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Comparison of different commercial solid-phase extraction cartridges used to extract heterocyclic amines from a lyophilised meat extract

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Abstract

Heterocyclic amines are a group of potent mutagenic compounds which are generated when muscle meat is cooked. Since they are possible human carcinogens, these mutagens have received considerable attention in recent years, and several analytical techniques have been developed for their quantification. Although the purification step is one of the most important, there are a great number of variables influencing the recovery of the amines, especially when real samples are analysed. In this work we studied the influence of sample spiking mode on the recoveries. Furthermore, on the basis of a previously developed clean-up method, the effect of changing commercial source and structure of the sorbents used in two solid-phase extraction steps was examined. This purification method was applied to the quantification of the heterocyclic aromatic amines present in a lyophilised meat extract by means of liquid chromatography–mass spectrometry.

Keywords: Solid-phase extraction; Food analysis; Meat; Amines, heterocyclic aromatic

1. Introduction

Nowadays it is widely accepted that diet is one of the most important factors in the origination of cancer in the human population [1]. In 1977, Nagao et al. found important mutagenic activity in smoke condensate and charred parts of broiled fish and beef [2]. Since then, more than 20 highly mutagenic compounds, grouped under the denomination of heterocyclic amines (HAs), have been isolated mainly from various protein-rich heated foods, such as cooked meat and fish [3,4] or flavors [5]. Furthermore, some of these mutagens have been found in other matrices, including cigarette smoke condensate [6], wine [7] and environmental samples [8].

Besides being among the most potent mutagenic/genotoxic substances ever tested by both in vitro and in vivo studies [9,10], the HAs examined have been shown to be carcinogenic in long-term animal experiments developed with rodents and primates [11,12]. Moreover, several epidemiological studies suggest that consumption of meat and cooking procedures are related to an increased risk of some types of human cancer [13,14].

On the basis of this evidence, it seems possible that HAs are potential human carcinogens. To determine the importance of the consumption of these compounds for human health, it is essential to quantify the amounts present in foods by means of reliable and quantitative methods, which is not easy due to the low level of concentration of the heterocyclic amines (0.1–50 ng/g) and the high complexity of the matrix. Various purification meth-

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ods based on liquid–liquid extraction [15], extraction with blue cotton [16], solid-phase extraction (SPE) with disposable columns [17,18] or immunoaffinity purification [19] can be found in the literature, mainly followed by different separation and determination techniques such as gas chromatography–mass spectrometry (GC–MS) [20,21], gas chromatography with nitrogen–phosphorus selective detection (GC–NPD) [22], liquid chromatography–mass spectrometry (LC–MS) [23,24], liquid chromatography tandem mass spectrometry (LC–MS–MS) [25], liquid chromatography with electrochemical (LC–ED) [26,27], ultraviolet (LC–UV) [28,29] and/or fluorescence [30,31] detection system, capillary electrophoresis–mass spectrometry (CE–MS) [32], capillary electrophoresis with UV detection (CE–UV) [33,34] or micellar electrokinetic chromatography with electrochemical detection (MEKC–ED) [35].

Since the degree of selectivity in the detection depends on the clean-up efficiency of the sample preparation procedure, the use of detectors with poor specificity, such as UV, requires exhaustive purification process [36,37], but frequently a decrease of analyte recovery and thus of accuracy of the results occurs. Therefore, more selective detection systems such as MS are suitable when higher recoveries but less exhaustive clean-up are obtained. In this work, we applied a simplified purification procedure described previously [38,39] to the analysis of the HAs present in a lyophilised meat extract using MS detection. Some factors affecting the extraction efficiency, including the spiking mode and the change of sorbent trademark and structure in the PRS and C_{18} steps, were studied in order to achieve maximum recoveries and optimal clean-up efficiency, which would provide suitable limits of detection in the MS analysis.

2. Experimental

2.1. Chemicals

The solvents and chemicals used were HPLC or analytical grade, and the water was purified in an Elix-Milli Q system (Millipore, Bedford, MA, USA). All the solutions were passed through a 0.45-μm filter before injection into the HPLC system.

The compounds studied were 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (7,8-DiMeIQx), 2-amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline (TriMeIQx), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1), 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-2), 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), purchased from Toronto Research Chemicals (Toronto, Canada), and 1-methyl-9*H*-pyrido[3,4-*b*]indole (harman) and 9*H*-pyrido[3,4-*b*]indole (norharman), which were from Sigma (Steinheim, Germany). Stock standard solutions of 100 μg ml^{-1} in methanol were prepared and used for further dilution. TriMeIQx was used as internal standard (2 μg ml^{-1} methanolic solution).

Diatomaceous earth extraction cartridges (Extrelut-20) and refill material were provided by Merck (Darmstadt, Germany); PRS sodium form (500 mg) and endcapped C_{18} (100 mg) Bond Elut cartridges were from Varian (Harbor City, USA), PRS hydrogen form (200 and 500 mg) and endcapped, non-endcapped and monofunctional C_{18} (100 mg) Isolute cartridges were from IST (Hengoed, Mid-Glamorgan, UK). Coupling pieces and stopcocks were from Varian. PRS cartridges were preconditioned depending on the case (Table 1), and C_{18} cartridges were preconditioned with methanol (5 ml) and water (5 ml). A lyophilised meat extract was prepared from a commercial meat extract (Bovril) which was previously spiked with IQ, MeIQx, 4,8-DiMeIQx and PhIP as described in Refs. [38,40].

