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Analysis of heterocyclic amines in food products: interlaboratory studies

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Abstract

A feasibility study and two interlaboratory exercises on the determination of selected heterocyclic amines (HAs) in beef extract, organised in the framework of a European project, are presented. The aim of these exercises was to improve the quality of the laboratories and to evaluate the performance of a standardised analytical method and also the methods currently used by each of the participants for the analysis of these compounds. Three lyophilised portions of a commercial beef material previously spiked with HAs at different concentration levels ranging from 10 to 75 ng g^{-1} were used as laboratory reference materials (lot A, B and C). Firstly, a feasibility study was carried out using a test standard solution and the beef extract (lot A), which contained only five HAs. Then, two interlaboratory exercises were carried out using the laboratory reference materials lot B and lot C, containing 10 selected HAs at two different concentration levels, 75 and 10 ng/g, respectively. The results obtained by all participant laboratories using the proposed method showed satisfactory agreement and the CV(%) between-laboratories obtained were from 8.3 to 24.1% for lot B and from 8.7 to 44.5% for lot C. The standardised method evaluated in these collaborative studies is therefore proposed for the analysis of HAs in food material. Moreover, LC–MS is recommended as the most suitable technique for the analysis of a large number of HAs in food samples.

Keywords: Food analysis; Inter-laboratory studies; Heterocyclic aromatic amines

1. Introduction

The contribution of the heterocyclic amines (HAs) to the mutagenic activity detected in some cooked meat and fish products has been known for more than 25 years [1,2]. These compounds are produced during the cooking of meat and fish products [3,4]and might contribute to the aetiology of human cancer [5]. In recent years, a growing number of studies have been reported on heterocyclic aromatic amines (HAs) in food samples, their chemistry, the formation of new mutagenic HAs and their biological activity and potential human toxicity [6–8]. To date, more than 20 HAs have been identified and isolated as potent mutagens from various food samples, and most of their structures have been elucidated [9]. Moreover, attempts have been made to develop or improve analytical methods for the determination of HAs in food [10,11]. Chromatographic techniques, such as liquid chromatography with selective detectors [12–15] or coupled to mass spectrometry [16,17], are needed to achieve high selectivity and sensitivity. The establishment of a standard methodology for HAs analysis has been attempted by means of interlaboratory exercises, although results have been unsatisfactory [18], mainly due to the lack of reference materials and validated analytical methods. In addition, the analysis of HAs is difficult because their concentration levels in food samples are very low (0.1–50 ng g^{-1}) and the complexity of the matrix often involves the use of extensive and laborious procedures for sample preparation. Consequently, reference materials and collaborative interlaboratory exercises are needed for the establishment of a standard methodology.

Here we present the results of a feasibility study and two interlaboratory exercises on the determination of selected heterocyclic amines in three meat extract (ME) laboratory

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reference materials which include the HAs most frequently found in foods. These exercises were conducted between October 2000 and November 2002 in the framework of a European project (QLK1-CT99-001197) with the aim of improving the quality of the participant laboratories and to detect problems and sources of error in the analytical procedures for the analysis of HAs. A common analytical procedure based on the Gross method [19] with several modifications [20], and some other methods were evaluated with the aim of proposing a standardised method for the analysis of HAs in food samples.

2. Experimental

2.1. Chemicals

The HAs studied were 2-amino-1,6-dimethylimidazo[4,5-*b*]pyridine (DMIP), 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), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-9*H*-pyrido-[2,3-*b*]indole (AαC), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*] indole (MeAαC), 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), and they were obtained from Toronto Research Chemicals Inc. (Toronto, Canada).

For the preparation of the test solution (TS; laboratory reference material) individual stock standard solutions of each HA (75 $\mu g\,g^{-1}$) in methanol were used. Methanol of HPLC grade was supplied by Merck (Darmstadt, Germany). Amber glass capillary-bottles Certain® of 4.5 ml were obtained from Promochem (Wesel, Germany)

2.2. Participants

The interlaboratory exercises were performed by four institutes in the framework of the European project "Heterocyclic Amines in Cooked Foods—Role in Human Health" from the EU programme Quality of Life and Management of Living Resources (QLK1-CT99-001197). The participants were the Department of Analytical Chemistry of the University of Barcelona (Spain), the Department of Applied Nutrition and Food Chemistry, Center for Chemistry Chemical Engineering of Lund University (Sweden), the Department of Biochemistry and Food Chemistry of Graz University of Technology (Austria), and the Institute for Analytical Chemistry of Vienna University (Austria). Two independent analysis were performed by each institute in order to generate eight sets of results for each intercomparison exercise. This part of the project was co-ordinated by the Department of Analytical Chemistry of the University of Barcelona (Spain), which also performed the preparation and the homogeneity and stability control of the laboratory reference materials as well as the statistical treatment of the data.

2.3. Reference materials

The HAs used in the interlaboratory exercises were chosen on the basis of their presence in cooked foods, their mutagenic/carinogenic activity and their commercial availability [6,7,10,11].

In order to evaluate the validity of the chromatographic determination method of each participant, a preliminary study based on the analysis of HAs in a test solution was performed. A test standard solution (1.2 $\mu g\,g^{-1}$) containing all the HAs was prepared from individual stock HA standard solutions by dilution in methanol. Two capillary-bottles Certain® of 4.5 ml filled with c.a. 4 ml of this solution were sent to each participant as a test solution of unknown concentration for the preliminary feasibility study.

