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Condensation nucleation light scattering detection for biogenic amines separated by ion-exchange chromatography

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Abstract

A sensitive method of analysis for biogenic amines, putrescine, cadaverine, histamine and an amino acid precursor, histidine is described herein using ion-exchange chromatography and condensation nucleation light scattering detection. The method was successfully used for the analysis of biogenic amines in fish samples. The method offers a number of advantages: fast elution of analytes with no need for mobile phase conductivity suppression, no derivatization and no electrochemical activity for the analyte's detection. The 3σ detection limits for these compounds were found to range from 8 to 20 ng/ml. © 1999 Published by Elsevier Science B.V. All rights reserved.

Keywords: Condensation nucleation light scattering detection; Detection, LC; Food analysis; Fish; Biogenic amines

1. Introduction

Biogenic amines have been found to occur in a wide variety of foods [1–6]. The major factor leading to the formation of these amines is the presence of certain bacteria able to decarboxylate free amino acids in the foods. Histamine, whose presence is a good indicator of decomposition, is a product of histidine when the latter is decarboxylated by microorganisms present in spoiled foods [2,3], and is a well-known inflammatory substance released from most cells. The most frequent foodborne intoxication known as 'scombroid poisoning' is caused by histamine [2]. Biogenic diamines like putrescine (1,4-diaminobutane) and cadaverine (1,5-diaminopentane) were also found in tumor cells and their detection in organ transplant recipients has been the basis for monitoring the extent of tissue rejection [7].

Various reversed-phase liquid chromatographic systems have been developed for analysis of biogenic amines, but because of a lack of a suitable chromophoric or fluorophoric group, alkyl amines cannot be directly detected with the required sensitivity by common spectrophotometric detectors such as UV absorbance or fluorescence, without derivatization to form 2-chloroethylnitrosourea (CENU), dansyl, fluorescamine or benzoyl derivatives [2–6]. Generally, derivatization methods suffer from various drawbacks, such as tedious sample preparation, interference from by-products, long analysis times and the risk of indeterminate errors [7,8]. Furthermore, some postcolumn derivatization methods form unstable derivatives and cause peaks

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to be broadened and diluted by the derivatization reagents [2].

Electrochemical detection has been applied to the direct detection of non-chromophoric compounds with electrochemical activity using ion chromatography as the method of separation [1,7,9,10]. Since in electrochemical detection the compound to be detected must interact with the electrode surface, it is prone to many electrochemical reaction product interferences [8]. Pulsed amperometric detection (PAD), when applied to amines, requires formation of a metal oxide (e.g. AuO) on an electrode surface to catalyze the amine oxidation which causes a large background signal [7,11]. Recently, integrated pulsed amperometric detection (IPAD) [11], integrated voltammetric detection (IVD) and integrated square wave detection (ISWD) [7] have been developed for biogenic amine detection with the goal of alleviating these problems.

Condensation nucleation light scattering detection (CNLSD) offers an alternative detection method for these substances. CNLSD is a universal detection method which relies only on the analyte's involatility relative to the mobile phase [12]. It is also universal in the sense that it has been interfaced to many different separation methods such as HPLC [13–15], ion-exchange chromatography [16], capillary electrophoresis [17–19], capillary electrochromatography [20] and microbore supercritical fluid [21] and liquid chromatography [22]. CNLSD is an aerosol-based detection method like evaporative light scattering detection (ELSD) except for the addition of a condensation growth step in CNLSD prior to the light scattering detector cell [12]. With CNLSD, the desolvated aerosol from the column effluent is introduced to a cooled and vapor saturated environment which causes the condensation of vapor onto the dry particles, amplifying the particles size, and greatly increasing the scattered intensity. For capillary separations, sub-pg detection limits [18,20] or even macromolecule (proteins) counting [14] have been reported. In earlier work using ion-exchange chromatography for separations of metals, mass concentration limits of detection (LODs) as low as 3 ng/ml were reported [16].

In this work, CNLSD is used for detection of underivatized amines that have been separated by ion-exchange chromatography (IEC). We will describe the development and optimization of the IEC–CNLSD method for biogenic amines and application of the technique to the determination of these species in fish samples.

