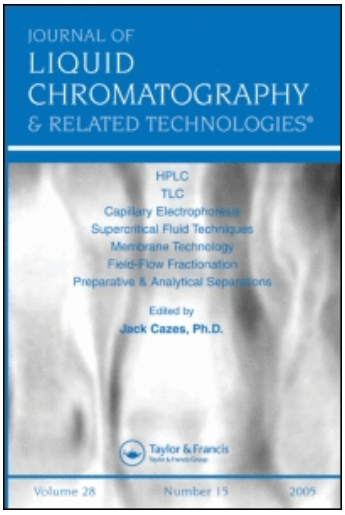

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Focused Microwave-Assisted Extraction and HPLC with Electrochemical Detection to Determine Heterocyclic Amines in Meat Extracts

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Abstract: A method for the determination of 11 heterocyclic amines (HAs) in meat extracts by high performance liquid chromatography with electrochemical detection is proposed. The chromatographic method is optimized to obtain both polar and less polar amines in the same chromatographic run, which is a difficult task when using electrochemical detection. The extraction of the meat extracts is carried out using a focused microwave system, a technique applied for first-time in the extraction of these analytes. The microwave extraction process takes only 5 minutes, an improvement on the extraction times for methods commonly applied to heterocyclic amines in food samples. This screening method allows us to determine HAs in meat extracts with average recoveries of 81.2%, 76.4%, 66.7%, 60.3%, and 63.8%, for granulated meat extract, non-granulated meat extract, chicken extract, and soup cubes (brands A and B), respectively. The proposed method has an intermediate precision ranging from 6.1 to 12.8%, and detection limits between 0.16 and 2.68 ng.

Keywords: Heterocyclic amines, High performance liquid chromatography, Electrochemical detection, Focused microwave-assisted extraction, Meat extracts

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INTRODUCTION

Heterocyclic amines (HAs) are compounds that are formed naturally in proteinaceous foodstuffs when exposed to heat during the cooking process at common household cooking temperatures.[1–3] These compounds are known for their potent mutagenic response and they have also been linked to cancer in laboratory rodents and non-human primates.[4,5] Some of these amines have been classified as possible human carcinogens (class 2B) and one of them as probable human carcinogen (class 2A), by the International Agency for Research on Cancer (IARC).[6] Therefore, a reliable method is needed to quantify these analytes in cooked foods in order to assess the risks to human health by their intake. It is widely recognized that diet is a major life style factor contributing to the risks of cancer.[7–9]

The determination of heterocyclic amines has been carried out using various techniques such as high performance liquid chromatography (HPLC) with ultraviolet (UV),[10] electrochemical (ECD),[11] fluorescence (FD),[12] and mass spectrometry (MS)[13] detection, as well as gas chromatography (GC) with mass spectrometry (MS),[14] capillary electrophoresis,[15] and enzyme linked immunosorbent assay.[16] Although HPLC-MS and GC-MS are selective and sensitive techniques, they require sophisticated and expensive equipment that is beyond the reach of many laboratories. In this sense, HPLC-ECD is an option to be considered as an alternative by its selectivity and sensitivity. In addition, the ECD avoids the use of phase mobile additives like triethylamine.[17] Electrochemical detection of HAs is based on the oxidization of these compounds at the operating potential. Isocratic elution is usually employed in the combination with HPLC-ECD.[11,18,19] The isocratic elution has the disadvantage that two different methods (different runs) are required in order to determine polar and less polar amines.

Although, numerous data on the amounts of HAs in various cooked foods have been reported in the past,[20–22] it remains a challenging analytical task to obtain reliable data on their quantitative determination at low levels of concentration. Therefore, it is not too much to say that the cleanup procedures for complex samples like meat extracts greatly influence the reliable and accurate analysis for these compounds. Several enrichment steps have been performed before the chromatographic analysis when analyzing HAs. Liquid-liquid extraction (LLE),[23,24] and mainly solid-phase extraction (SPE),[11,25] are the most commonly used techniques. These methods have several disadvantages: they are tedious, labor intensive, and time consuming. More recently, the solid-phase microextraction[26–28] has also been utilized. The microwave-assisted extraction has been widely used to extract different analytes from a number of solid matrixes.[29,30] Most experiments have been carried out using cavity, or to a lesser extent, focused microwave systems. To our knowledge, this extraction technique has not been applied to the analysis of heterocyclic amines despite its well-known advantages.