Three portions of a commercial beef material (Bovril, Bestfoods España S.A., Spain) were spiked with HAs at different concentration levels ranging from 10 to 75 $ng\,g^{-1}$ [21]. For preparation, three batches of c.a. 2–5 kg of the beef material were mixed with different amounts of a

Table 1
Interlaboratory exercises

Preliminary exercise	Time period	October 2000–January 2001
	Objective	Determine the quality of the routine laboratory methods for the determination of HAs in a standard solution
	Sample	Test solution of HAs (~1.2 $\mu g\,g^{-1}$)
Feasibility study[a]	Time period	June 2001 to September 2001
	Objective	Determine the performance of the analytical method for the determination of HAs in a meat extract
	Sample	Meat extract lot A (~50 $ng\,g^{-1}$)
First interlab. Exercise	Time period	October 2001 to January 2002
	Objective	Determine the accuracy and precision of both a recommended method and an own method for the analysis of HAs in a meat extract (high level)
	Sample	Meat extract lot B (~75 $ng\,g^{-1}$)
Second interlab. Exercise	Time period	June 2002 to October 2002
	Objective	Determine the accuracy and precision of both a recommended method and an own method for the analysis of HAs in a meat extract (low level)
	Sample	Meat extract lot C (10 $ng\,g^{-1}$)

[a] A second round of the preliminary exercise using a test solution was also performed.

spiked standard solution of 130 μg g^{-1} using a mixture of methanol:water (1:99) as solvent. After homogenisation, the three meat extracts were lyophilised, grinded, sieved at 250 μm and bottled for the interlaboratory exercises. The three meat extracts were: lot A, containing IQ, MeIQ, MeIQx, PhIP and AαC at ∼50 ng g^{-1}; lot B and C, containing DMIP, IQ, MeIQ, MeIQx, 4,8-DiMeIQx, Trp-P-2, Trp-P-1, PhIP, AαC and MeAαC at a concentration of ∼75 and 10 ng g^{-1}, respectively. Details of the preparation steps and the homogeneity and stability studies of these laboratory reference materials are given elsewhere in this issue [21].

2.4. Design of the interlaboratory exercises

Before the analysis of the reference meat extracts, a preliminary exercise was organised in order to familiarize the participant laboratories with these exercises and to check their own determination method. For this purpose a test solution (1.2 μg g^{-1}) was sent to the participants. The overall results of this first exercise were encouraging, but errors of accuracy and poor agreement between laboratories were observed, mainly due to problems related with instrument calibration and lack of precision of chromatographic data. After discussion a critical discussion of the results of the first round a second round using the same test solution was performed to improve the quality of the determination method before proceeding to the analysis of meat extracts. In these two studies, all laboratories were required to use at least two detection systems.

The feasibility exercise was focused on the analysis of a meat extract lot A, containing only five HAs (∼50 ng g^{-1}). This material was sent to the participants in order to determine the quality of their analytical methods used for routine analysis. In addition, the use of an analytical method

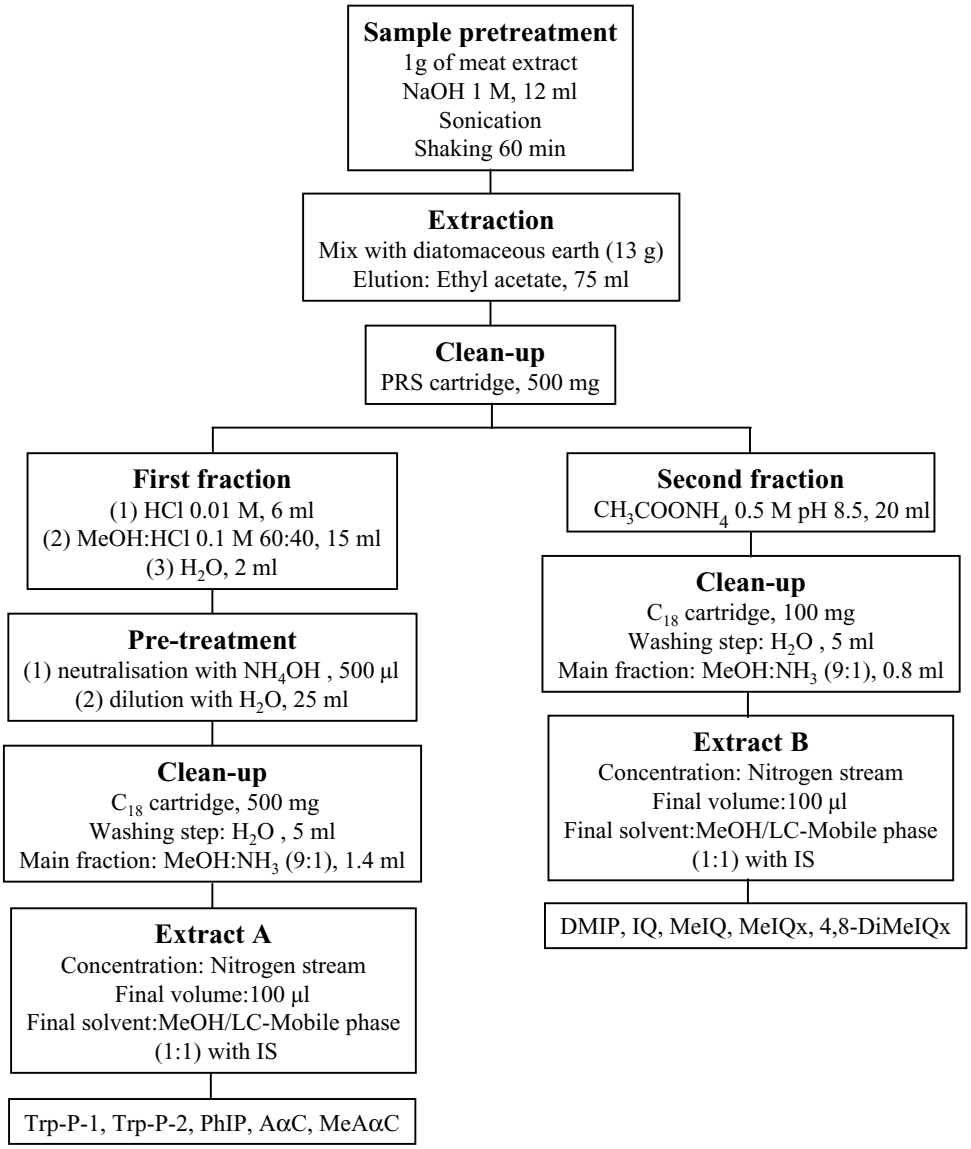

Fig. 1. Scheme of the extraction and clean-up procedures recommended for the analysis of HAs in meat extract.

