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Detection of heterocyclic aromatic amines in food flavours

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ABSTRACT

The successful determination of heterocyclic aromatic amines in trace amounts (ng/g) in processed flavours depends largely on the detection sensitivity and selectivity. As part of a systematic study of possibilities for the determination of these mutagenic amines, different detection modes in high-performance liquid chromatography were evaluated. Depending on the quality of the clean-up the identification of a peak by UV spectrophotometry (diode-array detection) might be necessary for a higher level of confidence. UV detection with gradient elution is the only method for the separation and determination of all the amines formed. However, electrochemical detection gives the best signal-to-noise ratio for isocratically eluted mutagenic amines.

INTRODUCTION

Heating of protein-rich food may lead to the formation of trace amounts of heterocyclic aromatic amines [1,2] (Fig. 1). These compounds are known to be mutagenic and potentially carcinogenic and they may be present in trace amounts in Maillard- and Amadori-type processed flavours. These browning-type food flavours are used in meat, cacao, bread and many other culinary flavours. They are very complex mixtures, with a high content of fat, and are commercially available as liquids, pastes or spray-dried powders.

As part of a systematic study of the determination of these heterocyclic amines in processed flavours, we evaluated the different possibilities for their detection in high-performance liquid chromatography (HPLC). Various methods for the determination and identification of these heterocyclic aromatic amines have been described. Liquid chromatography–mass spectrometry (LC–MS) [3–5], gas chromatography–mass spectrometry (GC–MS) [6] and HPLC with UV absorbance [7], electrochemical [8] and fluorescence detection have been successfully used, some for model systems only and others for cooked food products. Our objective was to find a simple but reliable method using HPLC as the separation technique and a suitable detection device for quantitative measurements of these mutagenic amines in processed food flavours.

LC–MS would be the preferred detection method for the highest selectivity and best on-line identification of mutagenic amines, but it is an expensive technique and may not be available in most laboratories. GC or GC–MS [9] is only possible with volatile derivatives of the mutagenic amines, otherwise these molecules tend to elute as broad, tailing peaks and therefore can not be detected in low concentrations. However, good derivatization reactions are possible only with some of the heterocyclic aromatic amines and a general method does not exist [10]. Incomplete derivatization leads to low sensitivity and non-reproducible results. Consequently the GC methodology was not developed further. In this paper we report the results obtained by using various HPLC detection methods; results of UV absorbance, electrochemical and fluorescence detection of these amines in food flavours are discussed.

EXPERIMENTAL

Materials

Aminoimidazoquinolines (IQ and MeIQ), aminoimidazoquinoxalines (MeIQx and DiMeIQx), aminopyridoindoles (Trp-P-1 and Trp-P-2) and aminopyridoimidazoles (Glu-p-1 and Glu-P-2)