Caffeine, MRFA (L–Met–Arg–Phe–Ala acetate·H_2O) and Ultramark 1621 (PEG mixture) were purchased from Sigma.

2.2. Instruments

LC analyses were performed by means of a Waters 2690 separations module (Milford, MA, USA), and a

Table 1
Scheme of the methods and characteristics of the cartridges

Method	PRS cartridge			C_{18} cartridge		
	Trademark	Characteristics	Preconditioning	Trademark	Characteristics	Preconditioning
1	Varian	Sodium form, non-endcapped, 500 mg, 40-μm particles, 60 Å porosity, 0.18 mequiv./g ion-exchange capacity	5 ml 0.1 *M* HCl 10 ml water 5 ml MeOH dried 7 ml DCM	Varian	Trifunctional, endcapped, 100 mg, 40-μm particles, 60 Å porosity, 2.1 μmol/m^2 surface coverage	5 ml MeOH 5 ml water
2	IST	Hydrogen form, non-endcapped, 200 mg, 50-μm particles, 60 Å porosity, 0.34 mequiv./g ion-exchange capacity	7 ml DCM	Varian	Trifunctional, endcapped, 100 mg, 40-μm particles, 60 Å porosity, 2.1 μmol/m^2 surface coverage	5 ml MeOH 5 ml water
3	IST	Hydrogen form, non-endcapped, 500 mg, 50-μm particles, 60 Å porosity, 0.34 mequiv./g ion-exchange capacity	7 ml DCM	Varian	Trifunctional, endcapped, 100 mg, 40-μm particles, 60 Å porosity, 2.1 μmol/m^2 surface coverage	5 ml MeOH 5 ml water
4	IST	Hydrogen form, non endcapped, 500 mg, 50-μm particles, 60 Å porosity, 0.34 mequiv./g ion-exchange capacity	5 ml 0.1 *M* HCl 10 ml water 5 ml MeOH dried 7 ml DCM	Varian	Trifunctional, endcapped, 100 mg, 40-μm particles, 60 Å porosity, 2.1 μmol/m^2 surface coverage	5 ml MeOH 5 ml water
5	Varian	Sodium form, non endcapped, 500 mg, 40-μm particles, 60 Å porosity, 0.18 mequiv./g ion-exchange capacity	5 ml 0.1 *M* HCl 10 ml water 5 ml MeOH dried 7 ml DCM	IST	Monofunctional, non-endcapped, 100 mg, 50-μm particles, 125 Å porosity, 1.9 μmol/m^2 surface coverage	5 ml MeOH 5 ml water
6	Varian	Sodium form, non endcapped, 500 mg, 40-μm particles, 60 Å porosity, 0.18 mequiv./g ion-exchange capacity	5 ml 0.1 *M* HCl 10 ml water 5 ml MeOH dried 7 ml DCM	IST	Trifunctional, non-endcapped, 100 mg, 50-μm particles, 60 Å porosity, 1.5 μmol/m^2 surface coverage	5 ml MeOH 5 ml water
7	Varian	Sodium form, non-endcapped, 500 mg, 40-μm particles, 60 Å porosity, 0.18 mequiv./g ion-exchange capacity	5 ml 0.1 *M* HCl 10 ml water 5 ml MeOH dried 7 ml DCM	IST	Trifunctional, endcapped, 100 mg, 50-μm particles, 60 Å porosity, 1.5 μmol/m^2 surface coverage	5 ml MeOH 5 ml water

Waters 2487 dual wavelength absorbance detector was used to record on-line UV chromatograms at 263 nm for qualitative purposes.

Determination and identification of the peaks in the sample were carried out with an LCQ (Finnigan MAT, San Jose, CA, USA) provided with an atmospheric pressure chemical ionisation interface and an ion-trap mass analyser. Optimal source working conditions to record positive ions were as follows: discharge voltage was 5 kV; heated capillary temperature was 150°C, and that of the vaporiser 450°C; nitrogen was used as drying gas at 72 l h^{-1} and as nebulizing gas at 360 l h^{-1}. For data acquisition in full scan mode, the mass spectrometer operated over a range of m/z 150.0–250.0 in centroid mode with a maximum injection time of 1000 ms, 1 microscan each cycle, automatic gain control in on mode and inject waveform off.

Efficiency of ion transference from source to the ion trap was automatically optimised by infusing methanolic solutions of IQ, 4,8-DiMeIQx and Trp-P-1. To prevent MS contamination when running LC–MS, a divert valve was used. MS calibration was carried out with the infusion of a mixture containing caffeine, MRFA and Ultramark 1621 into the APCI source.

A rotating shaker Rotary Mixer 34526 (Breda Scientific, Breda, Netherlands) was used for the sample homogenisation in the alkaline solution, and a Supelco Visiprep and a Visidry SPE vacuum manifold (Supelco, Gland, Switzerland) were used for manipulations with SPE cartridges and solvent evaporation, respectively.

