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Isolation by solid-phase extraction and liquid chromatographic determination of mutagenic amines in beef extracts

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Abstract

A solid-phase extraction method was successfully optimized for the isolation and preconcentration of five mutagenic amines, 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole, 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole, 2-amino-9H-pyrido[2,3-*b*]indole, 2-amino-3-methyl-9H-pyrido[2,3-*b*]indole and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, and two co-mutagens, 1-methyl-9H-pyrido[4,3-*b*]indole and 9H-pyrido[4,3-*b*]indole. Coupling of diatomaceous earth, propylsulphonyl silica gel, and octadecylsilane cartridges was used to separate selectively the imidazopyridine and indolpyridine derivatives from those of quinoxaline and quinoline. A method based on this sample preparation was applied to the determination of twelve heterocyclic amines and related substances in a commercial beef extract using HPLC with electrochemical and fluorescence detection. Good recovery values were obtained, ranging between 55 and 99%. The co-mutagens 1-methyl-9H-pyrido[4,3-*b*]indole (harman) and 9H-pyrido[4,3-*b*]indole (norharman) were found in the beef extract at levels of 110 and 53 ng g^{-1}, respectively, and 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2) and 2-amino-9H-pyrido[2,3-*b*]indole (AαC) were tentatively identified.

1. Introduction

It is now widely accepted that several groups of mutagenic and carcinogenic compounds can be produced during the cooking of certain foods, especially proteinaceous ones such as fish and meat [1,2]. Since the first report by Sugimura et al. [3] about the mutagenic properties of condensed smoke from broiled meat and fish, a great effort has been made worldwide to identify the substances responsible for mutagenic activity. It has been established that the basic fractions extracted from processed food samples show a potent mutagenic activity, and several heterocyclic amines (HAs) have been implicated. Most of these compounds are amines derived from imidazoquinoxalines, imidazoquinolines, imidazopyridines, and indolpyridines. At present, it is accepted that the heterocyclic amines may cause common cancers [4].

HAs and other related substances are present at low concentrations in complex matrices such as food extracts. The main analytical problem is to achieve an efficient isolation and preconcentration for their analysis, which is performed using various analytical techniques such as HPLC, with different detection systems: UV, fluorescence [5], electrochemical [6,7], MS [8,9], or GC–MS [10,11], or ELISA immunoassay [12]. Several methods of sample preparation suitable for the analysis of various home-cooked and commercial foodstuffs have been published

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using liquid–liquid extractions [11,13] or solid-phase extractions [5]. A good recovery for some specific mutagens has been reported, but for others low recoveries are obtained, and moreover, the sample matrix greatly influences the clean-up processes [14–16]. Therefore, further investigation in order to improve both the reproducibility and efficiency of the preconcentration methods for the mutagenic compounds occurring in processed foods is required.

In a previous study [6], high-performance liquid chromatography with electrochemical detection (HPLC–ED) was applied to the analysis of ten heterocyclic amines in a commercial beef extract. The sample preparation was carried out following the method proposed by Gross [5]. The recovery values obtained were satisfactory for imidazoquinolines and imidazoquinoxalines, but imidazopyridine and indolpyridine derivatives were not recovered. In this paper, the different steps for the isolation and preconcentration of all the pyridine derivatives are studied in order to improve on the recoveries reported previously. The method was applied to the determination of these compounds in a commercial beef extract using HPLC–ED and fluorescence detection.

2. Experimental

2.1. Chemicals

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-*f*]quinoxaline (4,8-DiMeIQx), 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2), 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1), 2-amino-9H-pyrido[2,3-*b*]indole (AαC), 2-amino-3-methyl-9H-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-9H-pyrido[4,3-*b*]indole (harman) and 9H-pyrido[4,3-*b*]indole (norharman), which were from Aldrich (Steinheim, Germany). Stock standard solutions of 100 μg ml^{-1} in methanol were prepared and used for further dilutions. Aniline (Carlo Erba, Milano, Italy) and 1-naphthylamine (Merck, Darmstadt, Germany) were used as internal standards (solutions of 1 μg ml^{-1} in methanol). Diatomaceous earth extraction cartridges (Extrelut; 20 ml) were provided by Merck. Bond Elut propylsulphonyl silica gel (PRS; 500 mg) and octadecylsilane (C_{18}; 500 and 100 mg) cartridges as well as coupling pieces and stopcocks were from Analytichem International (ICT, Basle, Switzerland). These cartridges were preconditioned with dichloromethane (4 ml) for PRS and methanol (10 ml) and water (10 ml) in the case of C_{18}.

Solvents and chemicals used were HPLC or analytical grade, and the water was purified in a Culligan system (Barcelona, Spain). All the solutions were passed through a 0.45-μm filter before injection into the HPLC system.