based on a modification of the Gross method [19,20] was proposed to the participants in order to evaluate its applicability as recommended method for the analysis of HAs in meat extracts.

The two following exercises were carried out to evaluate the skill of the laboratories. Two meat extracts spiked with 10 HAs at two concentration levels: high ($\sim$75 ng g^{-1}) for lot B and low ($\sim$10 ng g^{-1}) for lot C, were used. The timetable of the exercises, the objective and the type of material analysed are summarised in Table 1.

2.5. Analytical methods

Samples were analysed by two methods: a recommended method, tentatively proposed as common method, and the method currently used by each laboratory. Nevertheless, only two participants applied their own method. In order to improve the number of results in the interlaboratory exercises for statistical evaluation, the participants without own method were required to perform two independent determinations (different analysts, calibrations, instruments, etc.) using the recommended standardised analytical method. Therefore, different clean-up methods or different analysts are considered as independent laboratories for the statistical treatment of the results. The laboratories were numbered from 1 to 4 and the two independent methods performed by each participant were designated as A and B. The overall procedure for the recommended method is summarised in Fig. 1. The method proposed is based on the extraction of HAs from the meat extract by mixing a suspension of the material with sodium hydroxide solution with Extrelut material and eluting them using ethyl acetate. The extract is then purified using a PRS cartridge obtaining two fractions which were cleaned-up using C_{18} and contain the less polar amines Trp-P-1, Trp-P-2, PhIP, AαC and MeAαC (Fraction 1) and the polar amines DMIP, IQ, MeIQ, MeIQx and 4,8-DiMeIQx (Fraction 2), respectively. This method was used for the analysis of HAs in all meat extracts (labs 1A, 2A, 2B, 3A, 4A and 4B). In addition, two methods that yielded only one fraction containing all HAs were also applied (labs 1B and 3B). In Fig. 2 the overall procedures for these two methods are given. Sample treatment and the extraction of the HAs was similar to the recommended method, but the clean-up was carried out on a PRS cartridge in acidic form using ammonium acetate prior to the C_{18} clean-up (lab 1B) or on an OASIS cartridge with methanol with ammonia/water (lab 3B).

2.6. Chromatographic conditions

Liquid chromatography coupled to mass spectrometry (LC–MS) was the main technique used by the

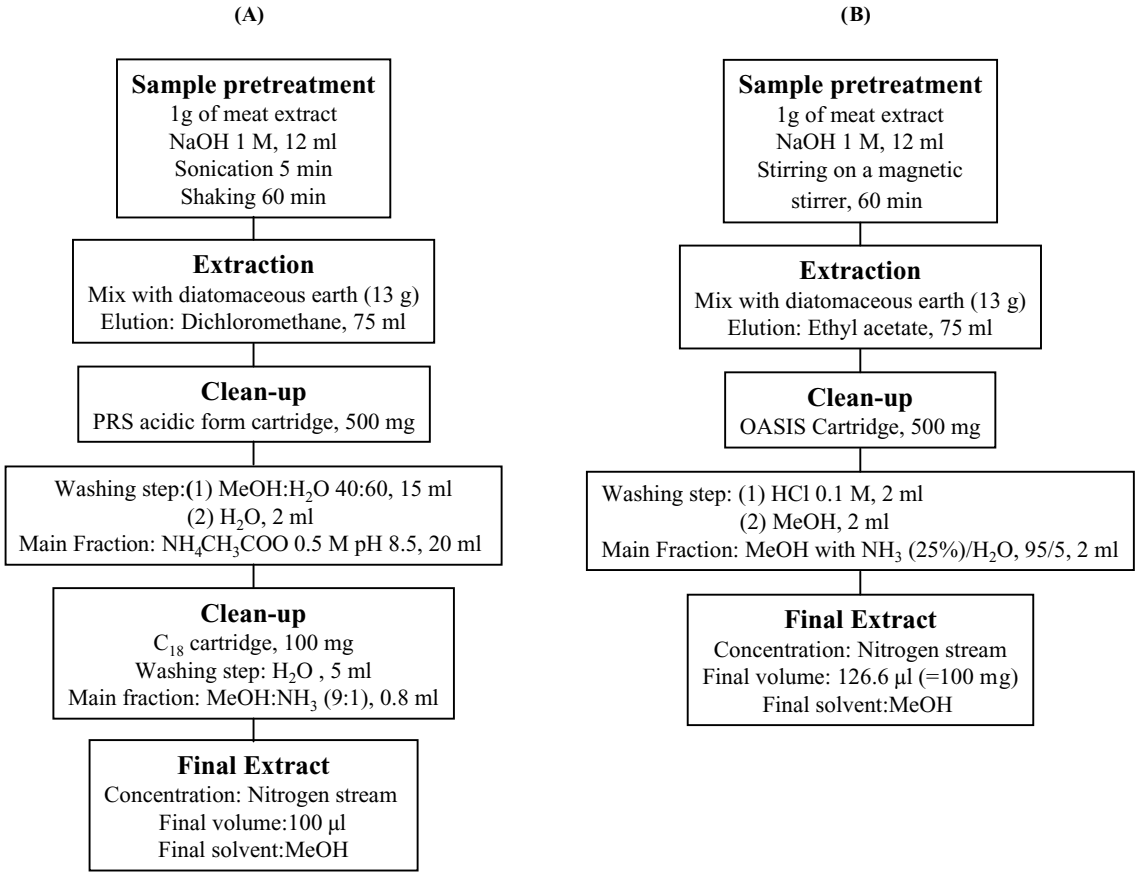

Fig. 2. Schemes of the extraction and clean-up procedures based on single extract used by (A) lab 1B and (B) lab 3B.

