Waters, Milford) equipped with an autosampler injection and pump systems (Waters, Milford, USA) was used. The autosampler vial tray was kept at 4 °C. The separation was performed using an ACQUITY UPLC® BEH C18 (Waters, Milford, USA) analytical column (50 × 2.1 mm i.d., 1.7 μm). An ultrasonic cleaner (Testlab, (model TB-04 TA, Buenos Aires, Argentina)), a centrifuge (U-320R-BOECO, Germany) and an electronic microbalance with a readability of 0.1 mg (Ohaus, model UMX2, Switzerland) were employed.

#### 2.2.1. Mass spectrometry conditions

Determinations were performed on a tandem triple quadrupole mass spectrometer with an electrospray ionization (ESI) source. For all compounds, the source was operated in a positive mode and the data was acquired in multiple reaction monitoring mode (MRM) of selected ions at the first (Q1) and third quadrupole (Q3). Source working conditions were as follows: capillary voltage, 2.7 kV; extractor voltage, 1.0 kV; source temperature, 150 °C; desolvation temperature, 350 °C; cone gas flow rate, 50 L h<sup>-1</sup>; desolvation gas flow rate, 400 L h<sup>-1</sup>. Ultrapure nitrogen was used as cone gas and argon was used as collision gases at a flow of 0.18 mL min<sup>-1</sup>, respectively. To choose the fragmentation patterns for the compounds in MRM mode, direct infusions (via syringe pump)

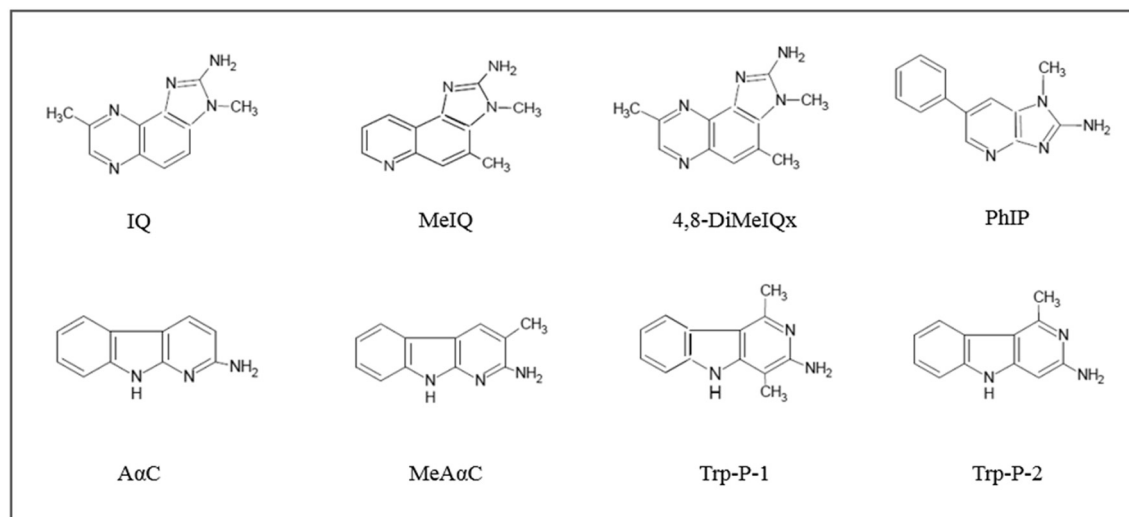


Fig. 1. Structures of the HAAs under study.

**Table 1**  
Parameters and diagnostic fragment ions used for the quantification and confirmation of the eight HAAs under study.

Analyte (MW)	Precursor ion (m/z)	Cone voltage (V)	Confirmation		Quantification	
			Product ion (m/z) (m/z)	Collision voltage (V) (V)	Product ion (m/z)	Collision voltage (V)
IQ (198)	199	25	184	32	154	32
MeIQ (212)	213	35	198	26	145	30
4,8DiMeIQx (227)	228	44	213	26	187	27
PhIP (224)	224	45	210	30	183	35
Trp-P-1 (211)	212	35	168	19	195	17
Trp-P-2 (197)	198	27	154	30	181	20
A $\alpha$ C (183)	184	25	140	25	167	24
MeA $\alpha$ C (197)	198	25	154/129	25	181	23

into the MS of the HAAs ( $0.5 \text{ mg L}^{-1}$ ) standards solution prepared as detailed in the previous section. The product ion scan mass spectra were recorded. The compound-dependent parameters were optimized and listed in Table 1. As can be seen, MeA $\alpha$ C and Trp-P-2 have the equal precursor and products ion. Nevertheless, these fragments did not interfere between them. The same was observed for the others HAAs under study. Data acquisition was carried out by MassLynx Mass Spectrometry Software (Waters, Milford, USA).