2. Experimental

2.1. Apparatus and chromatographic conditions

An overview of the IEC–CNLSD system is depicted in Fig. 1. Separations were performed with a 100×4.6 mm I.D. Alltech universal cation-exchange column (Deerfield, IL, USA) packed with polybutadiene–maleic acid coated silica. The mobile phase used was aqueous nitric acid with a small fraction (typically 10%) of acetonitrile.

A Beckman (Fullerton, CA, USA) model 110B single piston pump was used to deliver the eluent to the system at a flow-rate of 1 ml/min. A 30×4.6 mm I.D. Alltech scavenger column was placed on line before the injection valve to remove trace heavy metals in the acidic mobile phase. The injection valve was a Rheodyne (Cotati, CA, USA) model 7125 equipped with a 100-μl sample loop. The column effluent goes into the CNLSD system which uses a Meinhard (Santa Ana, CA, USA) type C nebulizer to convert the effluent to an aerosol. The aerosol is desolvated into dry particles as it passes along a 150°C heated drift tube (39×1.2 cm I.D.) followed by a 0°C West condenser (20×0.9 cm I.D.).

The signal (including the background signal) for CNLSD is dependent on the overlap of the particle size distribution of the aerosol with the condensation particle counter (CPC) detection efficiency curve [18]. A drawback of using higher acid concentration eluents is that the concentration of less volatile contaminants from the acid also increases. This leads to larger particles from the mobile phase and a higher background level in the chromatogram. One way to reduce the background is to use diffusion screens [13]. Diffusion screens remove the smaller-sized particles for which diffusional losses are enhanced. The small particles stick to the screen and are retained on impaction on the screen's surface. When a diffusion screen is used, the overlap of the particle size distribution and the diffusion screen penetration curve determines the response [18]. The