Table 2
Summary of the liquid chromatography techniques used for the determination of HAs in the test solution (TS) and meat extracts (ME)

Lab number	Exercise	Column type and particle size	Column dimensions (length × i.d., mm)	Mobile phase	Flow (ml/min)	Elution	Detection	Acquisition mode	Internal standard
1A	TS	Zorbax SB-C8, 3.5 μm (Agilent Technologies)	150 × 2.1	ACN/formic acid:ammonium formate, pH 3.7	0.3	Gradient	ESI–MS (ion-trap)	Full-scan	4,7,8-TriMeIQx
	ME	Symmetry C8, 5 μm (Waters)	150 × 2.1	ACN/formic acid:ammonium formate, pH 4.5	0.3	Gradient	ESI–MS (quadrupole)	SIM	4,7,8-TriMeIQx
		Semi-Micro TSKgel ODS-80TM C18, 5 μm (Toso Haas)	150 × 2.1	ACN/formic acid:ammonium formate, pH 4.0	0.3	Gradient	ESI–MS/MS (ion-trap)	product ion full-scan	4,7,8-TriMeIQx
							ESI–MS/MS (triple-Q)	MRM	4,7,8-TriMeIQx
1B	TS	Semi-Micro TSKgel ODS-80TM C18, 5 μm (Toso Haas)	250 × 4.6	ACN/formic acid:ammonium formate, pH 3.25	1	Gradient	APCI–MS/MS (ion-trap)	product ion full-scan	4,7,8-TriMeIQx
	ME	Semi-Micro TSKgel ODS-80TM C18, 5 μm (Toso Haas)	250 × 4.6	ACN/formic acid:ammonium formate, pH 3.25	1	Gradient	APCI–MS/MS (ion-trap)	product ion full-scan	4,7,8-TriMeIQx
							APCI–MS/MS (triple-Q)	MRM	D_3-IQ, D_3-MeIQx, D_3-PhIP, 4,7,8-TriMeIQx
2A	TS	Semi-Micro TSKgel ODS-80TM C18, 5 μm (Toso Haas)	250 × 4.6	ACN/acetic acid with TEA	1	Gradient	UV-DAD	Wavelength 220–360 nm	4,7,8-TriMeIQx
	ME	Semi-Micro TSKgel ODS-80TM C18, 5 μm (Toso Haas)	250 × 4.6	ACN/acetic acid with TEA	1	Gradient	UV-DAD and Fluor.	Variable wavelength	7,8-DiMeIQx and 4,7,8- TriMeIQx
		Zorbax SB-C8, 3.5 μm (Agilent Technologies)	150 × 4.6	ACN/acetic acid pH 3.5	1	Gradient	ESI–MS (ion-trap)	SIM	4,7,8-TriMeIQx
2B	TS/ME	Zorbax SB-C8, 3.5 μm (Agilent Technologies)	150 × 4.6	ACN/acetic acid pH 3.5	1	Gradient	ESI–MS (ion-trap)	SIM	4,7,8-TriMeIQx
3A	TS/ME	Semi-Micro TSKgel ODS-80TM C18, 5 μm (Toso Haas)	250 × 2	ACN/MeOH/acetic acid pH 5	0.3	Gradient	ESI–MS (quadrupole)	SIM	4,7,8-TriMeIQx
3B	TS	Semi-Micro TSKgel ODS-80TM C18, 5 μm (Toso Haas)	250 × 2	ACN/MeOH/acetic acid pH 5	0.3	Gradient	UV-DAD	Wavelength 262 nm	Not used
	ME	Semi-Micro TSKgel ODS-80TM C18, 5 μm (Toso Haas)	250 × 2	ACN/MeOH/acetic acid pH 5	0.3	Gradient	ESI–MS (quadrupole)	SIM	4,7,8-TriMeIQx
4A	TS	LiChrospher 60RP-Select B, 4 μm (Merck)	250 × 2	CAN/water/acetic acid:sodium acetate + trichloroacetic acid	0.3	Isocratic	Electrochem.	Simultaneous detection at 8 different potentials	4,7,8-TriMeIQx
	ME	LiChrospher 60RP-Select B, 4 μm (Merck)	250 × 4	ACN/water/acetic acid:sodium acetate + trichloroacetic acid	1.2	Gradient	Electrochem.	Simultaneous detection at 8 different potentials	7,8-DiMeIQx
4B	TS	LiChrospher 100RP-S, 5 μm (Merck)	250 × 4	ACN/MeOH/acetic acid: ammonium acetate pH 5.35	0.8	Gradient	UV and Fluor.	Variable wavelength	4,7,8-TriMeIQx
	ME	LiChrospher 100RP-S, 5 μm (Merck)	250 × 4	ACN/MeOH/acetic acid: ammonium acetate pH 5.35	0.8	Gradient	UV and Fluor.	Variable wavelength	4,7,8-TriMeIQx
		LiChrospher 60RP-select B, 4 μm (Merck)	250 × 2	ACN/acetic acid:ammonium acetate pH 5.75	0.25	Gradient	Fluorescence	Variable wavelength	Napththalene