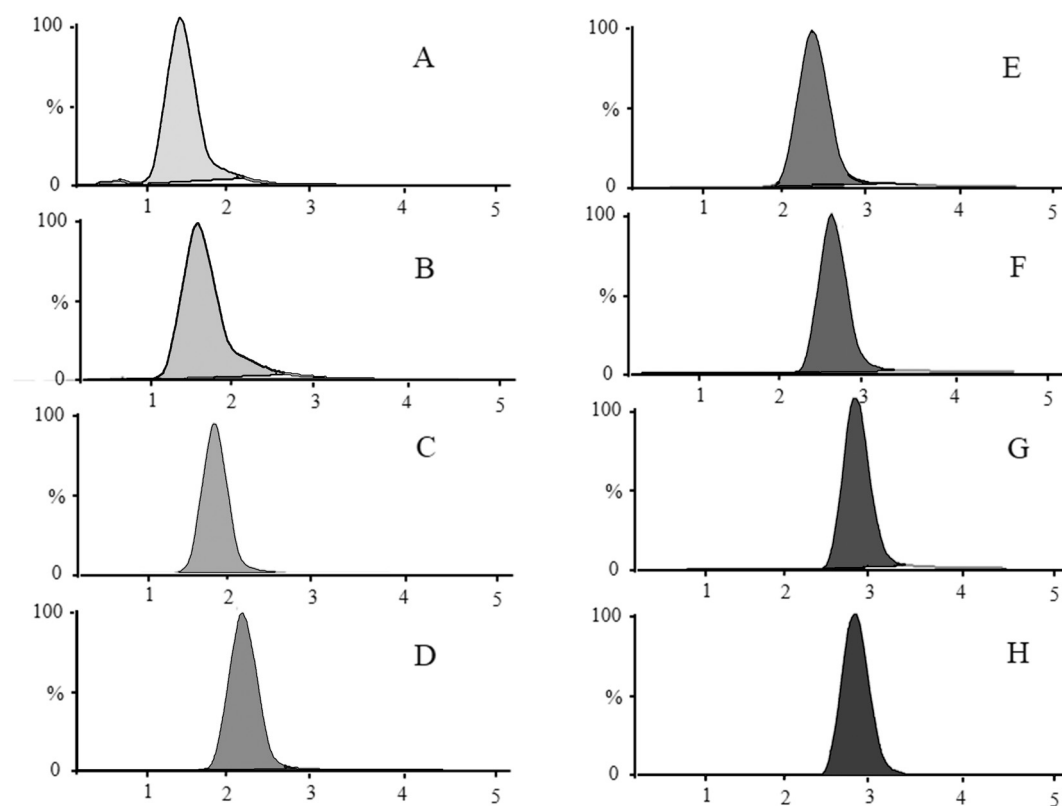
### 2.2.2. Chromatographic conditions

The separation was performed by injecting  $10 \mu\text{L}$  sample into an ACQUITY UPLC $\text{BEH C18}$  analytical column in reverse phase. The binary mobile phases consisted  $0.1\%$  (v/v) formic acid in water (A) and  $0.1\%$

(v/v) formic acid in methanol (B) delivered at  $0.25 \text{ mL min}^{-1}$ . The C18 gradient was started at an initial composition of  $90\%$  A and  $10\%$  B, then  $2.5 \text{ min}$  linear gradient to  $20\%$  A, held for  $2.5 \text{ min}$ . A return to the initial conditions was accomplished by a  $0.2 \text{ min}$  gradient to  $90\%$  A, where it was held for  $0.8 \text{ min}$ . Thus, all the HAAs studied were temporarily resolved within  $3 \text{ min}$ , being  $4.0 \text{ min}$  the total chromatographic run time (Fig. 2). The column was held at  $30 \text{ }^\circ\text{C}$ . Under these conditions, no sample contamination or sample-to-sample carryover was observed.

### 2.3. Aerosol collection

Samples were collected during a sampling campaign carried out between September and October 2016 (spring in the Southern



**Fig. 2.** Chromatograms of the eight HAAs determined by UHPLC-(+)ESI-MS/MS: A) IQ (tr: 1.73 min); B) MeIQ (tr: 1.82 min); C) 4,8-DiMeIQx (tr: 1.91 min); D) PhIP (tr: 2.14 min); E) Trp-P-1 (tr: 2.20 min); F) A $\alpha$ C (tr: 2.22 min); G) Trp-P-2 (tr: 2.25 min); H) MeA $\alpha$ C (tr: 2.25 min).

Hemisphere). The emission of 34 samples from different origins was examined. Thus, the collection from burning of different types of wood such as willow (*Salix humboldtiana*), espinillo (*Vachellia caven*), and pine, (*Pinus pinea*), among others native species from Argentina, was carried out. In addition, airborne particles were collected in urban areas impacted by traffic.

Fine-respirable ( $PM_{2.5}$ ) and coarse-inhalable ( $PM_{10}$ ) size ranges were obtained at one sampling site on a yard of a building at the National Commission of Atomic Energy (CNEA). The selected site is located at ~15 m distance from a highway ( $34^{\circ} 38'S$ ,  $58^{\circ} 28'W$ ). This site is placed approximately 200 km from the open sea and influenced mainly by emissions from urban areas.

$PM_{2.5}$  and  $PM_{10}$  samples were collected on quartz fiber filters (Sartorius Stedim Biotech, Germany) using a medium-volume air sampler (PQ200, BGI Incorporated, MA USA) equipped with a size-selective inlet. The average daily collected air volume was  $25\text{ m}^3$ . Before collection, each filter was placed in a stove at  $800^{\circ}\text{C}$  for 5 h to eliminate any impurity. Then, all filters were placed under constant temperature ( $-25^{\circ}\text{C}$ ) and relative humidity ( $<40\%$ ) for 24 h before weighing. Mass concentrations were obtained gravimetrically using an electronic microbalance. After sampling, all filters were wrapped in aluminum foil and stored in a dry and dark place at  $4^{\circ}\text{C}$  until analysis.