2.3. Sample preparation

A previously described purification method [38] was used to extract analytes from a lyophilised meat extract. Briefly, 1 g beef extract sample was homogenised in 12 ml 1 *M* NaOH with sonication, and the suspension was then shaken for 3 h. The alkaline solution was mixed with Extrelut refill material (14 g) and used to fill an empty Extrelut column. According to the selected SPE method (Table 1), a PRS column was preconditioned, and then coupled on-line to the Extrelut column. To extract the analytes from diatomaceous earth, 75 ml dichloromethane were passed through the tandem; the PRS cartridge was then dried and successively rinsed with 15 ml methanol–water (4:6, v/v) and 2 ml water. The cationic exchanger column was then coupled to the corresponding C_{18} column (see Table 1), and this tandem was eluted with 20 ml of 0.5 *M* ammonium acetate, pH 8.0. The sorbed HAs were finally eluted from C_{18}, after rinsing with 5 ml water, using 0.8 ml of methanol–ammonia (9:1, v/v). The solvent was gently evaporated under a stream of nitrogen and the analytes were redissolved in 50 μl of the internal standard in methanol. The final extract was analysed using the LC–MS method described in Section 2.4.

Quantification and recovery calculation of the amines in the beef extract was carried out by standard addition method. The meat extract was spiked with all the analysed compounds at three different levels (80, 160 and 320 ng g^{-1}) using the corresponding amount of a methanolic solution of the analytes, which was directly added to the sample. The solvent was allowed to evaporate for 1 h before processing the sample extraction step.

2.4. Chromatographic conditions

The amines were separated by means of reversed-phase LC using a TSK-Gel ODS 80T column (25.0× 4.6 mm I.D., 5 μm) (TosoHaas, Stuttgart, Germany) equipped with a Supelguard LC-8-DB precolumn (Supelco, Bellefonte, PA, USA).

Optimal separation was achieved with a ternary mobile phase at a flow-rate of 1 ml min^{-1}. Solvent A: 30 m*M* formic acid in water adjusted with ammonia solution to pH 3.25; solvent B: 30 m*M* formic acid in water adjusted with ammonia solution to pH 3.7; solvent C: acetonitrile. The gradient program was: 5–23% C in A, 0–18 min; 23% C in A, 18–21 min; 23% C in B, 21–25 min; 23–60% C in B, 25–33 min; 60% C in B, 33–40 min; return to the initial conditions, 40–50 min; 5 min post-run delay. In all cases the amount injected was 15 μl.

3. Results and discussion

3.1. Sample spiking

Extraction procedures are evaluated on the recovery achieved. Before the study on the different SPE cartridges, two important parameters influencing the recovery of the analytes were studied to ensure the accuracy of the results. The first was the time necessary to achieve a complete release of HAs from matrix by means of the initial alkaline treatment, and the second was the optimal spiking time, i.e. the time of contact between spiked analytes and sample, which is necessary to reproduce binding effects that occur in the case of native analytes. In each experiment, recoveries of all the analytes were calculated as described in Section 2, using Method 1 as extraction procedure (see Table 1).

To check the influence of the shaking time in the initial alkaline treatment, once the sample was homogenised in the sodium hydroxide solution, several periods (1, 3, 6 and 12 h) were studied using a spiking time of 24 h in all cases. Slightly lower recoveries were obtained for 1 h, and no significant differences were found between 3 and 12 h. Therefore, 3 h was fixed as the optimal shaking time. In the case of the contact time study, the periods tested were 1, 2, 6 and 24 h. In all cases, the shaking time for the dissolution step with 1 *M* NaOH was fixed at 3 h. Since no significant differences were observed between the recovery of each HA obtained at

different spiking times, 1 h was established as sufficient.

This study showed that both native and spiked analytes did not present differences in the extraction efficiency, which was quantitative with the treatment applied. This made standard addition a suitable method to determine extraction percentages without overestimation of the recoveries.

3.2. Efficiency of the clean-up and recoveries

The tandem extraction method chosen as reference, named as Method 1 in Table 1, is based on the use of Bond Elut PRS, preconditioned with diluted acid, and endcapped tridimensional Bond Elut C_{18}, both purchased from Varian [38]. The influence of the counter-ion in the ion-exchange sorbent was studied using the Bond Elut PRS in its original form, which presents sodium as a counter-ion [41], and alternatively the same sorbent was treated with an acidic preconditioning, thus hydrogen ions are introduced as counter-ions (Method 1). The effect derived from the use of different commercial sorbents was tested by changing either PRS or C_{18} cartridges. In the case of the cationic exchanger, in addition to the Bond Elut PRS cartridge provided by Varian, two different Isolute PRS sorbents supplied by IST were tested maintaining the same Bond Elut C_{18} sorbent in the tandem extraction. These Isolute PRS cartridges originally had a protonated form, i.e. with hydrogen ion as counter-ion. When Isolute PRS 200 mg was used, the preconditioning step consisted only in washing with dichloromethane (Method 2). The influence of the acidic preconditioning was checked with Isolute PRS 500 mg, which was treated only with dichloromethane (Method 3), and also with 0.1 *M* HCL (Method 4), in order to evaluate the effect of the acidic preconditioning on the Isolute PRS features. A more detailed description of the preconditioning step is presented in Table 1.

On the other hand, three different Isolute C_{18} cartridges provided by IST were compared to the Bond Elut C_{18} proposed as reference. The first is monofunctional non-endcapped Isolute C_{18} (Method 5), the second is non-endcapped tridimensional Isolute C_{18} (Method 6) and the third is endcapped tridimensional Isolute C_{18} (Method 7). In all cases, the C_{18} cartridges were preconditioned with 5 ml MeOH followed by 5 ml water.