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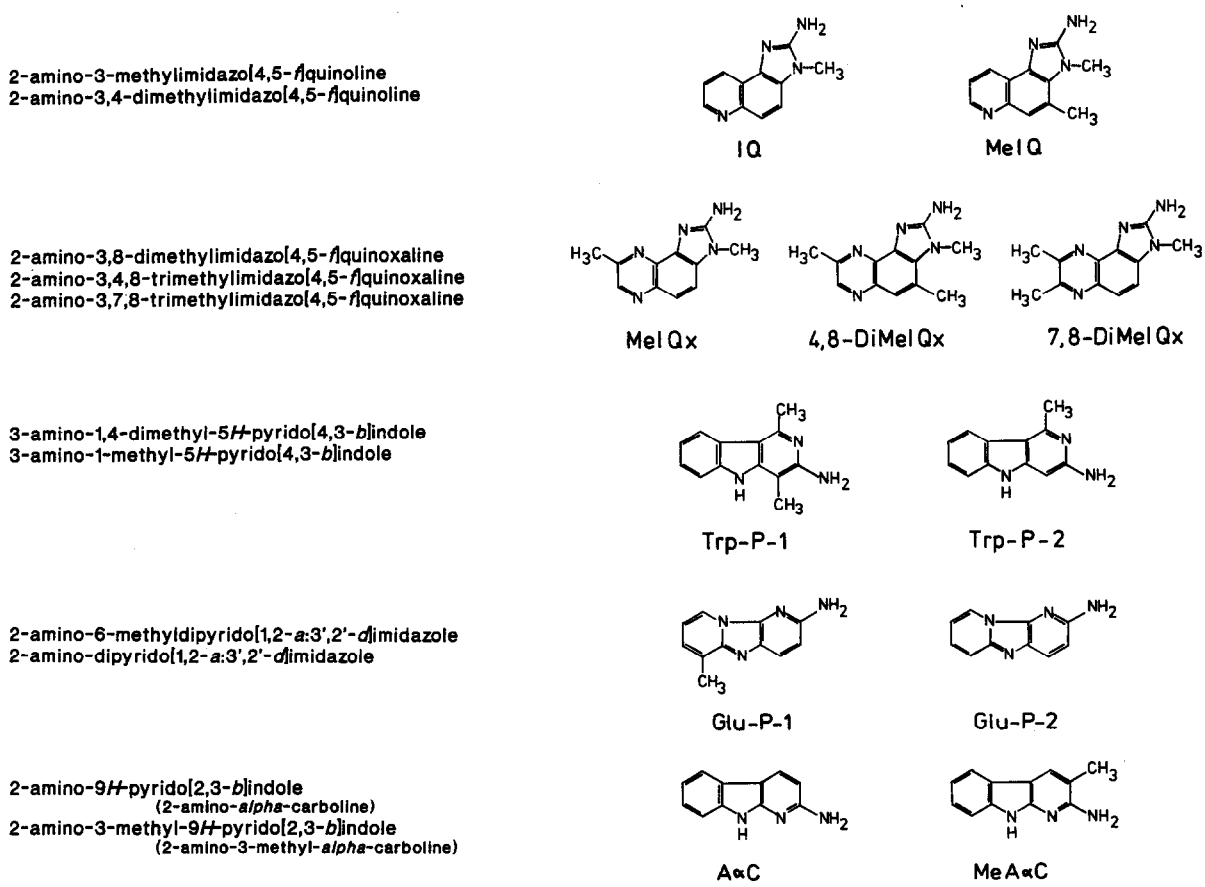

Fig. 1. Structures of heterocyclic aromatic amines.

were obtained from Toronto Chemical Research (Toronto, Canada). Their structures are shown in Fig. 1.

The reagents were of analytical-reagent grade (Fluka, Buchs, Switzerland) and the solvents (Rathburn Chemicals, Walkerburn, UK) were used without further purification. Water was purified in a UHP-2 system from Elgastat (Kleiner, Wohlen, Switzerland).

High-performance liquid chromatography

The column used for isocratic elution was a LiChrosorb RP-Select B, 5 μm (250 × 4.0 mm I.D.) from Merck (Darmstadt, Germany) operated at room temperature. Injections were made manually using a 10-μl loop injector (Valco, Schenkon, Switzerland). The mobile phase was a mixture of 0.1 *M* ammonium acetate solution (pH 4.5) (70–80%) and methanol–acetonitrile (1:2) (30–20%) pumped at 1.0 ml/min. Conditions for gradient elution are given in the legend to Fig. 4.

Detectors

The electrochemical detector was an LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN, USA), equipped with a glassy carbon electrode and operated in the oxidative mode. The applied potential was 950 mV and the typical offset was about 20 nA.

A Spectroflow 757 single-wavelength UV absorbance detector (Kratos, Ramsey, NJ, USA) with a cell volume of 12 μl was operated at a wavelength of 263 nm, with the highest sensitivity range of 0.005 a.u.f.s.

A Model 650-10LC fluorescence detector equipped with a flow cell of 15-μl volume was ob-

tained from Perkin-Elmer (Norwalk, CT, USA). The excitation wavelength was set at 360 nm and emission was measured at 450 nm.

An HP 1040A UV diode-array detector (Hewlett-Packard, Waldbronn, Germany) with an 8-μl cell was used. Two wavelengths were used to monitor the effluent simultaneously at 263 and 360 nm. Spectra of peaks were recorded from 210 to 400 nm.