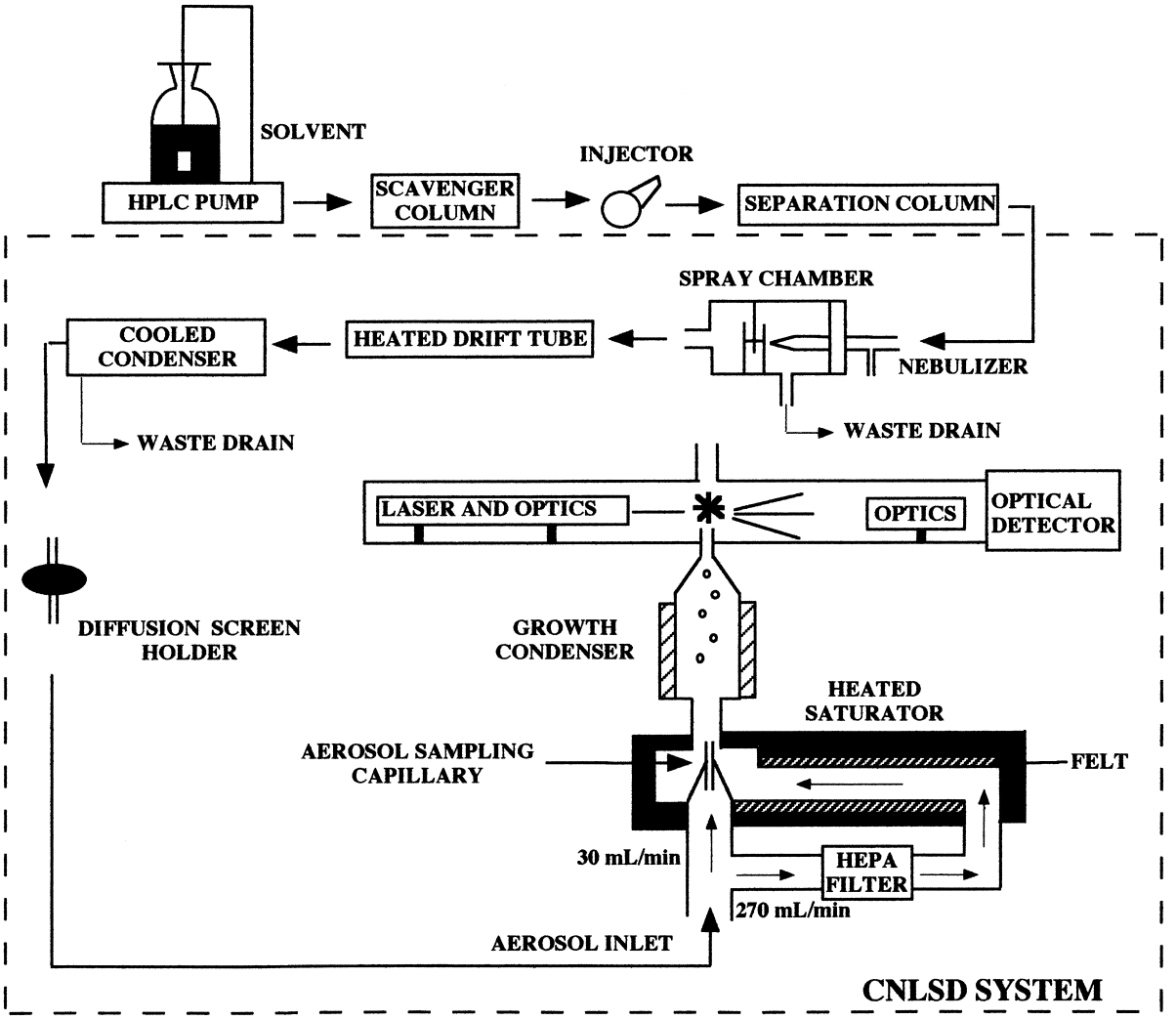

Fig. 1. Overview of the IEC–CNLSD system.

concentration dependent response is therefore controlled by the diffusion screen as all the particles penetrating the diffusion screen are detected by the CPC. Increasing the number of diffusion screens will increase the cut-off size for removal of particles from the aerosol stream entering the detector. So the number of diffusion screens used must be optimized such that a compromise between the analyte signal loss and the reduction in background noise is obtained [16]. The dry aerosol goes through diffusion screens, model 376060 particle sizer selector obtained from TSI (St. Paul, MN, USA) prior to the particle detector, a model 3025A condensation particle counter (CPC) from TSI.

The data were collected using a laboratory-written BASIC program that transfers data from the CPC to an IBM computer. The data files were then processed by KALEIDAGRAPH (Synergy software, PA, USA) on a Macintosh Quadra 610 Apple computer (Cupertino, CA, USA).

2.2. Chemicals

All solutions were prepared from reagent grade

Table 1
Signal-to-noise ratio of a 500 ng/ml putrescine standard with different numbers of diffusion screens

Number of diffusion screens	Signal intensity (no. of particles/ml)[a]	Background noise (no. of particles/ml)[a]	Signal-to-noise ratio
1	15 030	493	30
2	8809	104	85
3	4856	78	62

[a] Gas phase particle concentrations.