This paper offers a new focused microwave-assisted extraction method combined with HPLC-ECD for the determination of eleven heterocyclic

amines in commercial meat extracts. Furthermore, the chromatographic method is carried out to determine polar and less polar amines in the same chromatographic run by applying gradient elution with ECD and, therefore, reducing the chromatographic time. The novel combination with microwaves also results in a high reduction of the extraction time: around 5 minutes for the microwave extraction step. The proposed method is fast, effective, and allows us to determine low levels of HAs in highly complex matrixes like meat extracts. The optimization of the microwave extraction process was developed by means of an experimental design, in order to decrease the optimization time.

EXPERIMENTAL

Reagents

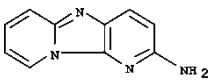
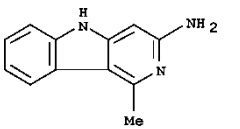
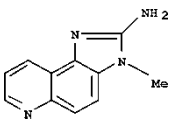
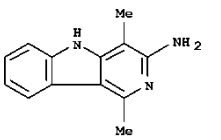
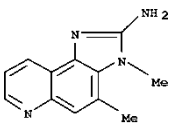
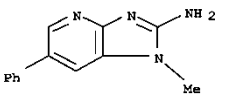
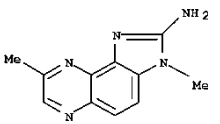
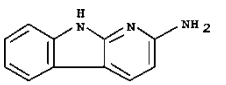
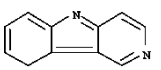
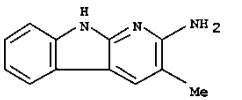
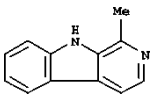
The studied amines were: 2-aminodipirydo[1,2-a:3′,2′-*d*]imidazole (Glu-P-2), 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo [4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (PhIP), 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC), purchased from Toronto Research Chemicals (Toronto, Canada), and 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC), 9*H*-pyrido[4,3-*b*]indole (NH) and 1-methyl-9 *H*-pyrido[4,3-*b*]indole (H), purchased from Aldrich-Chemie (Beerse, Belgium). Their structures can be observed in Table 1. Stock standard solutions of these amines were prepared containing 100 μg/mL of IQ; 420 μg/mL of MeIQ and Glu-P-2; 90 μg/mL of MeIQx; 620 μg/mL of NH; 140 μg/mL of H; 400 μg/mL of Trp-P-1; 380 μg/mL of Trp-P-2 and PhIP; 230 μg/mL of AαC and 200 μg/mL of MeAαC in methanol. The solutions were kept in the dark and refrigerated at 0°C. These stock standard solutions were used for the preparation of working standard solutions.

All chemicals and solvents were of HPLC or analytical-reagent grade. Water was purified using Milli-Q gradient A10 (Millipore, UK). All the solvents were filtered through a 0.45 μm Durapore® membrane filter (Millipore) before being used in the chromatographic system.

Instrumentation

Focused microwave-assisted extractions were performed at atmospheric pressure at the standard frequency of 2450 MHz using a CEM Focused Microwave™ Synthesis System apparatus, model Discover (CEM corporation, Matthews, NC, USA) equipped with an infrared temperature control system, stirring, and cooling options. The cooling was carried out by means of an air flow. The ChemDriver™ software (CEM) was used for data acquisition.

Table 1. Studied amines with their structures

Compound	Abbreviation	Structure	Compound	Abbreviation	Structure
2-Aminodipyrido[1,2-a:3′,2′-*d*]imidazole	Glu-P-2		3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole	Trp-P-2	
2-Amino-3-methylimidazo[4,5-*f*]quinoline	IQ		3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole	Trp-P-1	
2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline	MeIQ		2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine	PhIP	
2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline	MeIQx		2-Amino-9*H*-pyrido[2,3-*b*]indole	AαC	
9*H*-pyrido[4,3-*b*]indole	NH		2-Amino-3-methyl-9*H*-pyrido[2,3-*b*]indole	MeAαC	
1-Methyl-9*H*-pyrido[4,3-*b*]indole	H				