3.2.1. PRS cartridges

Results obtained in the study of PRS cartridges are given in Table 2, where the recoveries of the different analytes obtained with Methods 1–4 are detailed. For quinolines (IQ, MeIQ), α-carbolines (AαC, MeAαC) and δ-carbolines (Glu-P-1, Glu-P-2) slightly higher recoveries (58–94%) were obtained when using Isolute PRS 500 mg without acidic preconditioning (Method 3). However, this method gave low recoveries for Harman and the γ-carbolines

Table 2
Recovery values obtained with methods which differ in the PRS cartridge step and which use C_{18} from Varian

Compound	Method 1		Method 2		Method 3		Method 4	
	Recovery (%)	SD	Recovery (%)	SD	Recovery (%0	SD	Recovery (%)	SD
Glu-P-2	57.6	1.9	61.3	1.6	67.4	1.2	62.5	1.6
IQ	69.5	3.2	77.4	4.1	90.1	3.2	84.7	2.9
MeIQ	73.2	4.4	83.5	5.4	93.4	7.2	84.0	3.1
Glu-P-1	57.2	1.1	53.4	1.5	57.8	1.2	58.0	1.3
MeIQx	70.3	3.9	74.7	2.6	77.7	3.3	79.9	1.1
7,8-DiMeIQx	75.2	1.8	79.8	2.9	78.2	2.0	79.5	1.6
4,8-DiMeIQx	52.3	1.9	56.7	1.9	60.2	2.9	61.3	1.1
Norharman	54.1	3.3	59.4	1.8	61.9	4.4	55.1	2.3
Harman	49.6	5.2	49.2	2.1	34.9	8.3	23.5	5.0
Trp-P-2	49.8	1.0	54.6	3.3	15.6	1.9	10.1	2.5
PhIP	74.3	2.7	80.1	3.1	79.0	3.9	85.5	2.2
Trp-P-1	45.6	2.0	61.6	1.5	9.9	4.8	2.4	0.8
AαC	49.4	1.5	49.8	0.5	57.3	1.4	57.7	1.2
MeAαC	51.1	1.4	54.7	1.1	61.1	1.7	59.0	1.3

(Trp-P-1,Trp-P-2), ranging from 9 to 33%. The same effect, but more pronounced, was observed for these amines when the cationic exchanger was activated with diluted acid using Method 4 (3–24%). In general, good recoveries for all the compounds were obtained with the IST 200 mg cartridge (Method 2), achieving values above 50%.

Since PRS adsorbent is a strong cation exchanger, when normal-phase elution mode is used, HAs are retained through non-specific adsorption on the polar propylsulphonic acid (or sodium salt) residues as well as on the residual free silanol groups. The comparison of the results obtained with Bond Elut PRS [41] showed a different behaviour between sodium PRS and protonated PRS, achieving lower recoveries when the sodium form of the sorbent was used. This finding emphasizes the importance of the ion-exchange activation by introducing acidic groups in the propylsulphonate sorbent, which helps to the protonation of the amines and thus to the establishment of ionic interactions. On the other hand, the low recoveries obtained in the present study with protonated PRS (Method 3 and 4) could be attributed to a very strong retention of Trp-P-1, Trp-P-2 and H in the sorbent. The more efficient interaction between these HAs and the propylsulphonic group, which makes their reversed-phase elution difficult, could be explained by their higher basicity [42]. This hypothesis is supported by the fact that these analytes were recovered at higher levels (>45%) when using Bond Elut PRS (Method 1), which has fewer functionalized points per area (Table 1), and, in the case of Method 2, because the mass of the sorbent is 200 instead of 500 mg.

When the UV chromatograms were observed, only minor differences in the degree of sample purification were found. For example, when the PRS cartridges were used with preconditioning, an interference for AαC occurred. Therefore, the most suitable PRS cartridge for the analysis of this beef extract would be Isolute PRS 200 mg (Method 2), since all the analytes were recovered at high percentages without requiring an acidic activation, which simplified the procedure and avoided the AαC interference with UV detection.

3.2.2. C_{18} cartridges

In the study of C_{18} sorbents, higher recoveries and acceptable standard deviations were obtained using the IST cartridges based on monofunctional Isolute C_{18} (Method 5). As shown in Table 3, several HAs, specially quinolines (IQ, MeIQ), had recoveries more than 15% higher than those obtained with the reference method. Moreover, different selectivity was observed when changing the structure of the sorbent, as shown in Fig. 1, where the UV chromatograms obtained from a spiked meat extract using monofunctional Isolute C_{18} (Method 5) and tridimensional Isolute C_{18} (Method 6) are reproduced.

Table 3
Recovery values obtained with methods which use different C_{18} cartridges combined with PRS from Varian

Compound	Method 1		Method 2		Method 3		Method 4	
	Recovery (%)	SD	Recovery (%)	SD	Recovery (%)	SD	Recovery (%)	SD
Glu-P-2	57.6	1.9	70.4	1.3	65.8	0.5	62.8	2.1
IQ	69.5	3.2	93.4	3.2	84.7	3.0	84.0	2.0
MeIQ	73.2	4.4	94.3	3.7	81.9	4.9	83.3	2.0
Glu-P-1	57.2	1.1	57.5	4.0	57.3	0.8	57.6	1.6
MeIQx	70.3	3.9	84.0	2.8	77.1	1.7	79.5	2.1
7,8-DiMeIQx	75.2	1.8	84.6	1.9	76.3	1.6	81.1	1.9
4,8-DiMeIQx	52.3	1.9	63.3	1.8	56.3	0.9	59.6	0.8
Norharman	54.1	3.3	67.7	4.3	60.6	1.6	55.6	2.2
Harman	49.6	5.2	60.0	8.6	50.4	4.1	48.7	3.5
Trp-P-2	49.8	1.0	59.7	1.7	50.2	2.3	54.2	1.5
PhIP	74.3	2.7	88.3	3.3	77.5	2.4	86.5	2.0
Trp-P-1	45.6	2.0	49.0	3.1	34.7	3.7	42.7	2.2
AαC	49.4	1.5	61.6	2.1	52.9	0.7	53.4	1.3
MeAαC	51.1	1.4	59.8	1.6	52.5	1.6	55.6	1.4