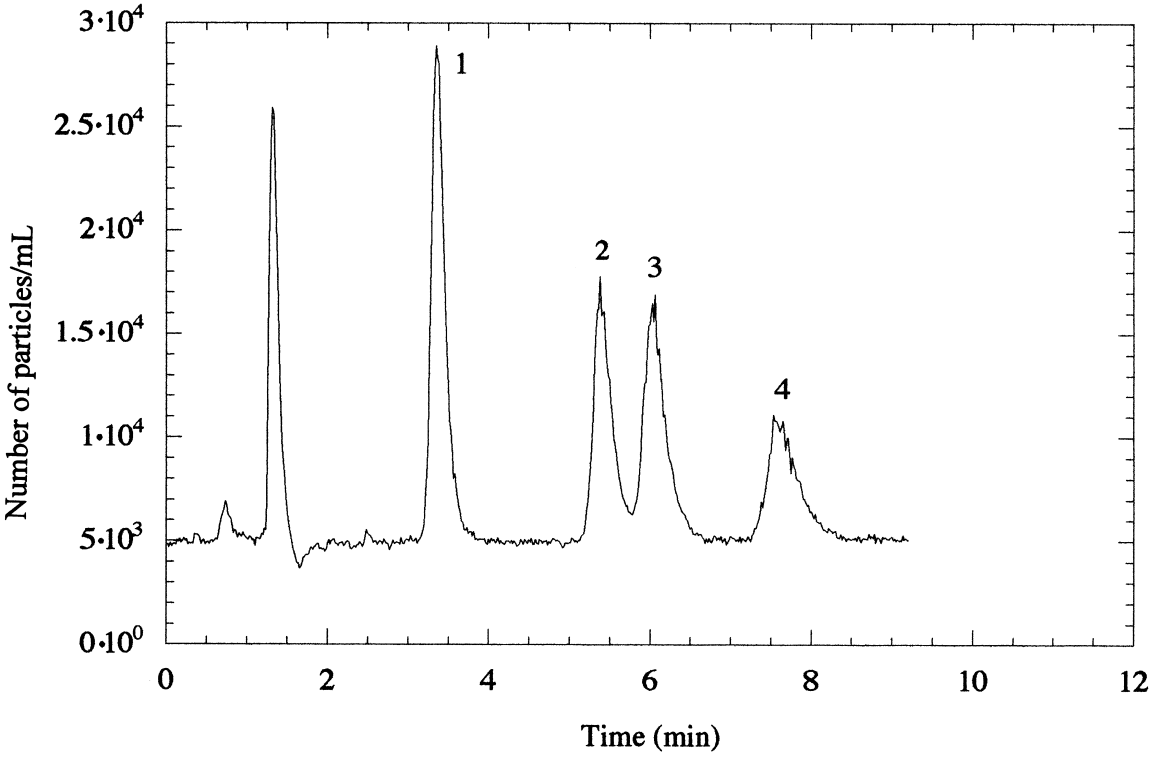

Fig. 2. Chromatogram of a mixture of 600 ng/ml each of the biogenic amine standard. Eluent: 10 m*M* nitric acid and 10% acetonitrile. Peak identification: 1=histidine, 2=putrescine, 3=cadaverine, 4=histamine, two diffusion screens. Signal: gas phase particle concentrations.

chemicals. Putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride and the amino acid histidine were obtained from Sigma (St. Louis, MO, USA). The nitric acid used was Ultrex ultrapure grade from J.T. Baker (Phillipsburg, NJ, USA) and the acetonitrile was an HPLC grade supplied by Fisher (St. Louis, MO, USA).

Individual stock solutions of 1000 μg/ml of each amine were prepared in Barnstead NANOpure water

Table 2
Estimated 3σ LODs of the amines[a]

Amines	ng/ml	ng	μ*M*[b]
Histidine	8	0.80	0.51
Putrescine	12	1.2	0.71
Cadaverine	12	1.2	0.69
Histamine	20	2.0	1.1

[a] 100-μl sample volume.
[b] Molecular mass used for calculations were of the dihydrochloride salts of biogenic amines.

(Dubuque, IA, USA). Standard solutions were prepared by dilution from the stock solution.

2.3. Extraction of the biogenic amines from fish samples

The tuna fish and mackerel sardine samples weighing 6.5 g each were first washed with deionized water. These were then ground and later homogenized by magnetic stirring for 45 min in an Erlenmeyer flask which contained a solution of 20 ml water and 10 ml 6 *M* HCl. The homogenates were then centrifuged for 20 min. A 100-μl aliquot of this diluted and syringe filtered supernatant was injected into the column for separation and later detection by CNLSD. The dilution factor utilized (1000×) was chosen for convenient sample handling that resulted in a satisfactory measurement of the species and samples investigated.

3. Results and discussion

3.1. Ion-exchange separation of standard biogenic amines

The biogenic amines contain amino groups which can be protonated by an acidic mobile phase and separated by ion-exchange and hydrophobic interactions with the stationary phase. As was useful for ion-exchange separation of amino acids with this column and CNLSD [23], a mobile phase made up of 5 m*M* nitric acid with 5% acetonitrile aqueous solution was first tried for the separation of these compounds. However retention times were long, degrading the detection sensitivity for these compounds. So the eluent strength was increased to 10 m*M* nitric acid with 10% acetonitrile. Using this mobile phase, optimization of the number of diffusion screens was conducted by determining the signal-to-noise (S/N) ratio of a 500 ng/ml putrescine