Standard solutions

The standard solutions used for the determination of the absolute sensitivity and for showing the detection selectivity were prepared in the mobile phase. The composition of the standard mixture was selected to show the best possible use of the detector.

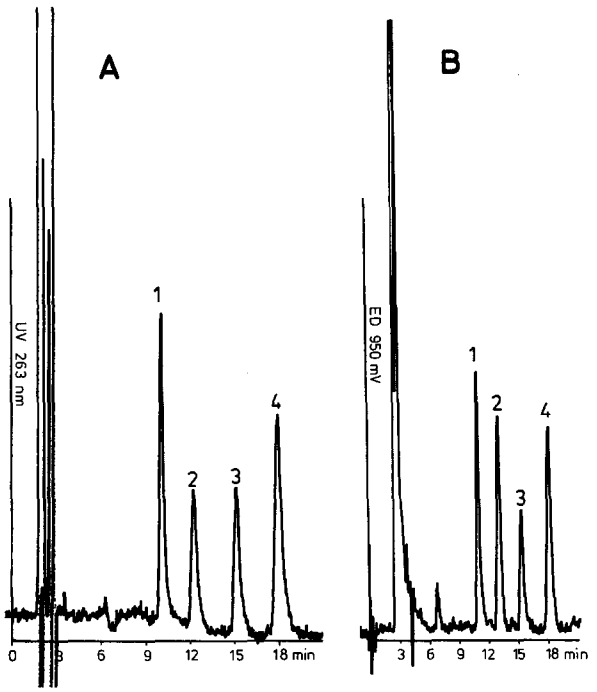

Fig. 2. HPLC of a standard solution of (1) MeIQx, (2) IQ, (3) 7,8-DiMeIQx and (4) 4,8-DiMeIQx. Column: LiChrosorb RP-Select B, 5 μm (250 × 4 mm I.D.). Mobile phase: mixture of 80% 0.1 *M* ammonium acetate solution (pH 4.5) and 20% methanol–acetonitrile (1:2). (A) UV detection at 263 nm: 1.35 ng of MeIQx, 1.13 ng of IQ, 1.04 ng of 7,8-DiMeIQx and 1.82 ng of 4,8-DiMeIQx. (B) Electrochemical detection at 950 mV: 270 pg of MeIQx, 226 pg of IQ, 208 pg of 7,8-DiMeIQx and 264 pg of 4,8-DiMeIQx.

Sample and sample preparation

A commercially available meat extract, as used in households and food industries, was chosen as a reference, to be able to compare the efficiency of the clean-up with results in the literature. The sample was a Maillard-type beef flavour. Maillard processed flavours contain large amounts of fat, which makes the sample preparation very difficult. Therefore, an efficient clean-up procedure is essential.

For the meat extract, the clean-up procedure described by Gross and Grüter [11,12] was used, with two solid-phase extraction steps. However, this clean-up is not sufficient for processed food flavours and additional steps had to be developed. To remove the large amount of fat we used Soxhlet extraction of the spray-dried product, placed the extract on Kieselgur (Extrelut; Merck) and extracted with diethyl ether prior to elution of the amines. Affinity chromatography on a copper phthalocyanine complex [13] (C.I. Reactive Blue 21; Hoechst, Frankfurt a.M., Germany), was an additional separation step to exclude other matrix components,