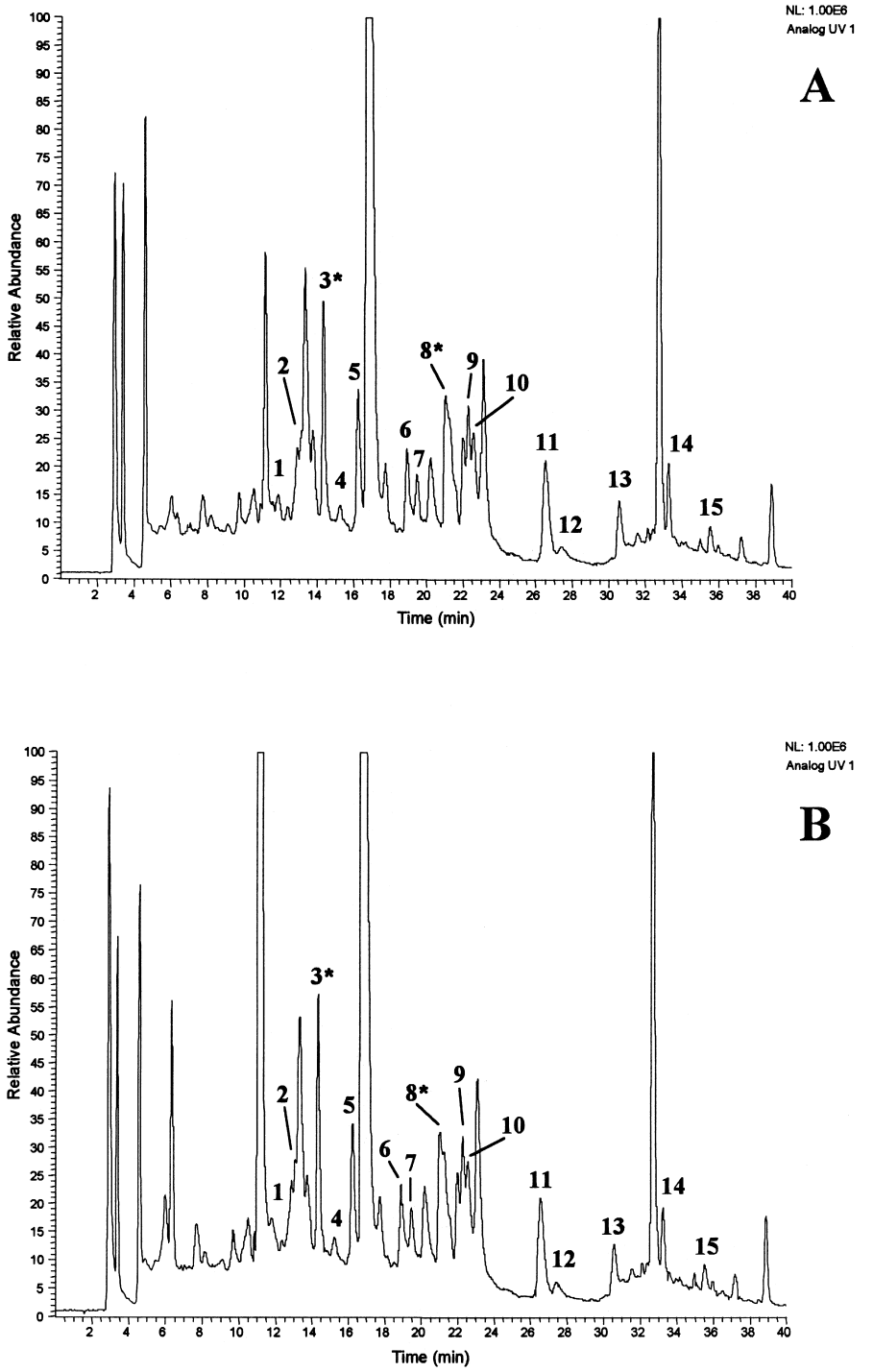

Fig. 1. Comparison of the UV chromatograms, acquired at λ=263 nm, obtained with Methods 5 (A) and 6 (B). The sample was spiked with 80 ng g^{-1}. Peaks: 1=Glu-P-2; 2=IQ; 3=MeIQ; 4=Glu-P-1; 5=MeIQx; 6=7,8-DiMeIQx; 7=4,8-DiMeIQx; 8=Norharman; 9=TriMeIQx (I.S.); 10=Harman; 11=Trp-P-2; 12=PhIP; 13=Trp-P-1; 14=AαC; 15=MeAαC. *=non-pure peak. Chromatographic conditions as in Section 2.