ACN: acetonitrile; TEA: triethanolamine; MeoH: methanol.

participants, but LC with electrochemical, fluorescence and UV-detection were also applied. In LC–MS, the compounds were identified by *m*/*z* values of their protonated molecule ion $[M + H]^+$ for MS experiments and using selected product ions for MS/MS determinations. For the other detection systems, the identification was performed from the retention times and the compounds were confirmed by standard addition. Most laboratories used the TSK-Gel ODS 80T-C8 column (5 μm, 250 mm × 4.6 mm i.d.) from Toso-Haas (Stuttgart, Germany), although the Symmetry C8 LC column (5 μm, 150 mm × 2.1 mm i.d.) from Waters (Milford, MA, USA) and the Zorbax SB-C8 column (3.5 μm, 150 mm × 2.1 mm i.d. and 4.6 mm i.d.) were also used successfully. Finally, Lichrosper 100RP-S column (5 μm, 250 mm × 4 mm i.d.) and LiChrospher 60RP-Select B column (4 μm, 250 mm × 4 mm i.d. and 2 mm i.d.) from Merck (Darmstadt, Germany) were used by lab 4B combined with UV, fluorescence and electrochemical detection. A range of mobile phase compositions was used to exploit the high selectivity of chromatographic systems. Table 2 summarises the chromatographic conditions for the TS and for ME. LC with UV, fluorescence and electrochemical detection were only used by three participants (labs 2A, 4A and 4B). The other laboratories applied LC–MS with electrospray as ionisation technique (ESI) and atmospheric pressure chemical ionisation (APCI) was only used by lab 1B. In addition, selected ion monitoring (SIM) and full-scan modes were used for acquisition in MS methods, while multiple reaction monitoring (MRM) and product ion full-scan were the acquisition modes for MS/MS. Ion-traps, quadrupoles and a triple-quadrupole were used. In all cases, quantification based on peak area was preferred and 4,7,8-TriMeIQx was selected as internal standard by the most of the laboratories. Only lab 1B proposed the use of the isotopically labelled compounds D_3-IQ, D_3-MeIQx and D_3-PhIP for the analysis HAs in meat extract lot B. For fluorescence detection (lab 4B) the internal standard was naphthalene.

2.7. Statistical treatment of the results

The results were discussed in separate meetings after statistical treatment. If it turned out in the critical discussion that some of the raw data might be wrong due to systematic errors (e.g. poor peak shape, calibration errors, insufficient chromatographic separation, incorrect peak assignment or extremely large or bimodal distribution of the data sets), these data have been eliminated from the data set. All results were treated using the software SoftCRM (version 1.0.2) [22] recommended by the Standard, Measurements and Testing Programme [23,24] for certification exercises.

Following the technical discussion the accepted results were subjected to a series of statistical tests:

- Kolmogorov–Smirnov–Lilliefors tests to assess the conformity of the distribution of laboratory means to normal distributions.
- Dixon and Nalimov tests to detect outlying values in the population of laboratory means.
- Cochran test to detect outlying values in the laboratory variances.
- Barlett test to assess the overall consistency of the variance values obtained in the participating laboratories.
- Snedecor *F*-test to check if the between-laboratory variance is significant.
- Scheffe *t*-test to estimate the two-by-two compatibility of individual data sets.

3. Results and discussion

3.1. General

In all exercises, each laboratory performed six independent replicate determinations of HAs using the recommended analytical method or its own method on three separate days and from different bottles. In addition, experiments on recovery, procedure blanks and detector linearity were carried out following a protocol discussed beforehand. The moisture content of the samples was determined from each bottle and day using a sample intake not lower than 1 g. Karl–Fisher and gravimetry were the methods used for the determination of moisture, which was 6.5%. For quantification of the HAs in the meat extract materials, standard addition was recommended in order to avoid matrix effects on the detection. Four spiking levels around 50, 100, 150 and 200%, of the native concentration of the HAs in the sample were proposed. For each replicate analysis, a standard addition curve was performed each day. In addition, participants were requested to use standards of high purity from traceable origin as far as possible and the calibration solutions were prepared by weight, using calibrated balances. The use of internal standards was mandatory. It was necessary to establish that internal standards selected were not present in the meat extract.

3.2. Preliminary exercises

A test standard solution containing twelve HAs (DMIP, IQ, MeIQ, MeIQx, 7,8-DiMeIQx, 4,8-DiMeIQx, TriMeIQx, Trp-P-2, PhIP, Trp-P-1 AαC and MeAαC) at a concentration level around 1.2 μg g^{-1} of each amine was sent to the participants to determine the quality of the chromatographic determination methods. The results are summarised in Table 3. The statistical treatment of the raw data of the exercise revealed a high spread of results due to systematic errors detected during the exercise. The main problems were related to calibration and quantification methods. For instance, in some cases the concentration of the samples was outside the working calibration range and poor regression coefficients were obtained. In addition, high standard deviations were obtained by some participants and there were large important differences within laboratories in the results obtained

Table 3
Results obtained on the first and second preliminary exercises for the determination of HAs in a test solution