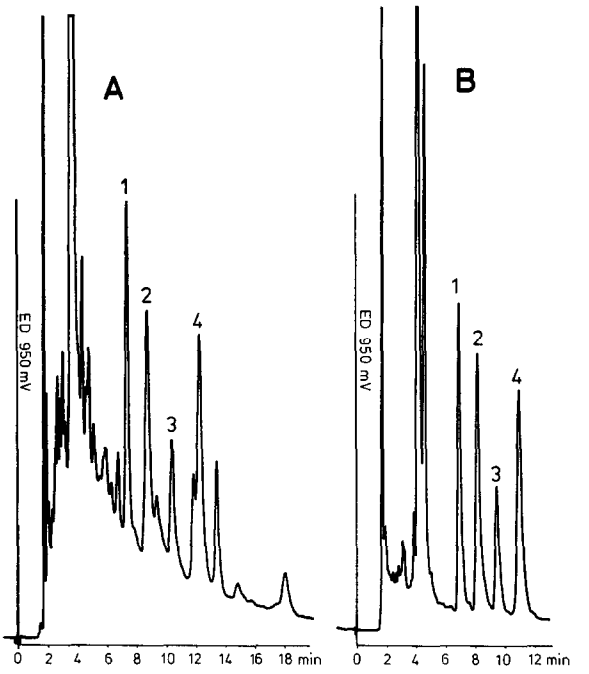

Fig. 3. Analysis of processed meat flavours spiked with 40 ng/g of (1) MeIQx, (2) IQ, (3) 7,8-DiMeIQx and (4) 4,8-DiMeIQx. Column and mobile phase as in Fig. 2. Electrochemical detection at 950 mV. (A) Beef flavour; (B) chicken flavour.

leading to a cleaner sample extract. Further work on this important step is in progress.

RESULTS AND DISCUSSION

UV absorbance detection is by far the most popular method for monitoring the effluents from HPLC columns. As most of the heterocyclic aromatic amines have good absorbance characteristics between 260 and 270 nm, this was the first method to be evaluated (Fig. 2A). The sensitivity achieved with our instrumentation was about 200 pg, but the detection selectivity was too low for the determination of the amines in the matrix of processed flavours at the level of 5–10 ng/g. We therefore looked for alternatives.

Grivas and Nyhammar [14] compared the sensitivity of UV and electrochemical detection (ED) for IQ, MeIQ, MeIQx and 4,8-DiMeIQx and found a 2–3 times lower detection limit for ED. However, they applied ED to model reaction systems only. Takahashi *et al.* [8] used HPLC–ED for the determination of IQ and MeIQx in beef extracts with a detection limit of *ca.* 0.2 ng/g. A more recent paper