2.2. Instruments

HPLC was carried out with a Gilson Model 302 pump equipped with a Manometric Module Model 802C (Gilson, Villiers le Bel, France), and a Metrohm 656 electrochemical detector made up of a working electrode (glassy carbon electrode, Model 6.0805.010), a reference electrode (Ag/AgCl/KCl, 3M) and an auxiliary electrode (glassy carbon electrode, Model 6.0805.010) (Metrohm, Herisau, Switzerland). A Chromatopac C-R3A data processor (Shimadzu, Kyoto, Japan) was used in the HPLC–ED system. The fluorescence detection was performed using an Aminco-Bowman Series 2 luminescence spectrometer (SLM-Aminco, Urbana, IL, USA). An ODS/2-PM data system was used in this case to record the data. An Applied Biosystem Model 1000s (Foster City, CA, USA) photodiode-array UV detector (DAD) was used for the confirmation of the peaks of the samples. A Rheodyne 7125 injector equipped with a loop of 50 μl was used to introduce the sample. The amines were separated using a TSK-Gel ODS 80T column (5

μm, 25.0 cm × 4.6 mm I.D.) (Toso Haas, Stuttgart, Germany) and a Supelguard LC-8-DB precolumn (Supelco, Gland, Switzerland). As mobile phases 50 m*M* ammonium acetate (pH 4.0)–acetonitrile (90:10) and 50 m*M* ammonium acetate (pH 5.7)–acetonitrile (70:30) were used at a flow-rate of 1 ml min^{-1}. Separations were carried out at room temperature. The optimum working potential obtained from the hydrodynamic voltammograms [6] was +1000 mV. Fluorescence was monitored at 425 nm when excited at 300 nm, except for Glu-P-1 which was detected at 450 nm, being the excitation wavelength of 360 nm.

A Supelco Visiprep and a Visidry SPE vacuum manifold (Supelco, Gland, Switzerland) were used for manipulations with solid-phase extraction cartridges.

2.3. Analytical procedure

Sample preparation and clean-up were based on the method proposed by Gross [5], which includes different solid-phase extraction stages. The first step uses a diatomaceous earth cartridge (Extrelut) after homogenizing the sample in a sodium hydroxide solution. In the second stage, the analytes are eluted directly to a propylsulphonic cartridge (PRS) by coupling this to the first cartridge, using dichloromethane as eluent. The PRS was then washed with three different solvents: HCl solution, MeOH–0.1 *M* HCl mixture, and water. These fractions, which contained the imidazopyridine and indolpyridine derivatives, were collected and concentrated using a C_{18} cartridge (500 mg). The aminoimidazoquinoxalines and aminoimidazoquinolines retained in the PRS cartridge were eluted using ammonium acetate (0.5 *M*, pH 8.0) directly into another C_{18} cartridge (100 mg). Finally, the HAs retained in the C_{18} cartridges were eluted (methanol–ammonia) to give two final extracts. Each extract was evaporated to dryness under a stream of nitrogen and redissolved in a methanolic internal standard (IS) solution, 50 μl for the unspiked samples and 100 μl for the spiked ones.

Heterocyclic amines were determined using HPLC–ED. Good separations between all the compounds using isocratic mode (a gradient system is difficult to perform when an electrochemical detector is used) can only be achieved with two different conditions, as we reported previously [6]. So, IQ, MeIQ, MeIQx, 4,8-DiMeIQx, and Glu-P-1 were analyzed using a mobile phase of 50 m*M* ammonium acetate (pH 4.0)–acetonitrile (90:10) and aniline as internal standard, and Trp-P-1, Trp-P-2, harman, norharman, AαC, MeAαC, and PhIP were separated with 50 m*M* ammonium acetate (pH 5.7)–acetonitrile (70:30), using 1-naphthylamine as internal standard.

The analytes in the beef extract samples were quantified by the standard addition method. The spiked samples were prepared by addition of about 125, 250 and 500 ng of each standard at the beginning of the clean-up process, when 10 ml of NaOH was added. Recoveries were estimated from these additions with the HPLC–ED and HPLC–fluorescence systems when possible. These values were calculated from the slope of the regression line performed with the amount added versus the amount measured.

3. Results and discussions

3.1. Clean-up procedure optimization

The sample preparation and clean-up performed previously [6] gave recovery values ranging from 63 to 77% for both aminoimidazoquinolines and aminoimidazoquinoxalines, and 17% for Glu-P-1, but the other compounds were not recovered. For this reason the different steps of the clean-up were studied separately, to identify where the analytes were lost and to achieve better values for all the compounds. The optimization of all the steps of the clean-up procedure was carried out for the compounds Trp-P-1, Trp-P-2, harman, norharman, AαC, MeAαC, and PhIP, which were not recovered previously, and also for MeIQ, to check the global procedure. The analysis was performed by HPLC–ED using the mobile phase proposed for imidazopyridine and indolpyridine

derivatives, with 1-naphthylamine as internal standard. In Fig. 1 a chromatogram of a standard solution (1 μg ml^{-1}) under these conditions is given. In order to study the effect of different concentrations of heterocyclic amines in the clean-up procedure, the recoveries for each stage were established using standard solutions at three concentration levels (20, 100, and 200 ng).