HA	Target value ($\mu g\,g^{-1}$)	First preliminary interlaboratory exercise					Second preliminary interlaboratory exercise				
		Mean of lab means ($\mu g\,g^{-1}$)	S.D.	CV%	Labs	Percentage relative error	Mean of lab means ($\mu g\,g^{-1}$)	S.D.	CV%	Labs	Percentage relative error
DMIP	1.431	1.39	0.19	13.8	8	−2.9	1.48	0.03	2.1	8	3.4
IQ	1.841	1.72	0.18	10.3	8	−6.4	1.75	0.10	5.5	6	−5.2
MeIQ	1.440	1.36	0.19	13.7	8	−5.3	1.43	0.10	7.3	7	−0.8
MeIQx	1.355	1.41	0.17	12.1	8	4.1	1.40	0.12	8.6	7	3.3
7,8-DiMeIQx	1.354	1.36	0.09	6.4	8	0.1	1.38	0.09	6.3	7	4.6
4,8-DiMeIQx	1.417	1.47	0.13	8.9	8	3.7	1.44	0.14	9.7	7	1.3
Trp-P-2	1.144	1.15	0.15	12.8	8	0.1	1.08	0.09	8.2	6	−5.8
PhIP	1.172	0.99	0.24	24.0	8	−15.4	1.18	0.07	6.2	6	0.7
Trp-P-1	1.109	1.18	0.43	36.5	8	5.9	1.00	0.07	6.5	6	−9.6
AαC	1.487	1.52	0.27	17.9	8	2.0	1.54	0.03	2.2	6	3.2
MeAαC	1.550	1.44	0.31	21.4	8	−7.2	1.49	0.09	5.9	7	−3.7

with the two detection methods. After a thorough discussion of the results and to overcome these problems, a second exercise using the same test solution was organised before proceeding to the analysis of the meat extracts.

In the second round, the results (Table 3) agreed and low relative standard deviations (<10%) were obtained. Moreover, the means of the accepted laboratory means were in agreement with the target values, being the relative errors lower than 6%, except for Trp-P-1, which was 9.6%. Although for some amines the relative error was slightly higher than those obtained in the first exercise, the values of the second round were most reliable because the variability between laboratory means were lower. As an example, a comparison of the results for MeIQ in the first and second feasibility exercises is given in Fig. 3 where a better within- and between-laboratory precision and high accuracy can be observed in the second exercise.

3.3. Feasibility interlaboratory exercise

After the preliminary exercises, where all participants demonstrated their ability to perform accurate and precise HAs determinations in a test solution, a feasibility study of the analysis of five HAs in a food sample was carried out. For this purpose, the meat extract lot A containing only five HAs was used to demonstrate the proficiency of the participants. All laboratories carried out the analysis using the recommended method and lab 1B used an alternative determination method previously validated with the test solution. The results are summarised in Table 4. Generally, the results showed satisfactory agreement with acceptable between-laboratory precision lower than 28%. In addition, the within-laboratory coefficient of variation ranged from 2 to 30% and no significant differences between methods were detected.

3.4. First and second interlaboratory exercises

In order to evaluate the performance of the recommended analytical method, two interlaboratory exercises were performed. Two laboratory reference materials, meat extracts lot B and C, spiked at ∼75 and 10 $ng\,g^{-1}$ were used. For lot B, the results obtained by all participants agreed between them, except for some laboratories which gave values significantly lower than the others. Moreover, some laboratories gave high coefficient of variation (50–75%). The sources of error detected were related with the lack of linearity of the standard addition curve, low recoveries (<15%) of analytes and no use of internal standards on quantification. For meat extract lot C acceptable results were also obtained, although higher variability was observed. After detecting and removing the outlying mean values (Dixon and Nalimov tests) and variances (Cochran test), the results obtained for the first and second intercomparison exercises are given in Table 5, respectively. Generally, the results of the accepted data sets were in agreement between them and the coefficient of variation between-laboratories ranged from 8.3 to 24.1% for lot B and between 8.7 and 30% for lot C, except for DMIP, IQ and PhIP, which were higher (38–44%). These values are in agreement with the expected Horwitz CV(%) for samples at these concentration levels [25,26]. Moreover, the within-laboratory precision ranged from 4 to 38% for meat extract lot B and from 5 to 40% for lot C. As an example, the results obtained for MeIQ and Trp-P-2 in the second exercise (lot C) are given in Fig. 4, where satisfactory agreement between results can be observed. Regarding the laboratory means, no significant differences between laboratories were obtained. The concentrations determined on the meat extract lot B were between 63.6 and 71.9 $ng\,g^{-1}$, while for lot C the values ranged from 7.2 to 11.0 $ng\,g^{-1}$. Taking to account the results obtained in these two exercises, the common analytical procedure based on the Gross method [19] can be proposed as recommended method for the analysis of HAs in food samples. Regarding the methods that give one extract containing all the amines, only the one proposed by lab 1B (method (A) in Fig. 2) yielded results in agreement with those of the recommended method.

All the LC detection systems gave good results for the analysis of the test solution. In contrast, for meat extracts UV

