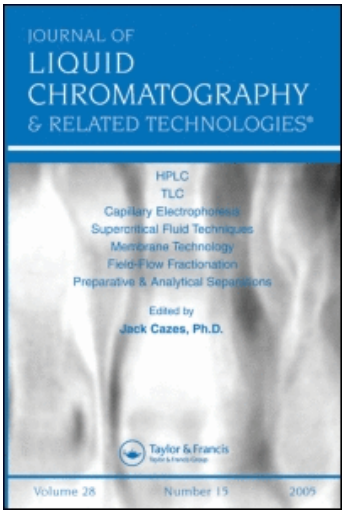

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SIMULTANEOUS DETERMINATION OF FOOD-RELATED AMINES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Ten amines found in marine foods, dimethylamine, trimethylamine, trimethylamine oxide, ammonia, urea, histamine, cadaverine, putrescine, spermine and spermidine were separated by HPLC using an ion-moderated partition column. Optimum resolution and sensitivity were obtained using 0.003N sodium hydroxide as the mobile phase and UV detection at 208 nm.

1. INTRODUCTION

A wide range of amines may be found in marine foods with the main interest being in those compounds which are associated with undesirable organoleptic changes to the food or induce harmful physiological responses in humans. Amines that have been related either positively or negatively to organoleptic changes include trimethylamine oxide and its decomposition products trimethylamine (TMA) and dimethylamine (DMA), ammonia which is

produced from the breakdown of various nitrogen containing compounds including urea, a range of decarboxylated amino acids including histamine from histidine, cadaverine from lysine and putrescine from ornithine and also the decomposition products of putrescine, spermine and spermidine (1-5). The major toxic amine is histamine which has been associated with scombroid poisoning and the US Department of Agriculture has proclaimed an upper safe limit of 50 mg histamine/100 g food (6).

A range of methods has been used to analyse these amines but many are colorimetric and are either specific for one compound or determine conjointly a mixture of compounds. Chromatographic methods can be used to separate amine mixtures with gas chromatography able to analyse the volatile bases readily (7). Non-volatile bases can also be analysed by gas chromatography but only after derivatisation to a more volatile compound (8).

The technique of high-performance liquid chromatography (HPLC) offers the possibility of analysing volatile and non-volatile bases simultaneously without the need to form derivatives and Gill & Thompson (9) developed such a system to give partial to full resolution of six amines (spermine, putrescine, cadaverine, histamine, TMA and DMA) on an ion-moderated partition column with detection by UV absorbance at 207 nm. In this paper we have examined this system and report an improved resolution, the separation of further amines and the sensitivity of each compound to detection.

2. MATERIALS AND METHODS

HPLC analysis used a modified partition column (HPX-72-0 in the -OH form, Biorad, Richmond) with a Waters U6K injector, Model 6000A pump, Model 730 data module and UV detectors Model 441 operating at 214 nm and Model 480 operating at 208 nm (Waters Associates, Milford). The wavelengths used for UV detection were selected following an examination of the UV spectrum of each compound in a spectrophotometer.

Standard solutions of the hydrochlorides of dimethylamine, trimethylamine, histamine, cadaverine, putrescine, spermine and spermidine, trimethylamine oxide dihydrate (Sigma Chemicals, St. Louis), ammonium sulphate (BDH Chemicals, Australia) and urea (Merck, Darmstadt) were prepared in deionized double distilled water. Each solution was initially prepared at 0.5 g/100 g and chromatographed in aqueous mobile phases with varying concentrations of sodium hydroxide at a flow rate of 0.6 ml min^{-1} to determine retention time although other concentrations were subsequently used with specific mobile phases which sometimes contained acetonitrile.

The limit of detection and linearity of response for each compound was determined at 208 and 214 nm using solutions containing a single compound at a range of concentrations. Since under the conditions used in our system noise was immeasurably small, the limit of detection was considered to be the amount that produced a peak height of 2 mm. The linearity of response was determined by calculating a linear regression from the set of values for peak height and amount injected with at least four values being obtained for each compound and using the correlation coefficient r^2 to determine to degree of linearity.