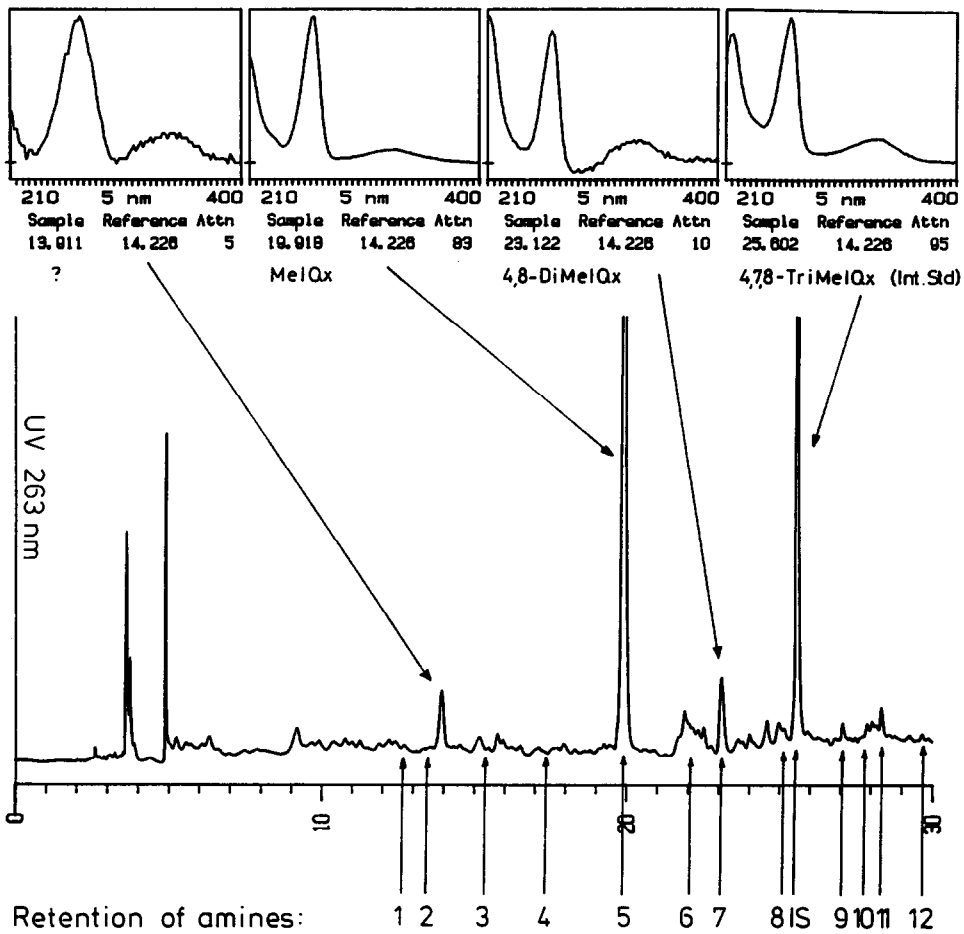

Fig. 4. Analysis of a natural meat extract. Column: TSK-gel, ODS-80 TM (250 × 4.6 mm I.D.). Mobile phase: A = 0.01 *M* triethylammonium phosphate (pH 3.2), B = 0.01 *M* triethylammonium phosphate (pH 3.6), C = acetonitrile, with tertiary gradient elution as follows: 0 min, 95% A, 0% B, 5% C; 15 min, 85% A, 0% B, 15% C; 16 min, 0% A, 85% B, 15% C; 20 min, 0% A, 75% B, 25% C; 30 min, 0% A, 45% B, 55% C. UV detection at 263 nm with spectra recording from 210 to 400 nm. The peak of MeIQx corresponds to an amount of 80 ng/g, 4,8-DiMeIQx was found in trace amounts (9 ng/g) and 4,7,8-TriMeIQx was used as internal standard. Peak identification: 1 = Glu-P-2; 2 = IQ; 3 = MeIQ; 4 = Glu-P-2; 5 = MeIQx; 6 = 4,8-DiMeIQx; 7 = norharman; 8 = harman; 9 = Trp-P-2; 10 = PhIP; 11 = Trp-P-1; 12 = A-α-C. Time scale in min.

by Billedeau *et al.* [15] confirmed the low sensitivity and reported not only on the optimization of the mobile phase for the separation of IQ, MeIQx, Glu-P-1, Trp-P-1 and Trp-P-2, but also on the effect of various potentials applied to the glassy carbon electrode on the detection sensitivity.

In our experiments, we found in addition to the expected low detection sensitivity of about 50 pg (Fig. 2B) a high detection selectivity for these amines in processed flavours (Fig. 3). For maximum detection sensitivity we applied a potential of 950 mV, using a glassy carbon electrode. This high potential reduces the selectivity to some extent, but gives the best results with our choice of mobile phase. However, it is impossible to use gradient elution in this high sensitivity range, which is a requirement for the determination of all eleven mutagenic amines in one run.

In addition to the limitations on the choice of the mobile phase, ED gives only the retention value for identification of a peak. UV detection with a diode-array instrument, capable of recording the UV absorption spectra of a peak, gives in addition to the retention the possibility of comparison of spectra for peak identification. Gross and Grüter [12] used this technique successfully in the analysis of meat extracts. Together with gradient elution and their suggested clean-up procedure, the determination of mutagenic amines in meat extract is feasible in the lower ng/g range (Fig. 4). For processed flavours, however, the detection selectivity is too low. Even with additional clean-up steps we were not able to meet a detection limit of less than 50 ng/g.

The imidazole-type amines show native fluorescence which can be used for highly sensitive and selective HPLC detection. The detection sensitivity is of the order of 1 pg and the limit of determination in processed beef flavour is below 0.5 ng/g. We therefore studied the possibilities of fluorogenic labeling of these heterocyclic aromatic amines. So far we have not been successful in derivatizing the common amino-group of the amines with a fluorescent reagent.

CONCLUSIONS

For a limited group of amines, ED is about five times more sensitive than UV detection. The main advantage of ED, however, is its selectivity. A good clean-up procedure is essential. The influence of the various steps in the sample preparation on the limit of determination of these heterocyclic aromatic amines in processed flavours is very high.

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