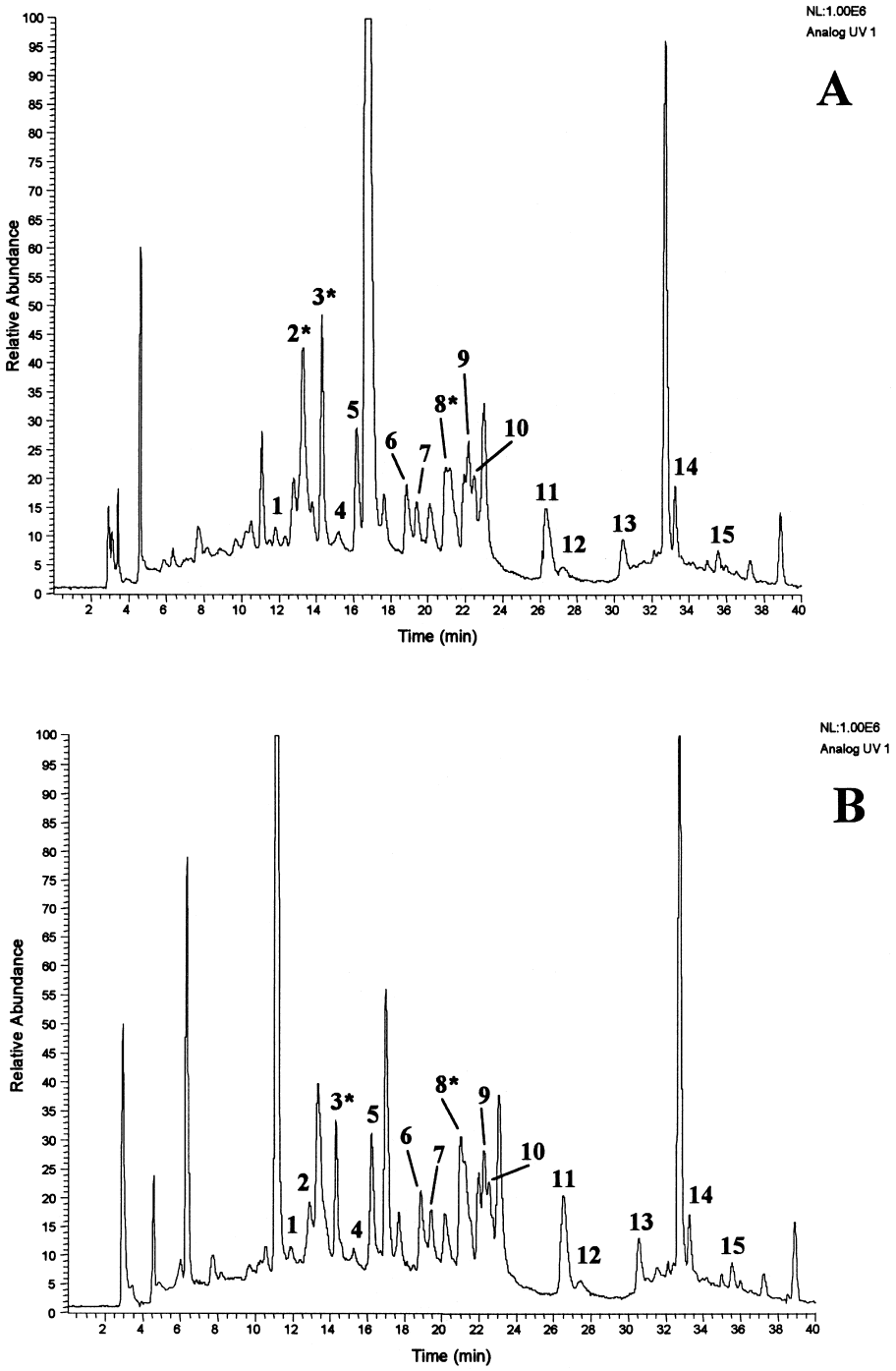

Fig. 2. Comparison of the UV chromatograms, acquired at λ=263 nm, obtained with Methods 1 (A) and 7 (B). The sample was spiked with 80 ng g^{-1}. Peaks: 1=Glu-P-2; 2=IQ; 3=MeIQ; 4=Glu-P-1; 5=MeIQx; 6=7,8-DiMeIQx; 7=4,8-DiMeIQx; 8=Norharman; 9=TriMeIQx (I.S.); 10=Harman; 11=Trp-P-2; 12=PhIP; 13=Trp-P-1; 14= AαC; 15=MeAαC. *=non-pure peak. Chromatographic conditions as in Section 2.

As a main difference, a major peak which elutes near Glu-P-2 is avoided when using monofunctional C_{18}.

Another outstanding effect is that derived from the change in trademark. Although equivalent cartridges of different commercial origin provide similar recoveries (Methods 1 and 7), interesting differences were found in the UV chromatograms of the spiked meat extract (Fig. 2): while Bond Elut tridimensional C_{18} (Method 1) gave an extract with a high peak in the tail of MeIQx, in the case of Isolute tridimensional C_{18} (Method 7) this interference was smaller, but two new major peaks appeared at 6.3 and 11.0 min. In addition to the difficulty of integrating the analytes peaks in UV chromatograms, the presence of major peaks coeluting very near the compounds of interest has marked effects on the limit of detection obtained in the determination with MS, as discussed below.