#### 2.4. Sample preparation and UAE procedure

One fourth of the whole filter area ( $\approx 4.34\text{ cm}^2$ ) was weighed and used for the HAAs extraction procedure. The quartz fiber filter sample was spiked with  $20\text{ ng g}^{-1}$  of a mixture of HAAs standards. Then, the filter was carefully cut into small pieces to increase the contact surface area between the filter sample and the extraction solvent; this step improved the extraction efficiency. The sample was transferred to a glass tube, where an optimized (2 mL) extraction solvent volume (MeOH) was added. Immediately, the mixture was gently shaken and then immersed in an ultrasonic bath for 10 min. The extraction was performed at 40 kHz frequency and 160 W, at room temperature. Phase separation was achieved by a centrifugation at 3000 rpm for 5 min. The supernatant was transferred into a glass vial for direct analysis by UHPLC-(+)ESI-MS/MS.

#### 2.5. Matrix effect (ME)

The matrix effect has an important impact on the qualitative and quantitative analyses, due to suppression or enhancement of the signal.

As known, the predominant cause is the presence of undesired components that co-elute in the chromatographic separation and upset the ionization effects [23,24]. Thus, the liquid chromatography/mass spectrometry response obtained from the standards may differ considerably from matrix samples.

In this work, after optimizing the determination parameters, the effects of the quartz fiber filter and the sample matrix over the compounds analytical responses were assessed by comparing the calibration curves slopes of the HAAs with the ones obtained for the mentioned matrices. The percentage of the quotient of the slopes (b) in the spiked and solvent samples was employed as an indicator of the extent of the matrix effect, which was calculated according to Eq. (1).

### 3. Results and discussion

The main parameters affecting the extraction conditions were optimized by full factorial design. The explored experimental parameters were evaluated as follows.

#### 3.1. UAE conditions

##### 3.1.1. Selection of the extraction solvent type

An appropriate extraction solvent is pivotal for potentiating extraction efficiency of the HAAs under study. In this context, ACN, MeOH and a mixture of them were employed as extraction solvents; as well, its effect on the efficient HAAs recovery was analyzed.

The results indicated (Fig. 3) that MeOH exhibited the best extraction efficiency, which was around 100% for all of the compounds. This is probably because of the polarity of this solvent which improves the mass transfer of all compounds [25]. Accordingly, MeOH was selected as an appropriate extraction solvent for subsequent experiments.

##### 3.1.2. Screening of the significant main variables on the UAE conditions

In order to evaluate the variables directly related to the UAE efficiency, a two-level-two-factors ( $2^2$ ) full factorial design was applied. This experimental design consisted of 4 runs and three replicates of the central point, which was performed in order to determine the influence of the factors and their interactions in the optimization of the principal steps of the extraction procedure. All the experiments were carried out randomly and run in triplicates ( $n = 3$ ) with the purpose of minimizing the effects of uncontrolled factors. According to the

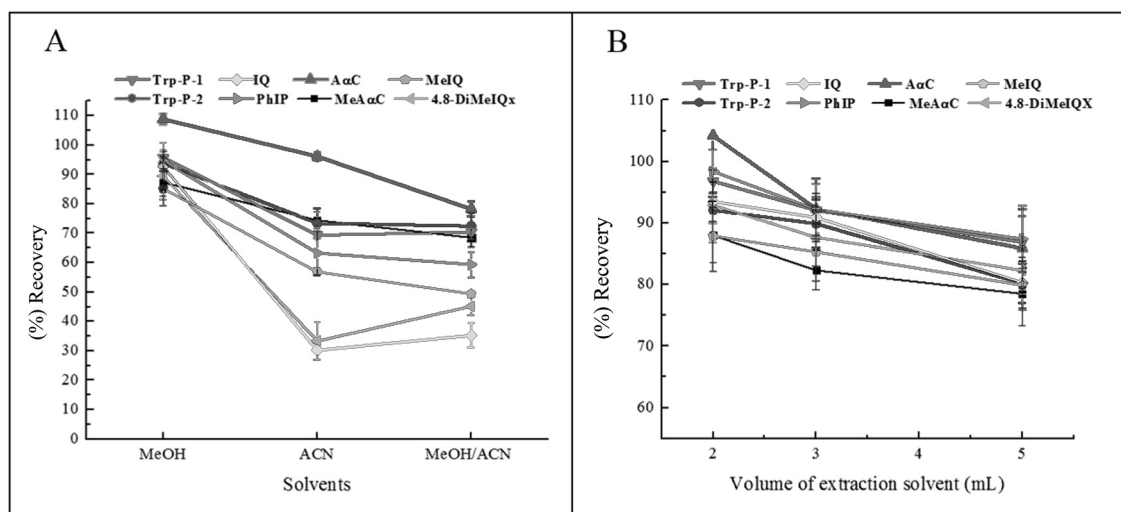


Fig. 3. Effect of the extraction solvents on the recovery of the HAAs from UAE-UHPLC-(+)ESI-MS/MS. Mixture standard concentration:  $20\text{ ng g}^{-1}$ ; extraction solvent volume: 2 mL centrifugation time and rate: 5 min, 3000 rpm; ultrasonic bath time: 10 min.

**Table 2**  
Full factorial design matrix.

Experiment	Experimental design		Recovery (%) <sup>a,b</sup>
	UA	ES	
1	+	+	51.7 ± 3.1
2	+	–	83.1 ± 2.0
3	–	+	48.7 ± 1.2
4	–	–	94.1 ± 4.0
5	0	0	70.3 ± 3.7
6	0	0	69.9 ± 2.6
7	0	0	56.6 ± 2.5

<sup>a</sup> Response resulted from the HAAs's average recoveries of the analytes under study.