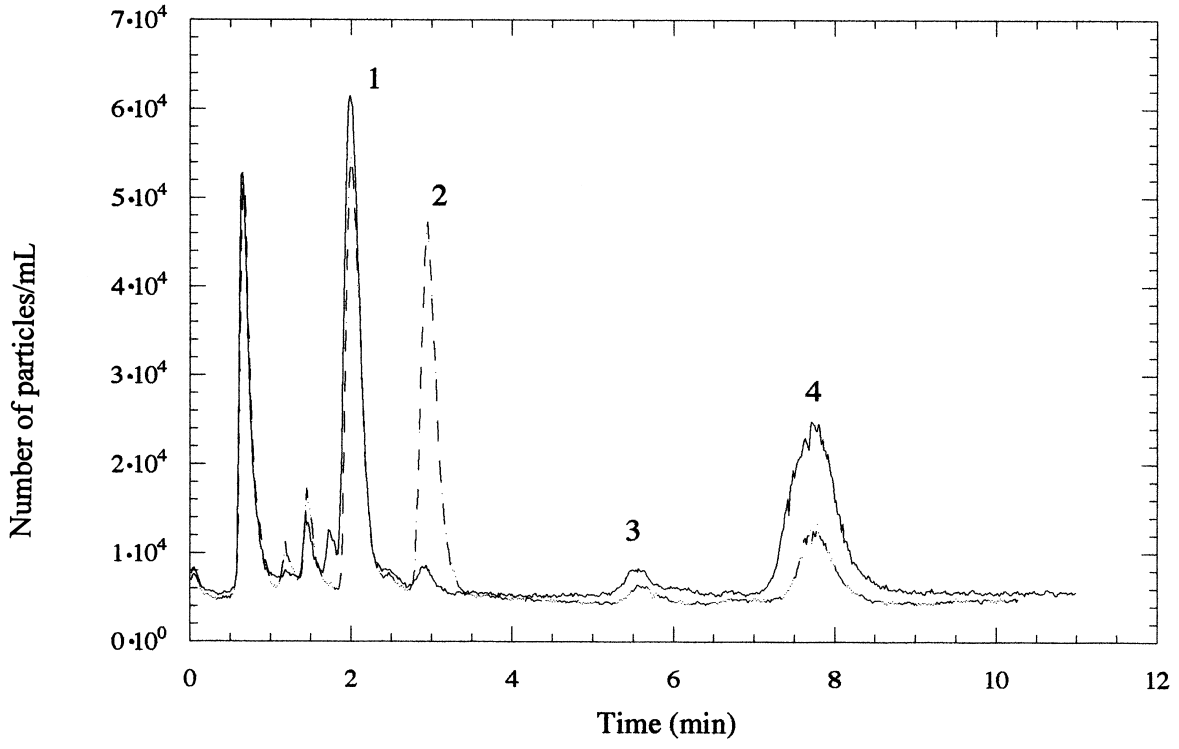

Fig. 3. Chromatogram of biogenic amines from tuna fish. Experimental conditions as in Fig. 2. Dotted line: refrigerated tuna extract. Peaks: 1=K^+ and Mg^{2+} ions, 2=histidine (4.10 mg/g), 3=putrescine (0.115 mg/g), 4=histamine (1.80 mg/g). Solid line: spoiled tuna extract. Peaks: 1=K^+ and Mg^{2+} ions, 2=histidine (0.165 mg/g), 3=putrescine (0.233 mg/g), 4=histamine (5.50 mg/g). Signal: same as in Fig. 2.

sample as a function of the number of diffusion screens. Table 1 shows the optimum S/N ratio of putrescine at two diffusion screens. Shown in Fig. 2 is a chromatogram of a 600 ng/ml sample of each of the biogenic amines under these conditions.

3.2. IEC–CNLSD for biogenic amine detection: figures of merit

Correlation coefficients equal to or greater than 0.995 were obtained for all peak area calibration curves, indicating good linearity. Slopes of calibrations for peak area were within 10% for histidine, putrescine, and cadaverine, while histamine showed a 35% lower slope. These results are consistent with previous studies which have shown similar mass response for most nonvolatile substances [13,15]. Good reproducibility was obtained with an average relative standard deviation of 2.0% for 0.9 μg/ml of each of the amines considered.