The HPLC equipment was a liquid chromatograph consisting of a delivery solvent ProStar 230 (Varian, California, USA) equipped with a Rheodyne valve (Supelco, Bellefonte, USA) of 20 μL. The detection of HAs was carried out using a ProStar 370 Electrochemical Detector (Varian). It was provided with a working electrode (glassy carbon), a reference electrode (Ag/AgCl, 2 M), and an auxiliary electrode (stainless steel). The analytical columns were two TSK GelODS-80^{TM} columns (5 μm, 150 mm × 4.6 mm i.d.) (Tosoh Biosep, Stuttgart, Germany), and a Pelliguard LC-18 guard column (Supelco). Data were acquired with the Star 5.51 chromatography workstation software (Varian).

The Statgraphic Plus (Statistical Graphics, Rockville) software package, version 5.1, was used for the statistical treatment.

Procedures

Meat extract samples were bought in a local supermarket. The extraction process of meat extracts was as follows: 6 mL of extractant phase (2.5% methanol in 0.05 M NaOH) were added to 1 g of meat extract sample (spiked or non-spiked) placed in a Pyrex® tube of 40 mL. After ensuring that an agitation bar was placed, the extraction tube was introduced into the microwave cavity. The optimum extraction was performed under a maximum radiation of 20 W (microwave oven power). Samples were exposed to microwaves until the temperature reached 80°C, and then kept to this temperature during 1 minute. The total microwave extraction time took around 5 minutes. The sample was kept stirring during the entire microwave extraction step. Afterwards, the tube was allowed to cool at room temperature. The supernatant was then separated and placed into a 100 mL decantation funnel. The funnel was placed in the freezer for 1 hour at − 18°C. This purification step was required for the removal of lipids and fats. Approximately 3 mL were then decanted, followed by centrifugation. An aliquot of 2 mL (already centrifuged) was diluted to 5 mL with deionized water and analyzed according to the optimal HPLC procedure.

The spiking process of the meat extract was as follows: the spiking solution was slowly added to form a dough, which was mechanically stirred for several minutes. Afterwards, it was stored in the dark for 24 hours. The spiked levels were: 25 μg/g for MeAαC; 20 μg/g for Glu-P-2, MeIQ and NH; 18 μg/g for IQ and AαC; 16 μg/g for MeIQx; 12 μg/g for PhIP; 10 μg/g for Trp-P-1; and 6 μg/g for H and Trp-P-2.

HPLC Analysis

The chromatographic separation of the HAs was carried out with a ternary mobile phase: 50 mM ammonium acetate (pH = 4.0):acetonitrile (90:10) (A solvent),

50 mM ammonium acetate (pH = 6.0):acetonitrile (70:30) (B solvent) and 50 mM ammonium acetate (pH = 6.0):acetonitrile (60:40) (C solvent), at a flow rate of 1 mL/min. The gradient program was: 100% A, from 0 to 27 min; 0–100% B, from 27 to 30 min; 100% B, from 30 to 44 min, 0–100% C, from 44 to 45 min; and 100% C, from 45 to 63 min; returned to initial conditions in 8 min, with 5 min of post-run delay. The columns and the detector were thermostated at 25°C.

The working potentials of the electrochemical detector were set at + 1000 mV for the polar fraction (from 0 to 34.3 min) and at + 950 mV for the less polar fraction (from 34.3 to 63 min).

RESULTS AND DISCUSSION

Chromatographic Separation

The chromatographic conditions were optimized to achieve an adequate resolution of the target HAs. The aim of the separation was to obtain both polar and less polar amines in the same chromatographic run, instead of using two different chromatographic methods.[11,18,19] In this way, an appropriate optimization of the gradient elution conditions was carried out. An adequate separation was obtained when using a ternary mobile phase, as has been described in the experimental section. These conditions are summarized in Table 2. The total chromatographic time was 63 minutes. When using 2 different chromatographic methods, and the same kind of column,[31] the total chromatographic time was 80 minutes: 50 minutes (first chromatogram for polar amines) plus 30 minutes (second chromatogram for less polar amines), and this is without considering the time required to reequilibrate the system between both methods. Therefore, a great decrease of the chromatographic time was obtained with the proposed method, which is very interesting for screening purposes. Figure 1 shows a representative chromatogram obtained under the optimal conditions.