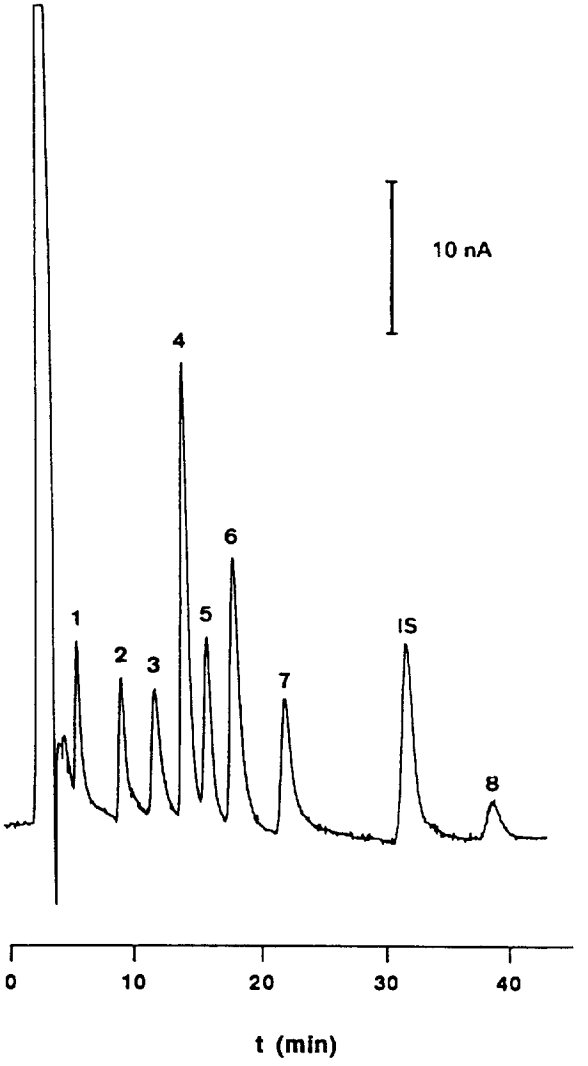

Fig. 1. HPLC–ED chromatogram of standard solution (1 μg ml^{-1}). Mobile phase: 50 m*M* ammonium acetate (pH 5.7)–acetonitrile (70:30). Peaks: 1 = MeIQ; 2 = Trp-P-2; 3 = Trp-P-1; 4 = harman; 5 = PhIP; 6 = norharman; 7 = AαC; IS = 1-naphthylamine; 8 = MeAαC.

Extrelut cartridge

In the first step 10 ml of 1 *M* NaOH was spiked with standard solutions of the amines, introduced in an Extrelut cartridge, and eluted using dichloromethane (DCM) at a flow-rate $\leq$1 ml min^{-1}. Different volumes of DCM were tested, and the percentages of recovery of the analytes in this step using 30, 45, 60, and 75 ml of DCM are given in Table 1. These values were calculated by comparing the ratio of the peak areas to an internal standard with those of a control sample (which represents 100% of recovery), as described in the literature [17]. The higher recoveries of the HAs were obtained when increasing the volume of DCM. Good recovery values can be reached with 60 ml of DCM except for AαC and MeAαC, which were never recovered at more than 50%. In order to increase these values, the sodium hydroxide solution spiked with the standards was mixed with the Extrelut packing as recommended by other authors [5]. In this case the elution was performed using 45 ml of DCM. The results of the recoveries are given in Table 1. Premixing increased the recovery values of all amines up to 70%, except for AαC, which did not exceed 50%. As a result, the procedure proposed for this step is to mix the diatomaceous earth with the sample and elute the analytes with 50 ml of DCM.

PRS cartridge

The retention of the amines in the PRS cartridge by coupling it directly to the Extrelut column is the second stage of the clean-up procedure. Firstly, the total retention of the analytes in the cartridge was checked using a DCM standard solution of the amines. Then, different cleaning steps using diluted HCl solution and then MeOH–0.1 *M* HCl mixture were evaluated. The PRS cartridge was rinsed with 6 ml of HCl at different concentrations: 0.1, 0.01, and 0.001 *M*. Regardless of the concentration of the HCl solution, a small fraction of the compounds was always eluted. So, in all the following assays, the cartridge was washed with 6 ml 0.01 *M* HCl as previously described [6], and the fraction eluted was collected for further analysis.

Table 1
Percentage of HAs recovered from the Extrelut cartridge at different volumes of dichloromethane

Analyte	% Recovery of the Extrelut step														
	ml DCM with 20 ng HA					ml DCM with 100 ng HA					ml DCM with 200 ng HA				
	30	45	45[a]	60	75	30	45	45[a]	60	75	30	45	45[a]	60	75
MeIQ	69	75	75	95	99	60	74	87	77	77	41	70	64	72	70
Trp-P-2	66	89	96	96	100	78	81	104	74	85	65	87	103	75	93
Trp-P-1	43	69	74	93	98	59	69	105	88	91	53	88	99	85	102
PhIP	30	55	65	97	99	32	34	68	79	81	64	73	69	76	75
Harman	77	91	91	91	97	90	91	107	91	90	89	89	98	92	102
Norharman	75	85	80	93	96	83	80	95	82	78	79	82	97	76	80
AαC	26	28	47	32	57	42	48	46	43	48	44	56	50	51	50
MeAαC	28	32	81	40	65	39	43	80	43	48	36	44	86	47	47

[a] Recovery value obtained when the diatomaceous earth is previously mixed with the sample.