<sup>b</sup> Experiments' recovery are shown as means ± standard deviation of three replicates ( $n = 3$ ).

experimental design, low, central, and high levels of the variables were designated as (–), (0), and (+), respectively. Ultrasonic assisted bath time (UA; maximum and minimum levels: 10 and 30 min; respectively, with 20 min as the central point), extraction solvent volume (ES; maximum and minimum levels: 2 and 5 mL; respectively, with 3.5 mL as the central point).

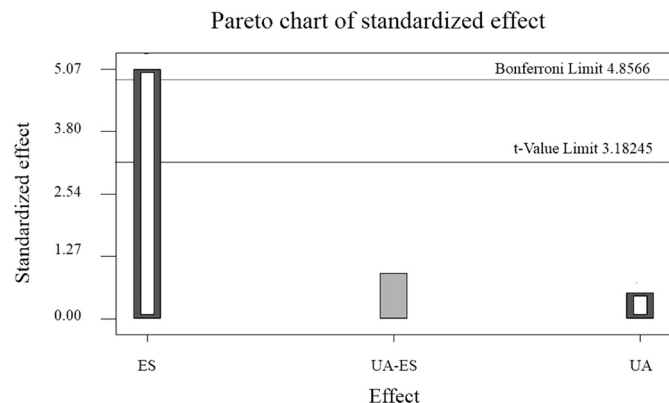
The experimental design matrix and the average percentage recovery of all analytes under study obtained for each experiment are listed in Table 2.

Statistical analysis of variance (ANOVA) and  $p$ -value (probability) was used to evaluate the statistical significance of the effects. The data analysis was performed using the software Design-Expert (Version 7.0.0, Stat-Ease Inc., Minneapolis).

Main effects and their interactions for the experimental design can be seen in the Pareto chart depicted in Fig. 4. This graphical representation demonstrated that the extraction solvent volume was statistically significant at 95% ( $\alpha = 0.05$ ) confidence level. As specified, neither the interaction between UA-ES nor UA had statistically significant effects on the percentage recovery. On the other hand, the extraction solvent volume has an important impact on the recovery efficiency, for this reason, the main goal was to develop a method that used the minimum extraction solvent volume. In this sense, the HAAs recovery was optimal when 2 mL of the MeOH was used as solvent for the extraction process.

### 3.1.3. Centrifugation time and rate

With the purpose to accomplish a clear supernatant, different centrifugation times (3–10 min) and rates (2500–5000 rpm) were assayed. An adequate separation during a reasonably short time was obtained after 5 min of centrifugation at 3000 rpm. After centrifugation at room



**Fig. 4.** Pareto chart of standardized effects for the independent variables: extraction solvent volume (ES, mL), ultrasonic assisted bath time (UA, min).

**Table 3**  
Linearity, linear range, detection and quantification limits, and relative standard deviation of the UAE-UHPLC-(+)ESI-MS/MS.

Compound	$r^2$	LR ( $\text{ng g}^{-1}$ )	LOD ( $\text{ng g}^{-1}$ )	LOQ ( $\text{ng g}^{-1}$ )	RSD (%) ( $n = 3$ )
IQ	0.999	3.27–30	1.08	3.27	8.0
MeIQ	0.996	0.58–30	0.19	0.58	6.7
4,8-DiMeIQx	0.998	9.76–50	3.22	9.76	5.4
PhIP	0.999	1.90–30	0.63	1.90	5.5
Trp-P-1	0.993	9.91–50	3.27	9.91	8.2
Trp-P-2	0.999	6.03–50	1.99	6.03	7.6
A $\alpha$ C	0.998	8.89–50	2.93	8.89	8.5
MeA $\alpha$ C	0.995	3.70–30	1.22	3.70	6.9

temperature, the supernatant was transferred into a glass vial and directly injected into UHPLC-(+)ESI-MS/MS.

### 3.1.4. Evaluation of matrix effect

As mentioned in Section 2.5, the effects of the quartz fiber filters material itself and the sample matrix over the HAAs signals were evaluated. Therefore, calibration curves from spiked filters, spiked matrix, and methanolic standard solutions were created. The slope values were used as indicators of the extent of the ion suppression or signal enhancement. According to our findings, neither signal enhancement nor suppression was observed for any of the HAAs under study. Consequently, quantification was carried by external calibration, which constitutes a remarkable advantage from the practical perspective.

### 3.2. Analytical figures of merit

The performance of the UAE-UHPLC-(+)ESI-MS/MS was evaluated. Thus, important figures of merit such as linearity, detection and quantification limits, selectivity, and precision were obtained. The system performance was investigated by extracting spiked quartz fiber filters. For this purpose, two collections of APM were considered: air samples directly collected from urban areas and samples collected during biomass burning of selected local trees. These samples were composed by three blank samples, and three replicates at 10  $\text{ng g}^{-1}$ , 20  $\text{ng g}^{-1}$ , 30  $\text{ng g}^{-1}$ , 40  $\text{ng g}^{-1}$  and 50  $\text{ng g}^{-1}$ . The linearity of the calibration curves for spiked quartz fiber filter was satisfactory with determination coefficients ( $R^2$ ) in the range of 0.993 to 0.999. The F-test demonstrated that linear regressions were statistically acceptable in the working ranges and this model showed goodness of fit.