Optimization of the Focused Microwave Extraction Process

The microwave-assisted extraction has turned out to be a highly useful analytical tool for the extraction of analytes from solid samples, mainly by its extraction efficiencies, as well as by the low extraction times achieved with this technique.[29,30] In this way, it seems interesting to make use of this technique for the analysis of HAs, in order not only to diminish the experimental times required in the tedious procedures described for their extraction,[32] but also to get appropriate extraction effectiveness from complex samples. The focused microwave was selected over the conventional microwave for having several advantages, such as the safety due to operate at atmospheric pressure or the

Table 2. Optimal chromatographic conditions for the studied amines and their retention times. The rest of chromatographic conditions as described in the experimental section

Time (min)	Mobile phase % A	% B	% C	Amine (Retention time ± SD[a])	E (mV)
0	100			Glu-P-2 (11.24 ± 0.66)	+1000
				IQ (16.80 ± 1.22)	
				MeIQ (22.22 ± 1.50)	
27	100			MeIQx (28.72 ± 1.54)	
30		100		NH (37.18 ± 0.38)	+950
				H (38.18 ± 0.20)	
				Trp-P-1 (39.33 ± 0.16)	
				Trp-P-2 (41.94 ± 0.20)	
44		100			
45			100	PhIP (45.66 ± 0.36)	
				AαC (52.95 ± 0.31)	
				MeAαC (60.16 ± 0.44)	
63			100		

[a]Standard deviation for n = 11.

possibility of using programmable addition of reagents at any time during the extraction.[33]

The optimization of the focused microwave-assisted extraction was carried out by means of the experimental design. The use of a factorial design to explore the variables that affect the microwave extraction allows a

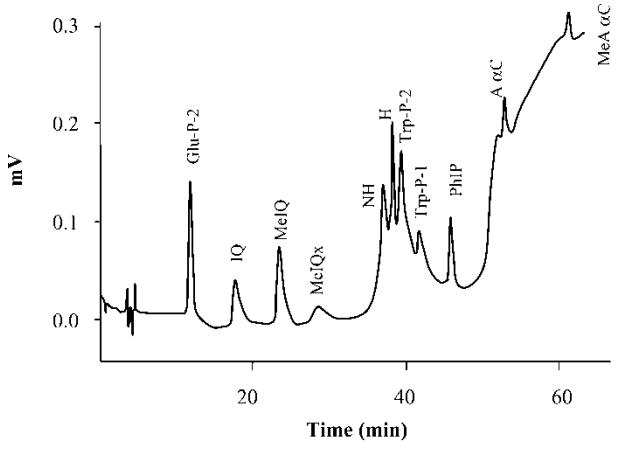


Figure 1. Representative chromatogram for the studied HAs, obtained by HPLC-ECD under the experimental conditions described in the text.

consideration of the overall number of experiments and possible interaction effects between the variables. The application of a statistical approach using a factorial design can both reduce the development time and provide less ambiguous data. Several variables could potentially affect the microwave extraction process, like the microwave oven power, the maximum temperature to be reached inside the extraction tube, and the hold time (that is, the fixed time where the maximum temperature is kept constant inside the extraction tube). Other variables implicated in the extraction process were kept constant: amount of sample (1 g of meat extract), extractant phase volume (6 mL), and the concentration of spiked amines (as described in the experimental section). A factorial design, 2^3, involving 8 experiments was selected. This model allows evaluating the effects of each variable, as well as the interaction effects between variables. The levels used for each variable were: 35 and 80°C for the maximum temperature, 20 and 150 W for the microwave power, and 0.5 and 4 minutes for the hold time. Higher temperatures were not selected in order not only to avoid losses of the extractant phase by volatilization, but mainly to prevent any HAs formation during heating. Higher powers for the focused microwave were not selected. Higher powers are usually associated to less reproducible results, due to the low irradiation times needed to reach the temperature. Furthermore, the main objective of the work was to decrease the extraction time, and for that reason higher hold times were not studied. The peak area of each amine was selected as the elemental response value of the design. Spiked granulated meat extract samples were employed as described in the experimental section. The experimental design matrix applied is shown in Table 3. The obtained results allowed classifying the amines in two different groups considering their behaviours under the factors considered. One group was formed by the amines Glu-P-2, IQ, MeIQ, MeIQx, H, and NH, group where some of the most polar amines are included. The second group was formed by the less polar amines Trp-P-2, PhIP, Trp-P-1, AαC, and MeAαC. The amines H and PhIP were chosen as representative examples of each group.