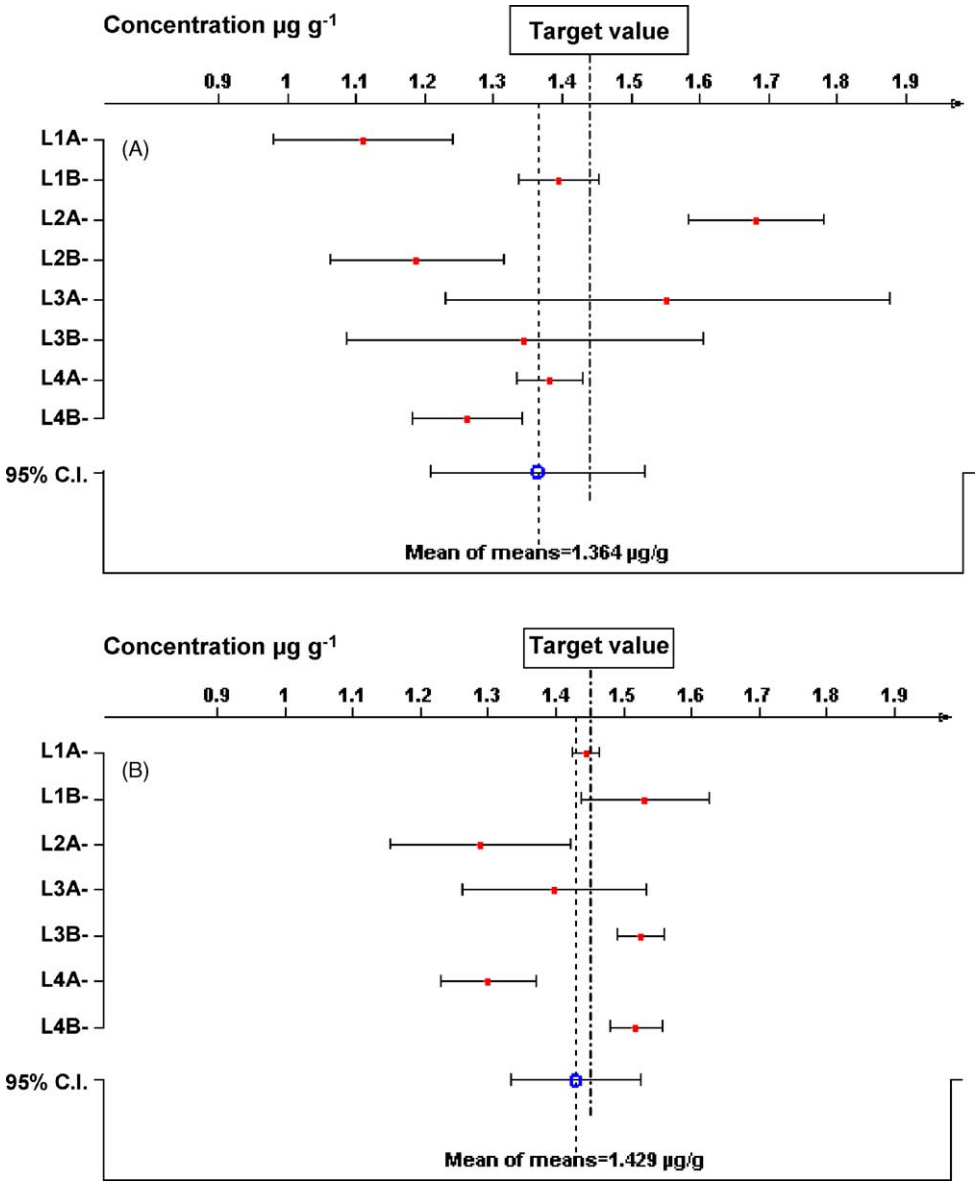

Fig. 3. Mean value of laboratory means and 95% confidential interval for the determination of MeIQ in the (A) first and (B) second feasibility interlaboratory exercises on the analysis of HAs in a test solution.

did not give satisfactory results due to interferences of the matrix. Moreover, electrochemical detection only could be used for the analysis of lot B, which had relatively high concentrations of HAs. In addition, for the analysis of meat extracts with LC-electrochemical detection, dichloromethane must be used as solvent instead of ethyl acetate in the first step of the clean-up procedure (diatomaceous earth) to prevent interferences. LC–MS always gave acceptable results, even for lot C, which contained the lowest HAs concentration. Finally, fluorescence detection appears to be a suitable

Table 4
Results obtained on the feasibility interlaboratory exercise for the analysis of HAs in meat extract lot A

HA	Mean of lab means ± S.D. (ng g^{-1})	Between-lab precision CV (%)	Within-lab precision range CV (%)	Data sets/individual data
IQ	39.6 ± 8.9	22.4	4.4–17.9	6/32
MeIQ	38.1 ± 4.8	12.7	6.1–19.9	5/30
MeIQx	43.2 ± 11.0	25.5	2.6–25.9	6/34
PhIP	40.4 ± 4.7	11.6	2.0–20.0	6/34
AαC	38.8 ± 8.9	22.9	5.0–30.0	6/34

Table 5
Results obtained on the interlaboratory exercises for the analysis of HAs in meat extracts lot B and C

HA	Meat extract lot B				Meat extract lot C			
	Mean of lab means ± S.D. (ng g^{-1})	Between-lab precision CV (%)	Within-lab precision range CV (%)	Data sets/ individual data	Mean of lab means ± S.D. (ng g^{-1})	Between-lab precision CV (%)	Within-lab precision range CV (%)	Data sets/ individual data
DMIP	68.1 ± 9.1	13.4	4.8–38.2	4/24	11.0 ± 4.1	37.5	13.4–21.2	5/30
IQ	64.5 ± 8.8	13.6	3.4–18.6	5/28	9.8 ± 4.4	44.5	9.1–35.0	5/30
MeIQ	63.6 ± 11.6	18.2	4.9–27.3	5/30	10.4 ± 1.1	10.9	11.1–30.1	4/24
MeIQx	64.5 ± 15.6	24.1	4.0–36.3	5/26	8.8 ± 1.5	17.4	7.7–27.8	4/24
4,8-DiMeIQx	71.6 ± 8.7	12.1	4.7–30.3	4/24	8.8 ± 2.7	30.0	5.2–36.3	6/36
Trp-P-2	68.6 ± 12.1	17.7	6.3–37.1	4/28	9.2 ± 1.1	12.4	9.2–36.2	5/30
PhIP	71.9 ± 9.5	13.3	5.1–22.3	6/36	9.6 ± 3.9	40.6	10.3–27.8	7/40
Trp-P-1	71.4 ± 10.6	14.9	6.6–32.3	5/30	7.2 ± 0.8	11.7	7.3–30.0	6/36
AαC	72.0 ± 6.6	9.2	4.2–26.1	5/30	8.6 ± 2.6	30.2	6.9–39.6	5/30
MeAαC	70.8 ± 5.9	8.3	6.4–28.1	4/24	8.5 ± 0.7	8.7	10.9–33.3	4/21