The second clean-up step in the procedure for removing the interfering substances was to flush the PRS cartridge with 15 ml of MeOH–0.1 *M* HCl (40:60) and with 2 ml water; in this step some of the analytes may be lost, as described by Gross and Grüter [14]. In this study, we took advantage of this fact to separate the analytes into two fractions, one retained in the PRS cartridge and the other removed using MeOH–0.1 *M* HCl followed by water as eluent. Therefore, different compositions of the MeOH–0.1 *M* HCl solution were studied, in order to increase both the retention of aminoimidazoquinolines and aminoimidazoquinoxalines, and the elution of imidazopyridine and indolpyridine derivatives. In Table 2 the percentages of elution of each amine eluted in this stage are summarized. For most of the amines an increase in the percentage of elution was observed when the percentage of methanol was higher; otherwise, the retention of MeIQ increased with the amount of methanol. This finding indicates that this procedure allowed an efficient separation of the two groups of amines using MeOH–0.1 *M* HCl (60:40) for elution of the PRS cartridge; extract A would contain IQ, MeIQ, MeIQx, and 4,8-DiMeIQx

Table 2
Percentage of amine eluted from the PRS cartridge using different percentages of methanol in the methanol–0.1 *M* HCl solution

Analyte	% Elution from the PRS cartridge step								
	% MeOH with 20 ng HA			% MeOH with 100 ng HA			% MeOH with 200 ng HA		
	40	50	60	40	50	60	40	50	60
MeIQ	56	45	0	40	28	29	0	1	0
Trp-P-2	100	82	100	100	89	100	89	99	93
Trp-P-1	100	86	100	100	97	98	98	98	99
PhIP	100	100	100	100	100	100	98	92	99
Harman	28	53	79	69	88	94	43	90	96
Norharman	58	83	81	58	92	89	92	86	96
AαC	100	100	100	100	100	100	100	100	100
MeAαC	100	100	100	100	100	100	100	100	100

Table 3
Percentage of amine recovered from the C_{18} cartridge

Analyte	% Recovery of the C_{18} cartridge step		
	20 ng	100 ng	200 ng
Trp-P-2	95	100	101
Trp-P-1	93	98	104
PhIP	80	85	110
Harman	80	95	92
Norharman	70	88	90
AαC	50	50	63
MeAαC	80	68	100

(strongly retained in the cartridge) as previously described [6], and extract B contained the other compounds, Trp-P-1, Trp-P-2, harman, norharman, PhIP, AαC, and MeAαC (eluted with high percentage, >75%).

C_{18} cartridge

The final step to evaluate was the extraction efficiencies obtained in the concentration using a C_{18} cartridge. In the procedure all the fractions collected from the PRS cartridge using 0.01 *M* HCl, MeOH–0.1 *M* HCl (60:40), and water were mixed and neutralized with 500 μl of concentrated ammonia solution and diluted with water to obtain a solution with less than 20% of methanol. To study the recovery of this step, a solution of the amines in the above-mentioned mixture of solvents was prepared and passed through a C_{18} cartridge (500 mg) at a flow-rate of 4–5 ml min^{-1}. The amines retained were eluted with 1.4 ml MeOH–$NH_{3\ (aq)}$ (9:1) to give the final extract B. The recovery values obtained are given in Table 3, and all are higher than 70%, except for AαC, showing that the losses in this step are low.

Global procedure

Before applying the procedure to the analysis of real samples, a study of the overall process, coupling the three main steps, was studied. Standard solutions at the three concentration levels were subjected to the total procedure. The recovery values and the relative standard deviations obtained for five replicates are given in Table 4. High recoveries (>60%) and low relative standard deviations (R.S.D. between 1 and 13%) were obtained, showing the suitability of the method for the analysis of imidazopyridine and indolpyridine derivatives in real samples. The procedure for the analysis of both groups of amines is summarized in Fig. 2.