Standard curves of peak height for the four amines shows good linearity over the concentration range of 0.4 to 1.0 μg/ml although there was a large difference in slope from one substance to another as suggested by the chromatogram in Fig. 2. For a S/N ratio of 3:1, the LODs were shown to be in the range of 8–20 ng/ml for these biogenic amines (Table 2). These values were much lower than the reported UV and fluorescence detection levels, which are above 100 ng/ml levels [2–4]. A laser-induced fluorescence detection method however was reported to give LODs at pg/ml levels [5], but product derivatization was required before detection. A nonderivatization electrochemical method has also been reported to provide sensitive detection [7,11]. Using ion-exchange separation and integrated pulsed amperometric detection, LODs were reported in the

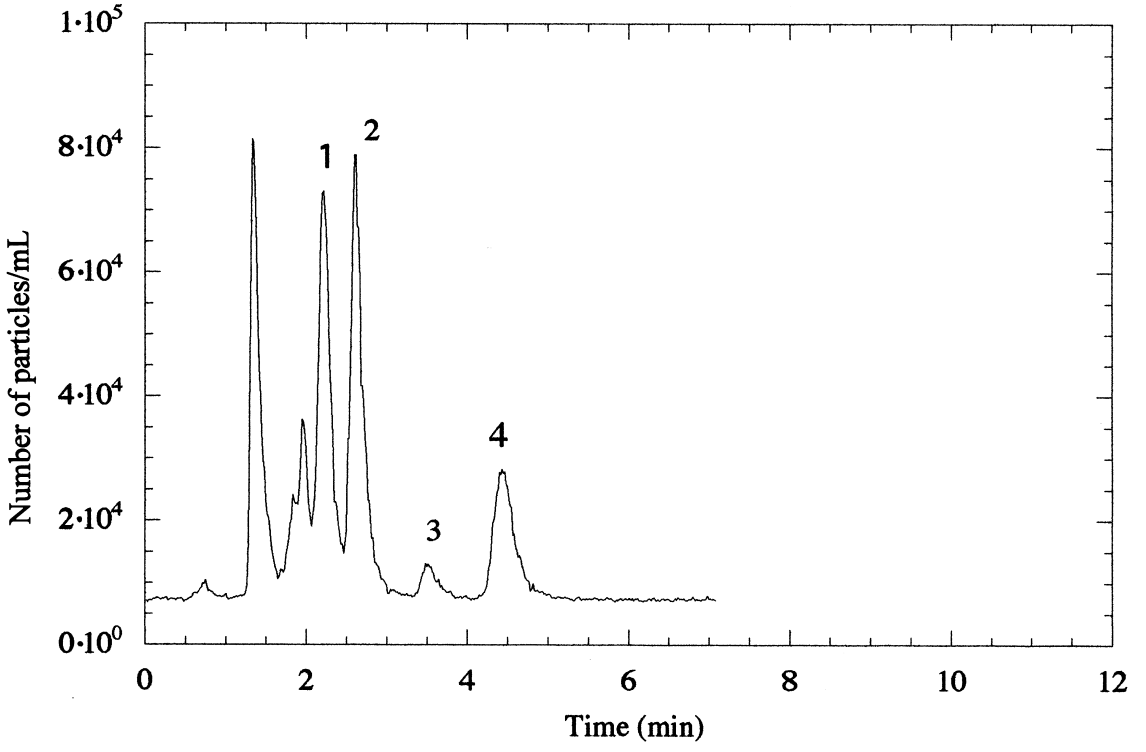

Fig. 4. Chromatogram of biogenic amines from refrigerated tuna fish. Eluent: 15 m*M* nitric acid and 10% acetonitrile. Peak identification as in Fig. 3. Signal: same as in Fig. 2.

range of 50 to 80 ng/ml levels for these amines [11]. Hoekstra and Johnson [7] reported LODs for 1,3-diaminopropane by LC–PAD, LC–IVD and LC–ISWD as 12, 2 and 1.2 ng/ml, respectively.

3.3. Application of the IEC –CNLSD method to fish samples

Fig. 3 is a chromatogram of diluted extracts of a refrigerated tuna and 2-day-old spoiled tuna extracts. Putrescine and histamine were present in the refrigerated tuna chromatogram (dotted line), indicating a start of decomposition, while a large peak for histamine and a very small peak for histidine were observed in the spoiled tuna chromatogram (solid line). The amount of histamine determined in the spoiled sample is slightly higher by ~0.7 mg/g than the one reported by Oguri et al. [4] for a 48-h decayed tuna fish sample analyzed by CE–UV.