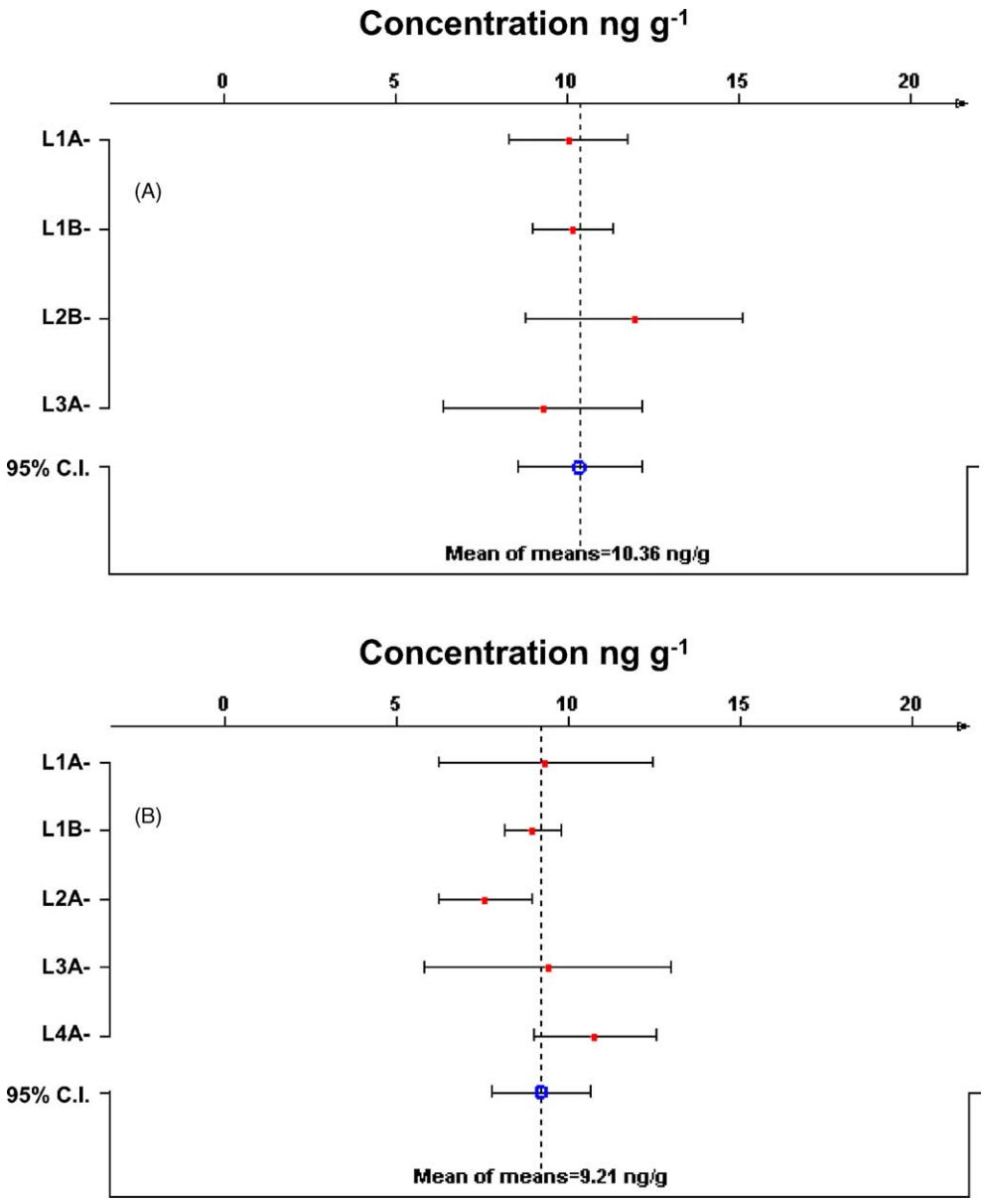

Fig. 4. Mean value of laboratory means and the 95% confidential interval for the determination of (A) MeIQ and (B) Trp-P-2 in the meat extract lot C.

technique for the analysis of some HAs in these materials, providing high sensitivity and selectivity at low cost. In conclusion, LC–MS can be proposed as the technique of choice for the analysis of HAs in complex food matrices. Moreover, LC–MS/MS seems to be the most suitable technique, at least for samples with very low amounts of amines, since the results obtained were never rejected in the intercomparison exercise.

4. Conclusions

The stepwise interlaboratory approach used in this study improved the quality of the analytical measurements of HAs and decreased between-laboratory differences. The suitability of the two-extract clean-up method for the analysis of HAs in meat extracts has been demonstrated and as a consequence it can be used as recommended method. Moreover, an analytical method based on a single extract has also provided good results and can be proposed as alternative method for the analysis of HAs in food samples. In addition, the use of LC–MS or LC–MS/MS is required for the determination of a large number of HAs in order to achieve satisfactory results, especially when the concentrations of the HAs in the sample are very low. On the other hand, stable isotope labeled standards are recommended in order to improve the quality of the quantification results. Nevertheless, at the moment only few isotopically labeled compounds are commercially available. Further collaborative studies using different food matrices and increasing the number of laboratories should be carried out in order to demonstrate the suitability of the analytical methods for the analysis of HAs in cooked food samples.

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