Table 3. Experimental design matrix

Run	Temperature	Hold time	Microwave power
1	(−)	(−)	(−)
2	(+)	(+)	(−)
3	(−)	(+)	(+)
4	(+)	(+)	(+)
5	(+)	(−)	(−)
6	(−)	(+)	(−)
7	(−)	(−)	(+)
8	(+)	(−)	(+)

(+): Maximum value, (−): Minimum value.

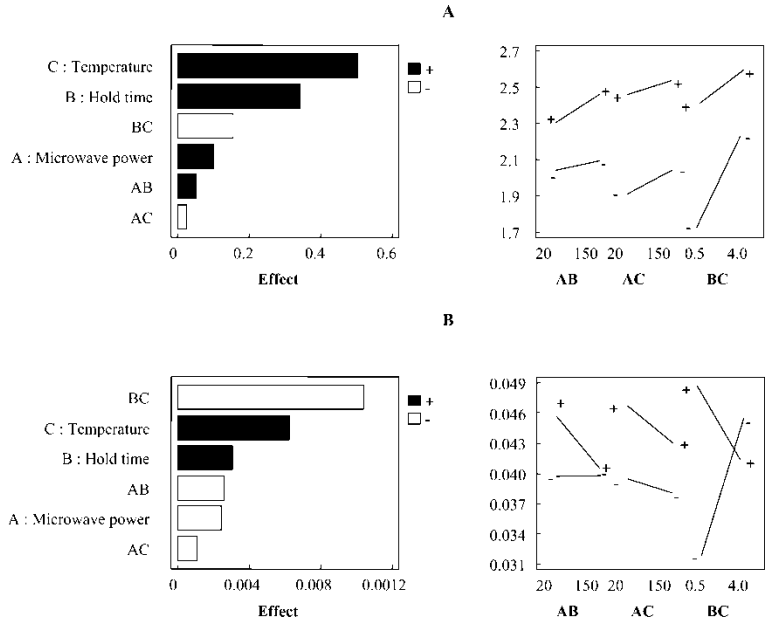


Figure 2. Pareto chart (left) and interaction effect plot (right) for the amines H (A) and PhIP (B) with the factorial design.

Figure 2(A) shows the Pareto Chart, which evaluate main effects and interaction,[34] obtained by analyzing the experimental results for the representative amines. In this kind of chart, bar lengths are proportional to the absolute value of the estimated effects, helping to compare the relative importance of the effects. It can be observed for less polar amines that temperature and hold time have a positive effect in the response, whereas the microwave power has a negative effect. On the other hand, all the factors have a positive effect for the first group of amines. Figure 2(B) shows the interactions among the factors. An interaction can be observed between the hold time and the temperature for both groups, being the most important factor for the less polar amines. For the less polar amines, the maximum is reached with the lowest level for the hold time and the highest level for the temperature. The maximum is also reached with the highest level for the temperature in the case of polar amines. Considering these results, the selected optimum temperature was 80°C, that is, the highest temperature value.

A series of experiments were carried out in order to study the effect of the interaction between the microwave power and the hold time, as well as to find the optimal values for these variables, focusing our attention in the less polar amines. The less polar amines usually present lower extraction efficiencies due to coeluting interferences.[11] Three different low values for the hold times were studied: 0.5, 1 and 1.5 min. This decision was made considering not only their