3.2. Application

The proposed clean-up procedure was applied to the determination of HAs in a commercial beef extract. In Fig. 3 a chromatogram of extract A for an unspiked and a spiked sample obtained by HPLC–ED is given. The percentage of recovery for each compound calculated using the

Table 4
Percentages of recovery in the global clean-up procedure ($n = 5$)

Analyte	% Recovery of the total process					
	20 ng		100 ng		200 ng	
	Mean	R.S.D. (%)	Mean	R.S.D. (%)	Mean	R.S.D. (%)
Trp-P-2	101	6	103	4	104	5
Trp-P-1	101	7	96	5	98	5
PhIP	86	10	90	6	94	9
Harman	77	7	102	6	94	9
Norharman	60	12	98	5	91	8
AαC	59	13	56	13	68	13
MeAαC	62	8	71	7	89	5

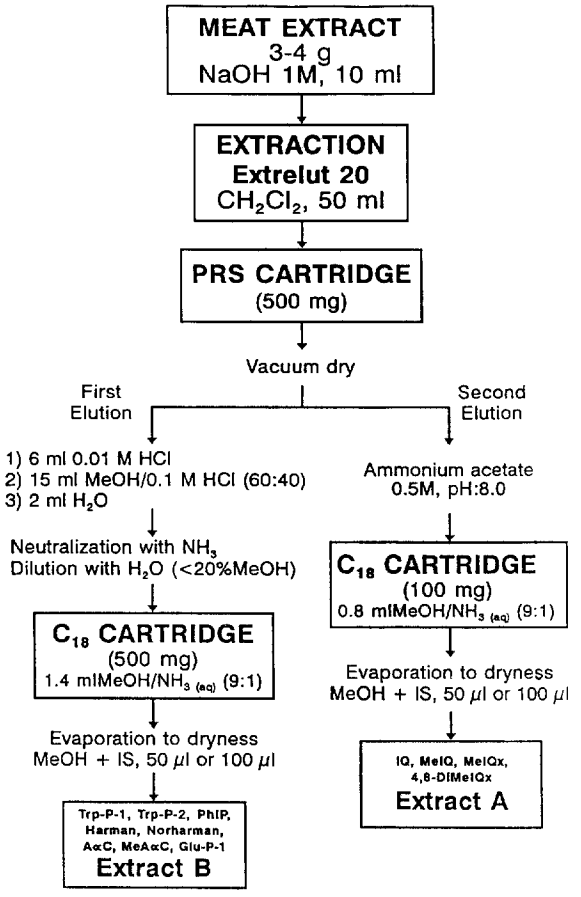

Fig. 2. Clean-up procedure scheme.

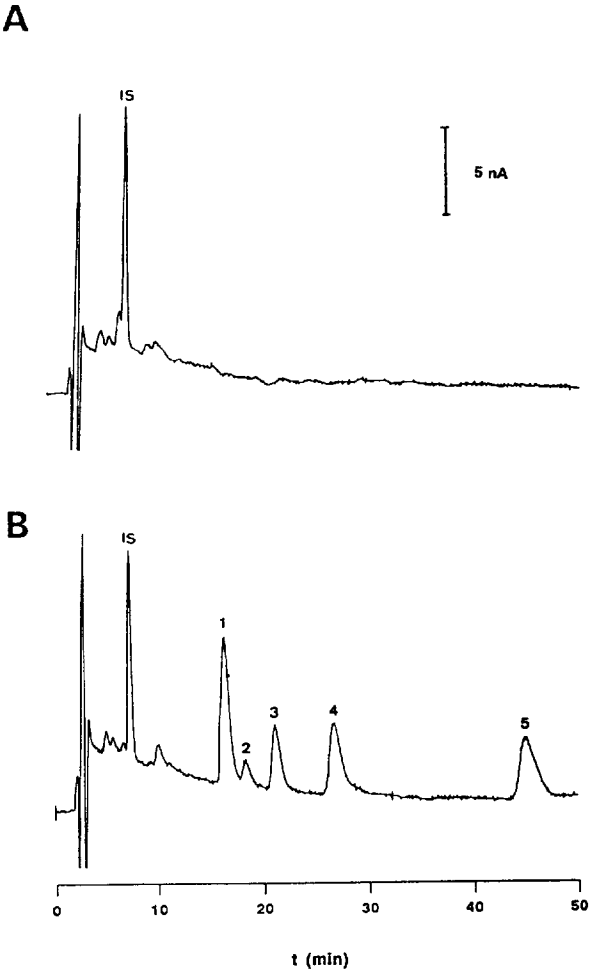

Fig. 3. HPLC–ED chromatogram of extract A of a beef extract sample. Mobile phase: 50 m*M* ammonium acetate (pH 4.0)–acetonitrile (90:10). (A) Extract A dissolved in 50 µl of IS solution, (B) extract A spiked before the clean-up (250 ng) dissolved in 100 µl of IS solution. Peaks: IS = aniline; 1 = IQ; 2 = Glu-P-1; 3 = MeIQx; 4 = MeIQ; 5 = 4,8-DiMeIQx.

standard addition method as described in the Experimental section, is shown in Table 5. Recoveries for IQ, MeIQ, MeIQx, and 4,8-DiMeIQx were higher than previously reported [6], which is due to the changes introduced to the original procedure, namely: an increase in the DCM volume in the Extrelut step and an increase in the percentage of methanol in the PRS stage. Glu-P-1 was recovered at 16% in extract A, a value that is in agreement with the published data [6]. In consequence, this amine should be found in extract B, although it was not possible to detect it by electrochemical detection, because of matrix interferences. Using fluorescence detection, Glu-P-1 could be detected in extract B without interfering peaks, as can be seen in Fig. 4, where the chromatogram obtained for extract B of an unspiked and a spiked sample using the mobile phase 50 m*M* ammonium acetate (pH 4.0)–acetonitrile (90:10) is given. The recovery for Glu-P-1 in extract B was 81%.