3.3. Limits of detection in MS

As observed previously [39], the coelution of compounds coextracted from the matrix greatly influenced the limits of detection (LODs) when real samples were analysed using MS detection, probably due to ionisation interferences or problems of ion trap saturation, which produced a decrease in the signal-to-noise ratio. This effect was also confirmed in this work when LODs for the various tandem extraction procedures were established. Detection limits based on a signal-to-noise ratio of 3:1 were calculated by spiking the beef extract at low concentrations levels (0.5–10 ng g^{-1}) with reference standards. Detection limits of the HAs already present in the sample were estimated from calibration curves taking into account the corresponding recovery values. Values obtained are shown in Table 4, where it can be seen that, in general, higher values were found for the HAs eluting in the first zone of the chromatogram, which is the least exhaustively purified. Moreover, in some cases significant differences were observed between methods. As an example, Fig. 3 shows that the trace at m/z 214, corresponding to MeIQx, obtained from the injection of the non-spiked extract purified using an Isolute tridimensional C_{18} cartridge (Method 7), has less background noise than that obtained when the Bond Elut tridimensional C_{18} cartridge was used (Method 1), producing a decrease in the LOD for MeIQx with Method 7. This can be explained by reference to the UV chromatograms in Fig. 2, where the peak in the tail of MeIQx is much higher using Method 1 than with Method 7. In addition, LODs for MeIQ obtained with Methods 3 and 4 were high due to the partial coelution of this analyte with an interference with the same m/z ratio. Since signals are not completely resolved, the integration was hindered at low levels of analyte concentration. From Table 4 it can also be deduced that high values can be related

Table 4
Limits of detection in the sample, obtained with MS detection

Analyte	Limits of detection (ng g^{-1})						
	Method 1	Method 2	Method 3	Method 4	Method 5	Method 6	Method 7
Glu-P-2	5.4	4.0	3.7	3.9	4.1	5.0	4.8
IQ	4.1	4.2	3.9	4.2	4.2	3.9	4.3
MeIQ	7.7	7.8	9.6	10.2	7.2	6.5	6.7
Glu-P-1	9.1	5.9	4.7	6.4	4.7	4.8	7.7
MeIQx	5.2	4.6	5.1	4.0	4.0	4.4	2.9
7,8-DiMeIQx	4.5	3.5	3.1	3.1	3.7	3.7	3.7
4,8-DiMeIQx	3.2	4.3	3.8	3.3	3.6	4.4	3.8
Norharman	3.5	3.6	3.1	3.1	2.8	3.3	3.4
Harman	3.7	3.8	4.2	5.2	3.0	3.2	3.2
Trp-P-2	2.6	2.3	11.7	10.0	2.3	2.4	2.0
PhIP	1.9	2.2	2.4	1.7	2.1	1.97	2.2
Trp-P-1	4.3	2.4	N.D.	>15.0	2.8	4.0	2.7
AαC	0.5	1.1	0.4	1.0	0.8	0.7	1.2
MeAαC	0.8	0.8	0.7	1.3	0.7	0.7	0.7