Additionally, the limits of detection (LOD) and quantification (LOQ) following the International Union of Pure and Applied Chemistry (IUPAC) recommendations were calculated as 3.3  $\text{Sy/b}$  and 10  $\text{Sy/b}$ ; respectively, under working conditions, where  $b$  is the slope of the regression curve and  $\text{Sy}$  the standard error of the blank [26,27].

The obtained LOD values were in the range from 0.19 to 2.64  $\text{ng g}^{-1}$  and for the LOQ were from 0.58 to 8.01  $\text{ng g}^{-1}$ ; respectively. Table 3 summarizes the analytical characteristics of the optimized method. Average intraday relative standard deviations (RSD (%)) values were from 5.4% to 8.5% at concentration levels from 10  $\text{ng g}^{-1}$  to 50  $\text{ng g}^{-1}$  for all HAAs.

The accuracy of the analytical method was evaluated based on the recovery of the HAAs from different collections of APM samples. Additions of known amounts of the HAAs under study at different concentration levels, from 0 to 50  $\text{ng g}^{-1}$ , to blank real sample matrices before processing them according to the above-mentioned procedure were carried out. The percentage recovery for the HAAs ranged from 83% to 109% as shown in Tables 4 and 5. From the analysis of the obtained results was possible to infer about the method's good precision and satisfactory recoveries. As an overview, Table 6 shows a comparison of the analytical performance among the proposed UAE-UHPLC-MS/MS

method and other works related to the determination of HAAs in outdoor air. As can be observed, the soxhlet extraction approach is efficient and has been applied to determination of HAAs, but the strategies related require large volumes of toxic solvents and time-consuming procedures, increasing considerably the overall sample treatment step. Therefore, the herein proposed procedure results to be an advantageous, green analytical alternative since the simultaneous extraction and sensitive detection of eight HAAs by using minimum solvents volumes, in a short period, with a very simple sample treatment, can be achieved.

### 3.3. Determination of the HAAs in APM

The amount of HAAs in the atmosphere depends on many factors such as temperature, season of the year, vapor and atmospheric pressures, concentration and properties of the collected material, among others. Different collections of APM samples from Argentina were analyzed as described in Section 2.4. All the eight studied compounds were detected in both analyzed particle sizes. It is important to notice that no literature reports have been found about HAAs in APM from biomass burning neither the presence of 4,8-DiMeIQx and Trp-P-1 in urban areas samples. Thus, this work constitutes a relevant contribution related to environmental monitoring. The data indicated that the content

of the HAAs and their distribution were different in the samples tested. As exhibited in Table 4, the HAAs levels were below the instrumental detection limits in airborne particulate matter sample from samples collected in a site impacted by traffic.

In this study, HAAs concentration levels resulted below the detection limits for samples collected in spring contrary to the findings of other authors that reported the highest levels in this season [28,29]. These differences may be attributed to different weather conditions and/or air quality of the urban sites under study [9].

On the other hand, in samples collected during biomass burning, all the HAAs were determined. The total HAAs concentrations in PM<sub>2.5</sub> were in the range from 4.1 to 240.9 ng m<sup>-3</sup>, meanwhile in PM<sub>10</sub> ranged from 23.0 to 355.5 ng m<sup>-3</sup> (Table 5). The results obtained for IQ, MeIQ, 4,8DiMeIQx, PhIP and Trp-P-2 indicated that levels were similar in both particle sizes, while for Trp-P-1, AαC, MeAαC the concentrations were two, three, and nine times higher in PM<sub>10</sub>; respectively. Thus, for some of these compounds, higher levels were present in bigger air particulate matter. It was observed that 4,8-DiMeIQx, PhIP and Trp-P-1 predominated in APM emitted during biomass burning, making this event an important source for these compounds. These findings could imply that these organic compounds have been formed partially or completely from the combustion of the raw material. The presence of HAAs in samples from biomass burning is scarce in the literature [2].

**Table 4**

Recoveries (%) obtained from the analysis of spiked in fiber filter samples from urban residential emission source using the proposed methodology. N.D.\*: not detected.