To determine recoveries for the other pyridine derivatives in extract B, a mobile phase with higher amounts of acetonitrile and higher pH was used. In Figs. 5 and 6 chromatograms of this extract obtained using electrochemical and fluo-

Table 5
Analysis of a beef extract

Analyte	Recovery (%)	Concentration[a] (ng g^{-1})
IQ	82 ± 7	nd[c]
MeIQ	99 ± 4	nd
MeIQx	87 ± 12	nd
4,8-DiMeIQx	78 ± 7	nd
Glu-P-1	81 ± 4	nd
Trp-P-1	91 ± 9	nd
Trp-P-2	74 ± 8	14 ± 5[b]
PhIP	55 ± 3	nd
Harman	70 ± 7	110 ± 20
Norharman	73 ± 9	53 ± 17
AαC	68 ± 4	2 ± 1[b]
MeAαC	70 ± 7	nd

[a] Value corrected by percent recovery.
[b] Identity not confirmed by DAD.
[c] nd = not detected.
Confidence intervals expressed as standard deviations [22].

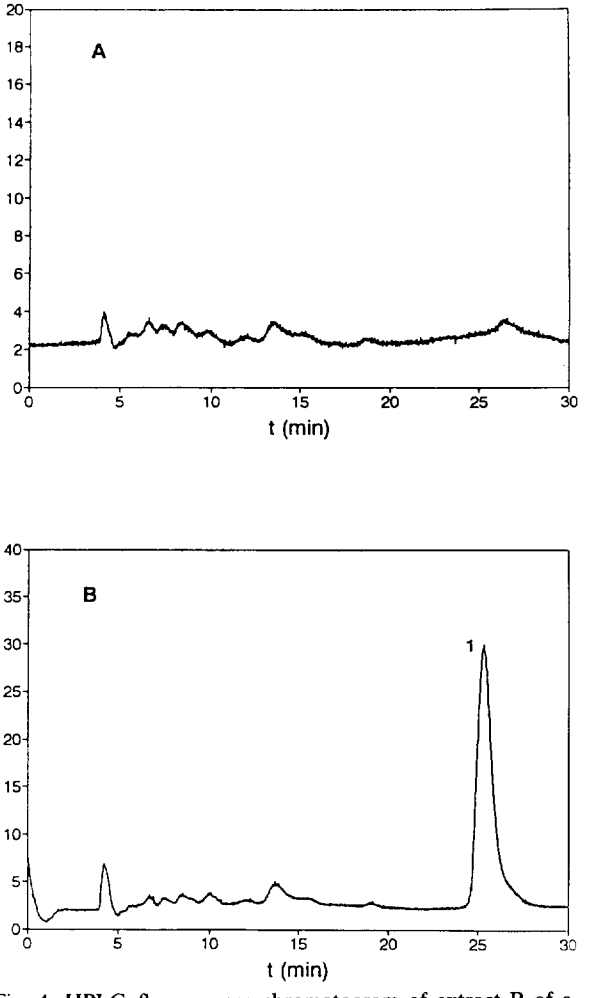

Fig. 4. HPLC–fluorescence chromatogram of extract B of a beef extract sample. Mobile phase: 50 m*M* ammonium acetate (pH 4.0)–acetonitrile (90:10). (A) Extract B in 50 μl IS solution, (B) extract B spiked before the clean-up (250 ng) dissolved in 100 μl IS solution. Peak: 1 = Glu-P-1.

rescence detection are given. Recoveries were calculated using both systems, except for Trp-P-1, which presented matrix interferences in electrochemical detection. The fluorescence detector was also used for the quantification of these amines without interfering peaks due to its higher selectivity. The recovery values achieved for the imidazopyridine and indolpyridine derivates in extract B were higher than 55%, as can be seen in Table 5, showing that if the proposed clean-up procedure is applied, these amines can be recovered with high efficiency.

The beef extract was analyzed using both detection techniques, and the results are given in Table 5. The identification of the compounds was performed comparing the retention times of the standards and the peaks of the sample using the corresponding mobile phase described in the Experimental section. Peak confirmation is necessary because generally the chromatograms of real samples present peaks that elute at the same retention times as the HAs. In order to confirm the identification of the HAs, the mobile phase in gradient mode proposed by Gross and Grüter [14] was used. The retention times for Trp-P-2, harman, norharman, and AαC agreed with the standards in both mobile phases. Furthermore, the identity of each suspected HA was studied by comparing the UV spectrum, obtained with a photodiode-array detector, with those of the standards as recommended in the literature [1,7,16] for complex samples. Recently, Jackson et al. [16] showed that many peaks are present in beef flavor samples and emphasized the importance of peak confirmation to rule out coeluting interferences. The presence of