Increasing the acid concentration from 10 to 15 m*M* nitric acid reduces the total chromatogram time from about 8 min to less than 5 min without increasing the background level and noise significantly (Fig. 4). This idea of increasing the acid concentration or doing a gradient elution could be very useful for routine use in columns which could tolerate lower pH mobile phase. This condition however was not routinely used in our experiment as the silica-based column could not tolerate very low pH mobile phase for lengthy times.

The chromatogram for an extract of canned mackerel is shown in Fig. 5. The Na^+ peak was very prominent as it is intentionally added for flavoring and serves as a preservative in canned foods. Although histidine was observed, biogenic amine species were not.

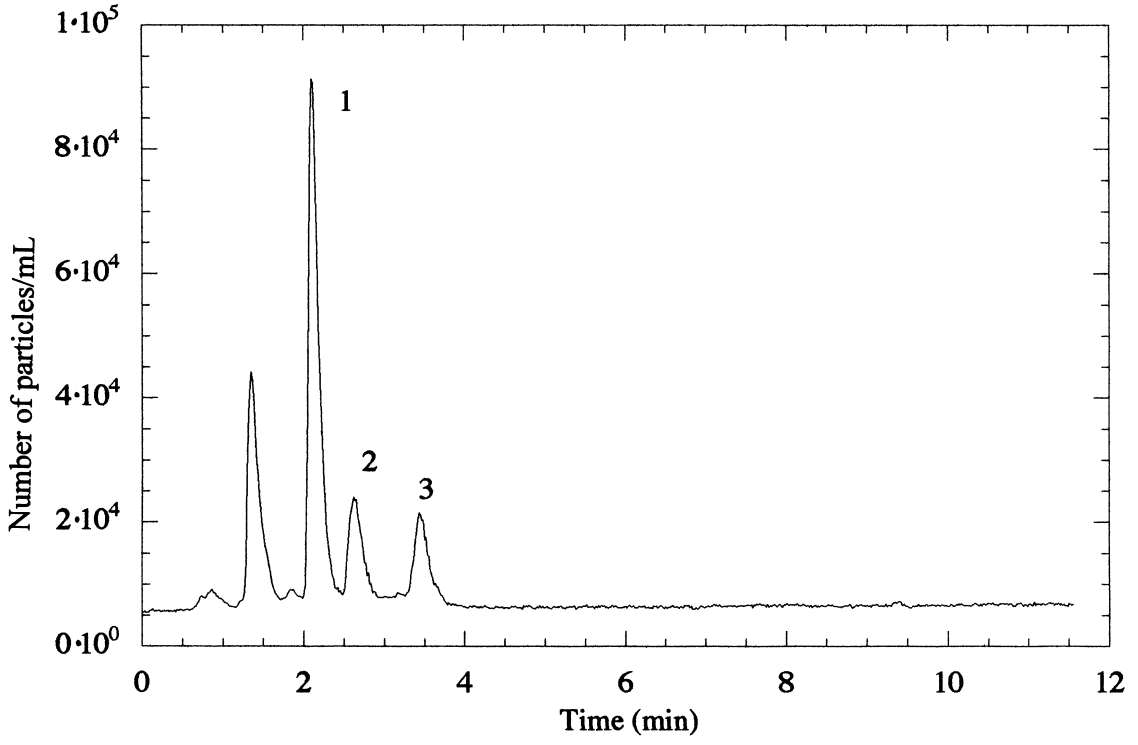

Fig. 5. Mackerel sardines chromatogram: experimental conditions as in Fig. 2. Peaks: 1=Na^+ ion, 2=K^+ and Mg^{2+} ions, 3=histidine. Signal: same as in Fig. 2.

4. Conclusions

Detection of biogenic amine compounds separated by ion-exchange chromatography with LODs at the low ng/ml level was obtained using CNLSD. This is a universal detection method for many species separated by cation exchange. In addition, no conductivity suppression of the mobile phase or derivatization of analytes was needed. A high acid concentration of 10 m*M* could be used as the mobile phase, resulting in short elution times for the analytes and fast analysis times. High sensitivity for the direct determination of biogenic amines was attained without the need for derivatization. The LODs were comparable to the best reported electrochemical detection limits with the added advantage that no complicated electrochemical activity of the eluents and analytes was involved. This method is applicable to the direct analysis of these amines from food sample extracts.

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