The ability of the HPLC system to resolve a mixture of amines was determined using the method developed by Morgan & Denning (10). A solution containing all ten compounds was prepared using the appropriate concentration of each compound to give a similar peak height and chromatographed. The degree of separation of an individual pair of adjacent peaks (P) was translated to a numerical value by calculating the ratio between the depth of the valley below the straight line connecting the maximum point of the two peaks (f) and the height of the straight line above the baseline at the valley (g), i.e. P=f/g; the value of P could therefore range from 1.0 for peaks that were completely separated to 0.0 for peaks that co-eluted. The overall degree of resolution of all compounds, referred to as the chromatographic response function (CRF), was calculated by summing the nine

individual P values with a score of 9.0 representing perfect separation of all compounds.

3. RESULTS AND DISCUSSION

A preliminary study examined the UV spectra of each compound and found that maximum absorption occurred at 200-206 nm for all compounds except histamine which was at 230 nm. Many compounds are known to absorb strongly around 200 nm and hence it was decided to use 208 and 214 nm as the wavelengths for HPLC detection as a compromise to minimise interference that would occur when food samples were analysed while retaining reasonable sensitivity.

Solutions of single compounds were chromatographed and it was found that all ten compounds had a different retention time when the mobile phase was sodium hydroxide at 0.003N or higher but with 0.001N sodium hydroxide, putrescine and cadaverine had the same retention time. Table 1 shows the effect of sodium hydroxide concentration in the mobile phase on retention times and degree of separation of adjacent peaks when all comopunds were injected simultaneously. The overall degree of resolution was greatest in 0.003N sodium hydroxide which had a CRF value of 6.68 with the lowest resolution of adjacent peaks being 0.54 between trimethylamine oxide and putrescine. This may be compared with a corresponding CRF value of 3.76 for 6 peaks obtained by Gill and Thompson (9). A chromatogram of the separation is given in Fig. 1 and illustrates that all peaks are resolved sufficiently to allow the HPLC system to be of practical use for resolving all ten compounds.

The use of 0.005 and 0.007N sodium hydroxide gave a similar CRF value to 0.003N but each mobile phase had a pair of peaks resolved at <0.5 (spermidine and trimethylamine oxide), while 0.001N sodium hydroxide had a lower CRF value of 5.63 and did not give any resolution of trimethylamine oxide, putrescine and cadaverine (i.e. P=0.0). Chromatograms showing

TABLE 1

Effect of sodium hydroxide concentration in the mobile phase on resolution of ten standard amines.

Compound	Sodium hydroxide concentration (N)							
	0.001		0.003		0.005		0.007	
	RT	P	RT	P	RT	P	RT	P
Spermine	6.4		6.6		6.7		6.8	
Spermidine	6.8	0.51	7.1	0.69	7.3	0.59	7.3	0.61
Trimethylamine oxide	7.7	0.80	7.7	0.65	7.7	0.46	7.7	0.31
Putrescine	7.7	0.00	8.2	0.54	8.4	0.77	8.5	0.85
Cadaverine	8.1	0.00	8.8	0.60	9.1	0.74	9.3	0.72
Dimethylamine	8.7	0.54	9.4	0.63	9.8	0.59	9.9	0.54
Trimethylamine	10.3	0.88	10.5	0.85	10.5	0.72	10.5	0.60
Ammonia	11.2	0.79	11.2	0.72	11.2	0.76	11.2	0.72
Urea	15.0	1.00	15.0	1.00	15.0	1.00	15.1	1.00
Histamine	38.9	1.00	40.7	1.00	41.1	1.00	41.0	1.00
C R F		5.63		6.68		6.63		6.45

RT = Retention time (minute)
P = Peak separation (= f/g)

CRF = Chromatographic Response Function $= \sum_{i=1} (Pi)$

the resolution with 0.001, 0.003 and 0.007N sodium hydroxide are given in Fig. 1. The retention time of compounds increased slightly as the concentration of sodium hydroxide increased. While the use of 0.003N sodium hydroxide gave the best overall resolution of the ten compounds, a few were significantly better separated in another concentration; resolution of trimethylamine oxide and putrescine increased with 0.001N and resolution of trimethylamine oxide and putrescine, and also of putrescine and cadaverine with 0.007N