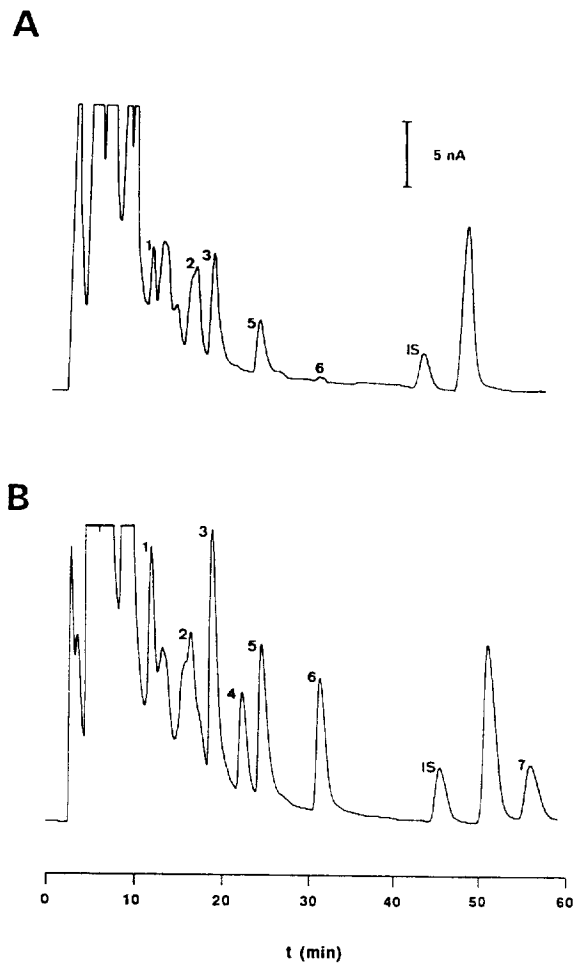

Fig. 5. HPLC–ED chromatogram of extract B of a beef extract sample. Mobile phase: 50 m*M* ammonium acetate (pH 5.7)–acetonitrile (70:30), at 0.8 ml min^{-1}. (A) Extract B in 50 μl IS solution, (B) extract B spiked before the clean-up (250 ng) dissolved in 100 μl of IS solution. Peaks: 1 = Trp-P-2; 2 = Trp-P-1; 3 = harman; 4 = PhIP; 5 = norharman; 6 = AαC; IS = 1-naphthylamine; 7 = MeAαC.

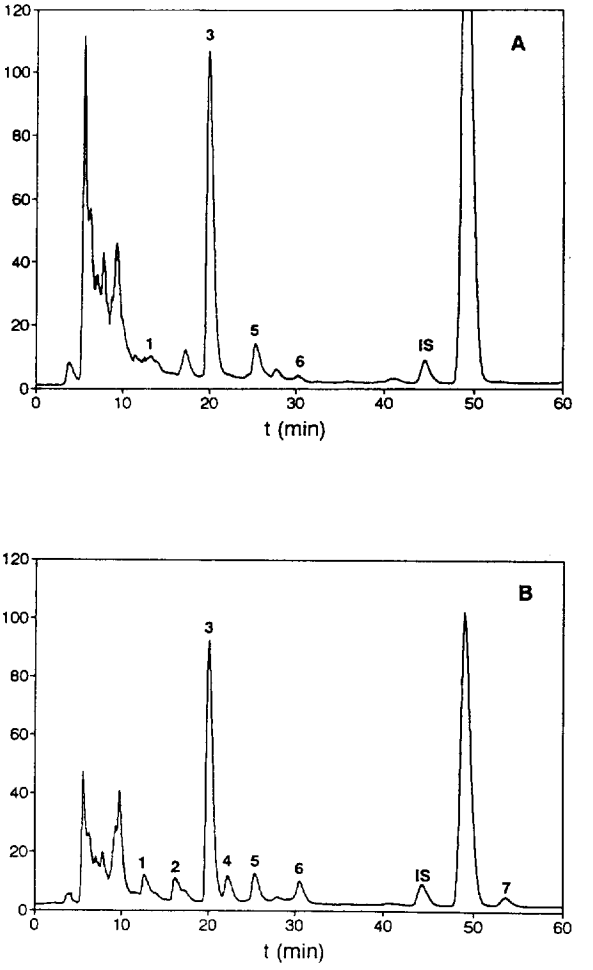

Fig. 6. HPLC–fluorescence chromatogram of extract B of a beef extract sample. Mobile phase: 50 m*M* ammonium acetate (pH 5.7)–acetonitrile (70:30), at 0.8 ml min^{-1}. (A) Extract B in 50 μl IS solution, (B) extract B spiked before the clean-up (250 ng) dissolved in 100 μl of IS solution. Peaks: 1 = Trp-P-2; 2 = Trp-P-1; 3 = harman; 4 = PhIP; 5 = norharman; 6 = AαC; IS = 1-naphthylamine; 7 = MeAαC.

harman and norharman in the samples was confirmed with photodiode-array detection. For Trp-P-2 and AαC the detector sensitivity was not high enough to confirm their presence in the sample, although they have been detected using electrochemical and fluorescence detectors.