APM HAAs	Urban areas emission (PM <sub>2.5</sub> )					Urban areas emission (PM <sub>10</sub> )				
	Sample concentration (ng m <sup>-3</sup> )	Added (ng g <sup>-1</sup> )	Found (ng g <sup>-1</sup> )	Recovery (%)	RSD (%) n = 3	Sample concentration (ng m <sup>-3</sup> )	Added (ng g <sup>-1</sup> )	Found (ng g <sup>-1</sup> )	Recovery (%)	RSD (%) n = 3
IQ	N.D.*	0	–	–	–	N.D.*	0	–	–	–
	*	10	9.3	93	8.0	*	10	9.6	96	2.6
	*	20	19.4	97	2.2	*	20	19.5	97	0.9
	*	30	26.4	88	3.0	*	30	29.3	98	2.1
	*	40	36.4	91	7.1	*	40	36.7	92	6.0
MeIQ	*	0	–	–	–	*	0	–	–	–
	*	10	9.6	96	5.6	*	10	9.3	93	6.7
	*	20	17.8	89	3.2	*	20	19.7	98	2.8
	*	30	29.7	99	1.9	*	30	30.2	100	2.5
	*	40	37.6	94	2.1	*	40	36.7	91	3.8
4,8-DiMeIQx	*	0	–	–	–	*	0	–	–	–
	*	10	9.5	95	8.1	*	10	10.1	100	2.2
	*	20	18.8	94	6.7	*	20	19.4	97	3.0
	*	30	29.4	98	6.6	*	30	28.8	96	6.8
	*	40	39.5	99	4.0	*	40	37.9	95	5.9
PhIP	*	0	–	–	–	*	0	–	–	–
	*	10	9.5	95	3.5	*	10	9.7	97	2.2
	*	20	18.3	91	5.2	*	20	19.1	96	5.8
	*	30	29.9	100	2.4	*	30	28.5	95	3.1
	*	40	37.6	94	6.1	*	40	38.2	95	2.9
Trp-P-1	*	0	–	–	–	*	0	–	–	–
	*	10	9.7	97	1.9	*	10	9.7	97	5.4
	*	20	18.1	90	3.5	*	20	18.3	92	2.4
	*	30	28.7	96	2.3	*	30	31.2	104	6.8
	*	40	39.1	98	2.9	*	40	40.5	101	4.2
Trp-P-2	*	0	–	–	–	*	0	–	–	–
	*	10	9.4	94	2.6	*	10	9.93	99	2.9
	*	20	18.2	91	3.9	*	20	20.5	102	3.7
	*	30	28.5	95	1.9	*	30	28.6	96	9.6
	*	40	38.8	97	1.8	*	40	38.3	96	5.6
AαC	*	0	–	–	–	*	0	–	–	–
	*	10	10.2	102	1.7	*	10	9.5	95	5.9
	*	20	18.6	93	2.8	*	20	19.8	99	5.3
	*	30	29.7	99	2.5	*	30	30.4	101	3.2
	*	40	40.4	101	3.5	*	40	39.4	98	2.3
MeAαC	*	0	–	–	–	*	0	–	–	–
	*	10	8.8	88	4.5	*	10	9.7	98	2.2
	*	20	18.7	93	1.7	*	20	20.5	102	3.1
	*	30	26.7	89	3.8	*	30	29.7	99	5.7
	*	40	37.6	94	1.4	*	40	41.0	102	2.5

**Table 5**  
Recoveries (%) obtained from the analysis of spiked in fiber filter samples from simulated biomass burning emission source using the proposed methodology.

APM	Biomass burning emission (PM <sub>2.5</sub> )					Biomass burning emission (PM <sub>10</sub> )					
	HAAs	Sample concentration (ng m <sup>-3</sup> )	Added (ng g <sup>-1</sup> )	Found (ng g <sup>-1</sup> )	Recovery (%)	RSD (%) n = 3	Sample concentration (ng m <sup>-3</sup> )	Added (ng g <sup>-1</sup> )	Found (ng g <sup>-1</sup> )	Recovery (%)	RSD (%) n = 3
IQ	4.1	0	2.1	–	–	23.0	0	11.4	–	–	
	23.4	10	11.7	96	5.3	42.2	10	20.9	95	6.3	
	40.7	20	20.4	92	5.6	62.7	20	31.1	98	4.4	
	58.5	30	29.3	91	4.1	80.1	30	39.7	94	1.9	
	80.7	40	40.4	96	2.9	101.7	40	50.4	98	7.2	
MeIQ	36.4	0	18.2	–	–	46.4	0	23.0	–	–	
	54.2	10	27.1	89	5.7	63.1	10	31.3	83	4.8	
	71.0	20	35.5	87	4.5	82.5	20	40.9	90	3.1	
	88.0	30	44.0	86	3.8	102.9	30	51.0	93	2.6	
	104.2	40	52.1	85	5.3	122.0	40	60.5	94	5.7	
4,8-DiMeIQx	222.5	0	111.3	–	–	269.8	0	133.7	–	–	
	240.8	10	120.4	91	5.0	288.5	10	143.0	93	2.1	
	260.4	20	130.2	95	3.6	307.8	20	152.6	95	4.9	
	280.8	30	140.4	97	3.8	328.0	30	162.6	96	4.5	
	300.6	40	150.3	98	6.9	348.6	40	172.8	98	3.7	
PhIP	240.9	0	120.5	–	–	227.4	0	112.7	–	–	
	260.8	10	130.4	99	2.2	246.9	10	122.4	97	3.1	
	281.6	20	140.8	101	3.7	267.3	20	132.5	99	5.6	
	299.2	30	149.6	97	6.5	286.9	30	142.2	98	5.3	
	318.4	40	159.2	97	2.8	308.0	40	152.7	100	0.9	
Trp-P-1	153.5	0	76.8	–	–	355.5	0	176.2	–	–	
	173.2	10	86.6	98	2.2	374.8	10	185.8	96	2.0	
	194.0	20	97.0	101	1.4	391.9	20	194.3	91	7.3	
	209.2	30	104.6	93	6.1	413.1	30	204.8	95	4.7	
	230.6	40	115.3	96	2.8	434.7	40	215.5	98	6.4	
Trp-P-2	38.2	0	19.1	–	–	43.6	0	21.6	–	–	
	57.4	10	28.7	96	6.3	62.5	10	31.0	94	4.5	
	76.6	20	38.3	96	3.9	82.1	20	40.7	96	8.3	
	94.4	30	47.2	94	4.2	103.1	30	51.1	98	7.6	
	114.0	40	57.0	95	3.7	122.9	40	60.9	98	5.7	
AαC	63.1	0	31.6	–	–	185.7	0	92.1	–	–	
	80.2	10	40.1	85	3.0	207.8	10	103.0	109	3.2	
	102.4	20	51.2	98	5.6	225.3	20	111.7	98	4.9	
	118.2	30	59.2	92	4.1	245.1	30	121.5	98	5.8	
	144.8	40	72.4	102	4.4	268.9	40	133.3	103	2.8	
MeAαC	32.6	0	16.3	–	–	230.7	0	114.3	–	–	
	51.0	10	25.5	92	5.2	249.1	10	123.5	92	5.8	
	69.2	20	34.6	91	2.6	267.9	20	132.8	93	4.7	
	86.4	30	43.2	90	5.8	286.9	30	142.2	93	7.1	
	107.6	40	53.8	94	4.6	307.6	40	152.5	96	4.2	