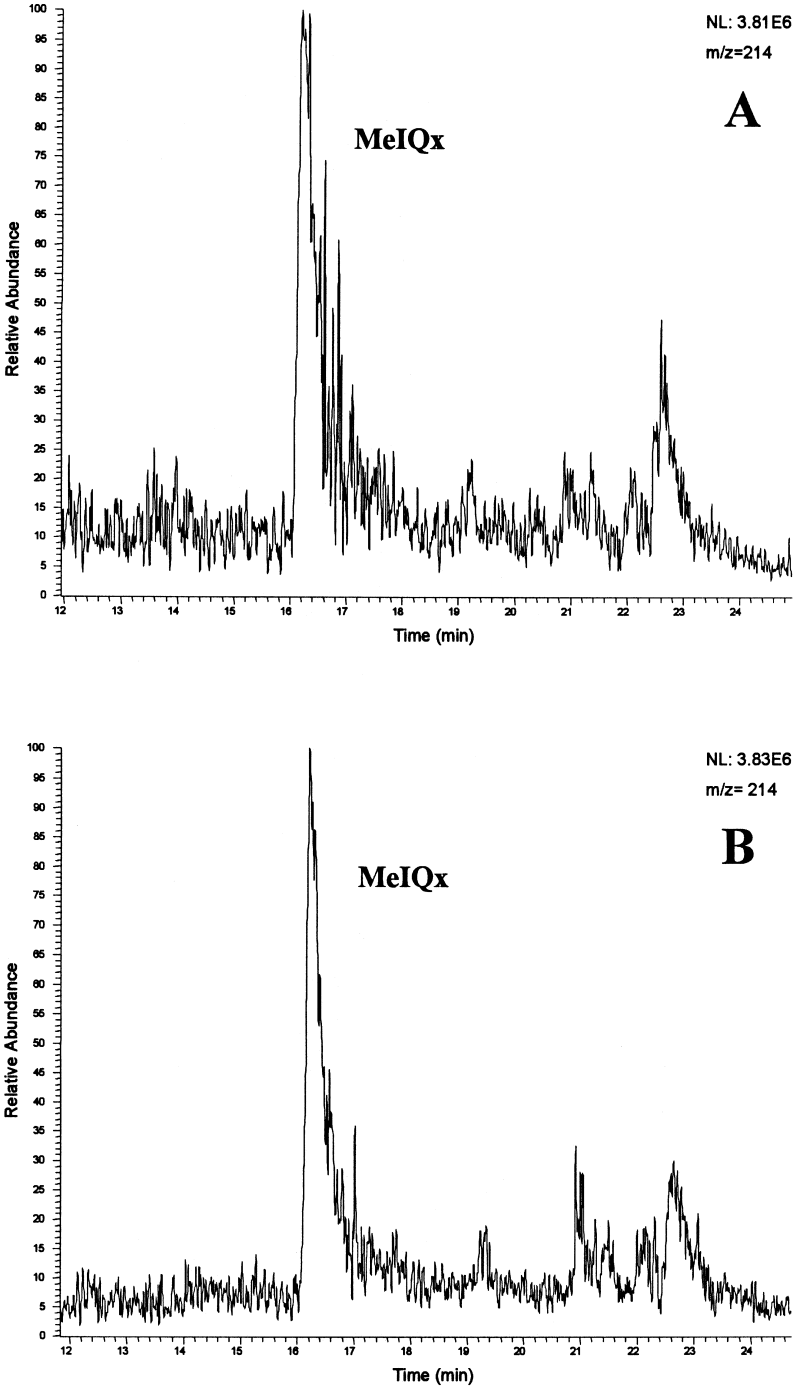

Fig. 3. Traces corresponding to m/z 214 (MeIQx) obtained with Methods 1 (A) and 7 (B).

Table 5
Quantification results in the analysis of the analytes present in the sample[a]

Analyte	Method 1		Method 2		Method 3		Method 4		Method 5		Method 6		Method 7	
	ng g^{-1}	SD	ng g^{-1}	SD	ng g^{-1}	SD	ng g^{-1}	SD	ng g^{-1}	SD	ng g^{-1}	SD	ng g^{-1}	SD
Glu-P-2	N.D.	–	N.D.	–	N.D.	–	N.D.	–	n.d.	–	N.D.	–	N.D.	–
IQ	32.5	7.3	37.4	2.7	31.2	4.2	37.4	7.5	37.5	3.2	35.1	2.8	34.4	4.9
MeIQ	N.D.	–	N.D.	–	N.D.	–	N.D.	–	N.D.	–	N.D.	–	N.D.	–
Glu-P-1	N.D.	–	N.D.	–	N.D.	–	N.D.	–	N.D.	–	N.D.	–	N.D.	–
MeIQx	41.4	2.6	45.9	3.9	46.1	2.1	39.9	1.3	40.2	1.4	39.6	2.7	40.1	1.5
7,8-DiMeIQx	N.D.	–	N.D.	–	N.D.	–	N.D.	–	N.D.	–	N.D.	–	N.D.	–
4,8-DiMeIQx	9.7	1.7	12.9	1.6	11.5	0.9	9.9	1.0	10.9	0.4	13.3	1.1	11.5	2.9
Norhaman	145.5	9.9	147.5	5.6	137.2	23.3	118.7	6.3	127.9	9.0	135.1	3.7	158.6	11.8
Harman	264.0	40.3	360.3	15.7	303.3	72.7	327.3	72.8	303.9	43.9	287.5	23.4	302.2	22.7
Trp-P-2	N.D.	–	N.D.	–	N.D.	–	N.D.	–	N.D.	–	N.D.	–	N.D.	–
PhIP	27.1	1.0	31.3	2.0	28.5	1.6	24.4	1.3	25.1	1.2	25.7	0.8	26.6	1.1
Trp-P-1	N.D.	–	N.D.	–	N.D.	–	N.D.	–	N.D.	–	N.D.	–	N.D.	–
AαC	N.Q.	–	N.D.	–	N.Q.	–	N.Q.	–	N.D.	–	N.Q.	–	N.Q.	–
MeAαC	N.Q.	–	N.D.	–	N.Q.	–	N.Q.	–	N.D.	–	N.Q.	–	N.Q.	–

[a] N.Q., below its limit of quantification; N.D., not detected.

to low recoveries, as in the case of Trp-P-1 and Trp-P-2 with Methods 3 and 4.

3.4. Analytical results

The analytes present in the sample were quantified with the LC–MS system described in Section 2, since unavoidable interferences produced when using UV detection prevented the quantification of some of the compounds, as was the case of IQ, MeIQ, Norharman or TriMeIQx. The results of the analysis are given in Table 5, which reveals that despite differences in the tandem extraction efficiency, similar values were obtained for the different methods tested. In addition to the four amines used to contaminate the original meat extract (IQ, MeIQx, 4,8-DiMeIQx and PhIP), the two comutagenic amines harman and norharman were detected and quantified. The rest of analytes were not detected except the α-carbolines, which were below their LOQs.

4. Conclusions

In this study, the effect of changes of commercial brand and structure of sorbents were studied on the basis of a previously developed clean-up procedure, but the conclusions can be generalised to the rest of procedures based on SPE proposed in the literature. For the PRS step, the most suitable cartridge was Isolute PRS 200 mg, due to the elimination of the acidic activation, which simplified the procedure, and because the recovery of all the analytes was higher than 50% without significant differences in the cleanness obtained, compared with the rest of cartridges. In the case of C_{18} adsorbents, higher recoveries were obtained when monofunctional Isolute C_{18} was used, but a better limit of detection was achieved for MeIQx, which is one of the most important analytes, with tridimensional endcapped Isolute C_{18}.

Differences between trademarks and structures suggest the necessity of a screening study previous to the analysis of a new sample, in order to select the most suitable sorbent for that case. Furthermore, successful application of a tandem extraction procedure greatly depends on the sample matrix composition. There is always a tandem extraction suitable for the analysis of HAs in meat extracts, but another purification procedure may be required for other samples.

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