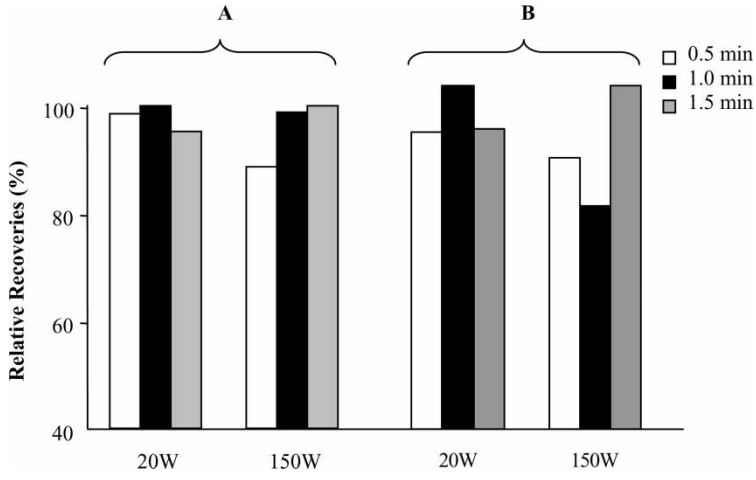


Figure 3. Extraction efficiencies for (A) Trp-P-1 and (B) PhIP at different hold times (0.5, 1 and 1.5 min) and powers (20 and 150 W), keeping constant the maximum temperature at 80°C.

negative effect in all the studied amines, but also in order to decrease the extraction time needed in the process. Two different powers were tested too: 20 and 150 W, keeping constant the already optimized temperature: 80°C. The obtained results can be observed in Figure 3, with the amine Trp-P-1 and PhIP as examples of less polar amines. A low influence of the hold time at low levels of the microwave power can be observed. It can also be seen that there is a slight increase in the response when increasing the hold time at high levels of the microwave power. This slight improvement can be attributed to the low irradiation times needed to reach the maximum temperature with high microwave powers and, hence, an extra radiation time (hold time) is quite useful. With respect to these results, the selected hold time was 1 minute and the selected microwave power was 20 W, in order to increase the effective microwaves action time and therefore to increase the extraction efficiency. Under these conditions, the total extraction time by microwaves was, approximately, five minutes. The short microwaves extraction times required with the proposed method should be highlighted.

Quality Parameters of the Analytical Method

The figures of merit were studied to evaluate the performance of the proposed procedure. Table 4 illustrates the slope, standard deviation of the regression, linearity of the calibration plots, intermediate precision, and detection limits of the optimized method for the studied HAs. All amines showed good linearity with correlation coefficients (R) ranging from 0.996 to 0.999. The studied working ranges were 6–16 ng (injected) for H, Trp-P-2 and Trp-P-1;

Table 4. Quality parameters of the analytical method

Amine	Slope ± SD[a]	Syx[b]	R	RSD[c] (%)	LOD (ng injected)
Glu-P-2	1948719.0 ± 48580.8	68632.2	0.998	7.4	1.98
IQ	2108842.8 ± 53472.4	62248.8	0.998	10.4	1.66
MeIQ	2161759.8 ± 34544.4	44388.0	0.999	6.9	1.15
MeIQx	1241836.7 ± 22312.0	18321.7	0.999	12.8	0.83
NH	496931.3 ± 25527.5	23687.8	0.996	10.4	2.68
H	2305158.0 ± 115999.3	46475.6	0.997	8.2	1.13
Trp-P-2	1740330.1 ± 40979.5	12841.2	0.999	12.5	0.41
Trp-P-1	896053.3 ± 5989.7	2480.6	0.999	7.7	0.16
PhIP	1130134.6 ± 33915.9	19799.0	0.999	6.1	0.98
AαC	66079.8 ± 23570.5	1904.4	0.996	12.7	1.62
MeAαC	72882.3 ± 2311.7	2778.6	0.997	8.4	2.14

[a]Standard deviation for n = 9.
[b]Standard deviation of the regression.
[c]Intermediate precision.

12–30 ng for PhIP; and 16–50 ng for the rest of HAs. Intermediate precision was evaluated by analyzing an intermediate standard by quadruplicate during three non-consecutive days under the optimized conditions. The obtained RSD ranged between 6.1 and 12.8%, showing the repeatability of the proposed method.

Detection limits (LODs) were calculated as described by Cuadros et al.[35] They oscillated between 0.16 ng (injected) for Trp-P-1 and 2.68 ng for NH. These LODs are slightly better than those reported by Galcerán et al. when using HPLC-ECD,[31] which oscillated between 0.19 and 3.31 ng (injected). Bermudo et al.[11] have obtained better results using SPE-HPLC-ECD with two chromatographic runs, with values varying between 0.01 and 0.08 ng.