#### 4. Conclusions

A rapid, sensitive and selective UAE methodology coupled with UHPLC-(+)-ESI-MS/MS was developed for ultra-trace extraction of eight HAAs in APM (PM<sub>2.5</sub> and PM<sub>10</sub>). UAE served as simple, fast, low-cost, and efficient multiple-sample extraction system. The HAAs were detected in both sizes of the atmospheric particles; in particular, in samples from biomass burning.

To the best of our knowledge, this study reports for the first time the presence of eight HAAs in airborne particles collected from different sources in the city of Buenos Aires, which provides information on the

presence of these compounds in urban atmospheres. From now on, more studies are necessary to support the herein proposed hypothesis to consider HAAs as potential markers of biomass burning. It is predicted that the results of this investigation will supply basic information about air quality and stimulates further studies to address HAAs nature and environmental impact.

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**Table 6**  
Comparison of the analytical performance of the proposed methodology with others already reported for HAAs determination.

Extraction procedure	Separation/detection technique	HAAs studied	Sample	LOD (ng m <sup>-3</sup> )	LOQ (ng m <sup>-3</sup> )	Ref.
Soxhlet	HPLC-UVD	IQ, MeIQ, PhIP, Trp-P-2, AαC, MeAαC	Outdoor air	0.093–0.609 <sup>a</sup>	n.m.*	[28]
Soxhlet	HPLC-FLD	PhIP, Trp-P-2, AαC, MeAαC	Outdoor air	1.8–8.4 <sup>b</sup>	*	[28]
Soxhlet	HPLC-UVD	IQ, MeIQ, PhIP, Trp-P-2, AαC, MeAαC	Outdoor air	0.28–1.82	*	[29]
Soxhlet	HPLC-FLD	PhIP, Trp-P-2, AαC, MeAαC	Outdoor air	0.005–0.025	*	[29]
UAE	UHPLC-MS/MS	IQ, MeIQ, 4,8-DiMeIQx, PhIP, Trp-P-1, Trp-P-2, AαC, MeAαC	Outdoor air	0.008–0.131	0.023–0.396	This work

\* n.m.: not mentioned.

<sup>a</sup> mg L<sup>-1</sup>.

<sup>b</sup> μg L<sup>-1</sup>.