Quantification was performed by electrochemical and fluorescence detection, and the results are given in Table 5. Values of 50 and 110 ng g^{-1} were obtained for norharman and harman, respectively. The estimated values for AαC and Trp-P-2 were from 2 to 14 ng g^{-1}, although the presence of these compounds could not be confirmed by photodiode-array detection. Other amines such as IQ, MeIQ, MeIQx, 4,8-DiMeIQx, Glu-P-1, and PhIP, which have been detected in some beef extracts [5–7,15–21], were not detected in this sample (Figs. 3–6).

4. Conclusions

The optimization of the PRS step in the solid-phase extraction Extrelut–PRS–C_{18} coupled cartridges allowed the effective separation of a complex group of twelve HAs and related substances into two well-defined groups: (A) IQ, MeIQ, MeIQx, and 4,8-DiMeIQx, and (B) Glu-P-1, Trp-P-1, Trp-P-2, harman, norharman, PhIP, AαC, and MeAαC. The proposed procedure causes an important change in the extraction selectivity of the PRS. Extract A contained the aminoimidazoquinoline and -quinoxaline derivatives, in agreement with the data of the literature. Otherwise, extract B included, together with the indolpyridine derivatives, the imidazopyridine ones (PhIP and Glu-P-1), in contrast to the results obtained with other extraction procedures [14,16]. One of the main advantages of this is that all the fluorescent compounds were eluted in the same fraction, simplifying their detection by the use of this highly selective and sensitive detection technique. Aminoimidazoquinoline and -quinoxaline derivatives (extract A) gave a clean chromatogram, and good detection limits using electrochemical detection were obtained. Indolpyridine and imidazopyridine derivatives (extract B) could be successfully analyzed using both electrochemical and fluorescence detection. The procedure developed was applied to the analysis of a commercial beef extract, giving good recoveries for the twelve HAs.

References

[1] M.G. Knize, J.S. Felton and G.A. Gross, J. Chromatogr., 624 (1992) 253.
[2] J.S. Felton and M.G. Knize, Mutat. Res., 259 (1991) 205.
[3] T. Sugimura, T. Kawachi, M. Nagao, T. Yahagi, Y. Seino, T. Okamoto, K. Shudo, T. Kosuge, K. Tsuji, K. Wadabayashi, Y. Iitaka and A. Itai, Proc. Jpn. Acad., 53 (1977) 58.
[4] T. Sugimura, M. Nagao and K. Wakabayashi, J. Natl. Cancer Inst., 86 (1994) 2.
[5] G.A. Gross, Carcinogenesis, 11 (1990) 1597.
[6] M.T. Galceran, P. Pais and L. Puignou, J. Chromatogr. A, 655 (1993) 101.
[7] R. Schwarzenbach and D. Gubler, J. Chromatogr., 624 (1992) 491.
[8] C.G. Edmonds, S.K. Sethi, Z. Yamaizumi, H. Kasai, S. Nishimura and J.A. McCloskey, Environ. Health Perspect., 67 (1986) 35.
[9] H. Milon, H. Bur and R. Turesky, J. Chromatogr., 394 (1987) 201.
[10] S. Vaimiotalo, K. Matveinen and A. Reunanen, Fresenius J. Anal. Chem., 345 (1993) 462.
[11] S. Murray, A.M. Lynch, M.G. Knize and N.J. Gooderham, J. Chromatogr. Biomed. Appl., 394 (1987) 201.
[12] M. Vanderlaan, B.E. Watkins, M. Hwang, M.G. Knize and J.S. Felton, Carcinogenesis, 9 (1988) 153.
[13] S. Murray, N.J. Gooderham, A.R. Boobis and D.S. Davies, Carcinogenesis, 9 (1988) 321.
[14] G.A. Gross and A. Grüter, J. Chromatogr., 592 (1992) 271.
[15] G.A. Gross, A. Grüter and S. Heyland, Food Chem. Toxicol., 30 (1992) 491.
[16] L.S. Jackson, W.A. Hargraves, W.H. Stroup and G.W. Diachenko, Mutat. Res., 320 (1994) 113.
[17] J.J. Richard and J.S. Fritz, J. Chromatogr. Sci., 18 (1980) 35.
[18] R.J. Turesky, H. Bur, T. Huyng-Ba, H.U. Aeschbacher and H. Milon, Food Chem. Toxicol., 26 (1988) 501.
[19] M. Takahashi, K. Wakabayashi, M. Nagao, M. Yamamoto, T. Masui, T. Goto, N. Kinae, I. Tomita and T. Sugimura, Carcinogenesis, 6 (1985) 1195.
[20] G.A. Gross, G. Philippossian and H.U.Aeschbacher, Carcinogenesis, 10 (1989) 1175.
[21] J.S. Felton, M.G. Knize, M. Roper, E. Fultz, N.H. Shen and K.W. Turteltaub, Cancer Res., 52 (Suppl.) (1992) 2103s.
[22] J.N. Miller, Analyst, 116 (1991) 3.