Analysis of Meat Extracts

Five different kinds of meat extracts were analyzed: granulated meat extract, non-granulated meat extract, chicken extract, and two different brands of meat soup cubes (A and B). The evaluation of the extraction efficiency was carried out in the optimized conditions with 3 spiked portions of meat extracts, as described in the experimental section. The obtained recoveries can be seen in Table 5. They oscillated between 36.0% for PhIP in the soup cube A and 109.5% for Trp-P-1 in the non-granulated meat extract, being the average extraction efficiency of 69.7% for all HAs in all the studied meat extracts. The average recoveries for all HAs in each meat extract sample were 81.2% for the granulated meat extract, 76.4% for the non-granulated extract, 66.7% for the chicken

Table 5. Extraction efficiencies obtained with some commercial meat extracts

	Recovery (%) ± SD[a]				
Amine	Granulated	Non-granulated	Chicken	Soup cubes A	Soup cubes B
Glu-P-2	77.6 ± 9.0	–	64.1 ± 4.1	–	–
IQ	77.2 ± 4.4	58.1 ± 6.5	72.5 ± 1.3	71.7 ± 1.7	63.8 ± 1.6
MeIQ	83.3 ± 12.7	81.9 ± 14.5	67.2 ± 1.3	57.9 ± 1.0	59.3 ± 1.5
MeIQx	101.3 ± 3.8	96.3 ± 4.4	86.2 ± 2.1	75.3 ± 1.4	73.2 ± 2.7
NH	85.1 ± 12.1	100.0 ± 2.3	81.0 ± 19.6	70.2 ± 2.0	65.4 ± 7.4
H	92.2 ± 12.6	62.8 ± 7.8	60.9 ± 18.6	47.6 ± 3.5	53.0 ± 3.2
Trp-P-2	57.5 ± 2.1	50.8 ± 6.7	–	–	–
Trp-P-1	71.7 ± 2.8	109.5 ± 8.8	71.3 ± 17.0	70.7 ± 12.4	93.7 ± 2.9
PhIP	77.3 ± 3.1	60.3 ± 7.3	55.4 ± 4.3	36.0 ± 5.0	52.0 ± 4.4
AαC	92.9 ± 2.6	80.4 ± 12.6	65.0 ± 3.3	53.0 ± 3.7	49.9 ± 6.1
MeAαC	76.9 ± 2.6	63.3 ± 8.1	43.5 ± 3.5	–	–
Average	81.2	76.4	66.7	60.3	63.8

[a]Standard deviation for n = 3.

extract, 60.3% for the soup cubes A, and 63.8% for the soup cubes B. In some cases, Glu-P-2 could not be appropriately quantified in the non-granulated meat extract, and in the soup cubes A and B, because of the presence of some impurities at its retention time. The same situation happens with Trp-P-2 in the soup cubes A and B, as well as in the chicken extract. Similar identification problems took place with MeAαC in the soup cubes A and B. Good recoveries higher than 51% were obtained for all HAs in the granulated and non-granulated meat extracts, and also with the chicken extract, with the exception of MeAαC in this last case. Lower recoveries were obtained with soup cubes A and B, ranging from 47.6 to 93.7%, with the exception of PhIP in the soup cube A. These differences reveal the high dependence of the extraction process with the nature of the matrix, especially for the less polar amines. It should be noted that the optimization of the method was carried out with the granulated meat extract. Hence, this sample generated the best extraction efficiencies under the optimized conditions. Some improvements in the extraction process with other kinds of samples, especially with soup cubes A and B, would be necessary if a higher sensitivity is required.

Finally, it should be also noted that the high recoveries obtained with PhIP, Trp-P-1, Trp-P-2, and AαC, amines, which usually interfere lead to problematic quantification.

CONCLUSIONS

The use of a microwave-assisted extraction procedure has been shown to be a valid alternative in sample preparation for the determination of heterocyclic amines in meat extracts prior to HPLC with electrochemical detection. The short extraction times achieved (five minutes), and the obtaining of both polar and less polar amines in the same chromatographic run, improve the analysis time usually required in methods commonly applied for the determination of these amines in food samples. The proposed method allows the determination of heterocyclic amines in different kinds of meat extracts, with average recoveries between 60.3 and 81.2%, and with limits of detection ranging from 0.16 to 2.68 ng. Furthermore, the method could also be adapted to carry out screening studies of heterocyclic amines in unknown samples.

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