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## Appendix A

$$ME = 100 - (b \text{ spiked} / b \text{ solvent}) \times 100 \quad (1)$$

## References

- [1] F. Oz, M.O. Yuzer, The effects of cooking on wire and stone barbecue at different cooking levels on the formation of heterocyclic aromatic amines and polycyclic aromatic hydrocarbons in beef steak, *Food Chem.* 203 (2016) 59–66.
- [2] B. Janoszka, U. Błaszczuk, L. Warzecha, M. Strózyk, A. Damasiewicz-Bodzek, D. Bodzek, Clean-up procedures for the analysis of heterocyclic aromatic amines (aminozaarenes) from heat-treated meat samples, *J. Chromatogr. A* 938 (2001) 155–165.
- [3] S. Manabe, N. Kurihara, O. Wada, S. Izumikawa, K. Asakuno, M. Morita, Detection of a carcinogen, 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine, in airborne particles and diesel-exhaust particles, *Environ. Pollut.* 80 (1993) 281–286.
- [4] S. Manabe, S. Izumikawa, K. Asakuno, O. Wada, Y. Kanai, Detection of carcinogenic amino- $\alpha$ -carbolines and amino- $\gamma$ -carbolines in diesel-exhaust particles, *Environ. Pollut.* 70 (1991) 255–265.
- [5] J.S. Felton, M.G. Knize, L.M. Bennett, M.A. Malfatti, M.E. Colvin, K.S. Kulp, Impact of environmental exposures on the mutagenicity/carcinogenicity of heterocyclic amines, *Toxicology* 198 (2004) 135–145.
- [6] IARC, Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins, World Health Organization, Geneva, 1993.
- [7] N.T. program, NTP 11th Report on Carcinogens, Report on Carcinogens: Carcinogen Profiles 11, 1, 2004.
- [8] W. Wang, L. Jing, J. Zhan, B. Wang, D.P. Zhang, H.W. Zhang, D.Q. Wang, Y. Yang, J. Zhao, Y.F. Sun, X.H. Bi, X.T. Wang, J.L. Feng, Nitrated polycyclic aromatic hydrocarbon pollution during the Shanghai World Expo 2010, *Atmos. Environ.* 89 (2014) 242–248.
- [9] J.P. Dawson, B.J. Bloomer, D.A. Winner, C.P. Weaver, Understanding the meteorological drivers of U.S. particulate matter concentrations in a changing climate, *Bull. Am. Meteorol. Soc.* 95 (2014) 521–532.
- [10] S. Fuzzi, U. Baltensperger, K. Carslaw, S. Decesari, H. Denier van der Gon, M.C. Facchini, D. Fowler, I. Koren, B. Langford, U. Lohmann, E. Nemitz, S. Pandis, I. Riipinen, Y. Rudich, M. Schaap, J.G. Slowik, D.V. Spracklen, E. Vignati, M. Wild, M. Williams, S. Gilardoni, Particulate matter, air quality and climate: lessons learned and future needs, *Atmos. Chem. Phys.* 15 (2015) 8217–8299.
- [11] J. Chen, C. Li, Z. Ristovski, A. Milic, Y. Gu, M.S. Islam, S. Wang, J. Hao, H. Zhang, C. He, H. Guo, H. Fu, B. Miljevic, L. Morawska, P. Thai, Y.F. Lam, G. Pereira, A. Ding, X. Huang, U.C. Dumka, A review of biomass burning: Emissions and impacts on air quality, health, and climate in China, *Sci. Total Environ.* 579 (2017) 1000–1034.
- [12] L. AlSioufi, A.M. Sánchez de la Campa, D. Sánchez-Rodas, Microwave extraction as an alternative to ultrasound probe for antimony speciation in airborne particulate matter, *Microchem. J.* 124 (2016) 256–260.
- [13] M. Dos Santos, D. Gómez, L. Dawidowski, E. Gautier, P. Smichowski, Determination of water-soluble and insoluble compounds in size classified airborne particulate matter, *Microchem. J.* 91 (2009) 133–139.
- [14] F. Fujiwara, M. Guiñez, S. Cerutti, P. Smichowski, UHPLC-(+)-APCI-MS/MS determination of oxygenated and nitrated polycyclic aromatic hydrocarbons in airborne particulate matter and tree barks collected in Buenos Aires City, *Microchem. J.* 116 (2014) 118–124.
- [15] M. Fontal, B.L. Van Drooge, J.O. Grimalt, A rapid method for the analysis of methyl dihydroxymalonate and galaxolide in indoor and outdoor air particulate matter, *J. Chromatogr. A* 1447 (2016) 135–140.
- [16] Y. Zhao, N.M. Kreisberg, D.R. Worton, G. Isaacman, D.R. Gentner, A.W.H. Chan, R.J. Weber, S. Liu, D.A. Day, L.M. Russell, S.V. Hering, A.H. Goldstein, Sources of organic aerosol investigated using organic compounds as tracers measured during CalNex in Bakersfield, *J. Geophys. Res. Atmos.* 118 (2013) 11,388–11,398.
- [17] N. Mastroianni, C. Postigo, M. López de Alda, M. Viana, A. Rodríguez, A. Alastuey, X. Querol, D. Barceló, Comprehensive monitoring of the occurrence of 22 drugs of abuse and transformation products in airborne particulate matter in the city of Barcelona, *Sci. Total Environ.* 532 (2015) 344–352.
- [18] M.I. Besser, J. Beltrán, V. Yusà, Design of experiment approach for the optimization of polybrominated diphenyl ethers determination in fine airborne particulate matter by microwave-assisted extraction and gas chromatography coupled to tandem mass spectrometry, *J. Chromatogr. A* 1323 (2014) 1–10.
- [19] S. Samy, M.D. Hays, Quantitative LC-MS for water-soluble heterocyclic amines in fine aerosols (PM 2.5) at Duke Forest, USA, *Atmos. Environ.* 72 (2013) 77–80.
- [20] Y. Picó, Ultrasound-assisted extraction for food and environmental samples, *Trends Anal. Chem.* 43 (2013) 84–99.
- [21] F. Chemat, N. Rombaut, A.-G. Sicaire, A. Meullemiestre, A.-S. Fabiano-Tixier, M. Abert-Vian, Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review, *Ultrason. Sonochem.* 34 (2017) 540–560.
- [22] M.C. Guamán-Balcázar, W. Setyaningsih, M. Palma, C.G. Barroso, Ultrasound-assisted extraction of resveratrol from functional foods: cookies and jams, *Appl. Acoust.* 103 (Part B) (2016) 207–213.
- [23] N.B. Cech, C.G. Enke, Practical implications of some recent studies in electrospray ionization fundamentals, *Mass Spectrom. Rev.* 20 (2001) 362–387.
- [24] F. Gosetti, E. Mazzucco, D. Zampieri, M.C. Gennaro, Signal suppression/enhancement in high-performance liquid chromatography-tandem mass spectrometry, *J. Chromatogr. A* 1217 (2010) 3929–3937.
- [25] E. Barceló-Barrachina, E. Moyano, L. Puignou, M. Galceran, Evaluation of reversed-phase columns for the analysis of heterocyclic aromatic amines by liquid chromatography-electrospray mass spectrometry, *J. Chromatogr. B* 802 (2004) 45–59.
- [26] L.A. Currie, Detection and quantification limits: origins and historical overview, *Anal. Chim. Acta* 391 (1999) 127–134.
- [27] J. Uhrovčík, Strategy for determination of LOD and LOQ values—some basic aspects, *Talanta* 119 (2014) 178–180.
- [28] X. Dong, D. Liu, S. Gao, Determination of heterocyclic amines in atmospheric particles by reversed phase high performance liquid chromatography, *Chin. J. Anal. Chem.* 37 (2009) 1415–1420.
- [29] X. Dong, D. Liu, S. Gao, Seasonal variations of atmospheric heterocyclic aromatic amines in Beijing, China, *Atmos. Res.* 120 (2013) 287–297.