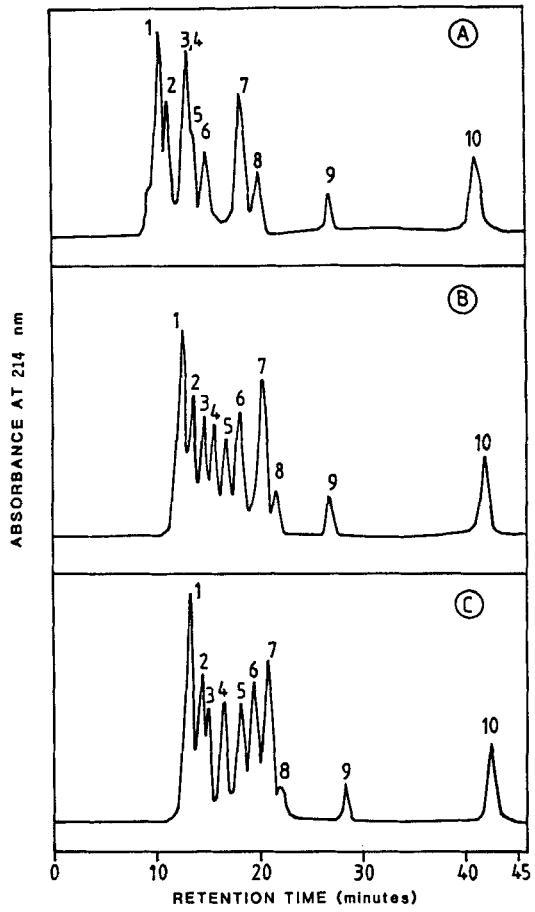

Figure 1 Chromatographic separation of standard amines using different sodium hydroxide concentrations as mobile phase. (A) 0.001 N, (B) 0.003 N, (C) 0.007 N.

Peak identity : (1) spermine, (2) spermidine, (3) trimethylamine oxide, (4) putrescine, (5) cadaverine, (6) dimethylamine, (7) trimethylamine, (8) ammonia, (9) urea, (10) histamine.

TABLE 2

Limit of detection of amines with HPLC using UV detection at 208 nm and 214 nm.

Compound	Limit of detection (μg)[a]	
	208 nm	214 nm
Spermine	0.25	0.50
Spermidine	0.20	0.37
Trimethylamine oxide	5.01	4.11
Putrescine	0.20	0.36
Cadaverine	0.42	0.57
Dimethylamine	0.18	0.24
Trimethylamine	0.06	0.05
Ammonia	2.20	4.50
Urea	7.91	20.80
Histamine	0.05	0.04

[a] Limit of detection was amount that produced a peak height of 2 mm.

sodium hydroxide. Given that not every amine will be in all food samples that require analysis, it may be beneficial to use a different mobile phase to 0.003N sodium hydroxide if these pairs of compounds are of particular interest.

Acetonitrile was added to 0.003N sodium hydroxide at 1, 2 and 3 ml/100 ml and the mixture of amines chromatographed. The addition of acetonitrile resulted in a small decrease in the CRF and the only pair of peaks that showed improved separation was cadaverine and dimethylamine in 3% acetonitrile which had a separation of P=0.80. The addition of acetonitrile caused a slight decrease in the retention time of all compounds.

The limit of detection of each compound at 208 and 214 nm is given in Table 2. This shows that most compounds were detected at lower levels at 208 nm except for trimethylamine and histamine showed little difference between the two wavelengths. There was also a wide difference between sensitivities of the various compounds to detection; histamine and trimethylamine showed the greatest sensitivity being detectable to <0.1 μg at 208 nm, dimethylamine, spermine, spermidine and putrescine could be detected at about 0.25 μg while trimethylamine oxide, urea and ammonia could not be detected until about 2-8 μg was present. The response of peak height to amount injected was linear over the range examined for all compounds with the correlation coefficients (r^2) always greater than 0.990; the concentration range examined extended to 15-20 times the limit of detection.

The HPLC method offers a simple, rapid method where a large range of amines can be readily resolved in a single sample injection with the total time of analysis being 40 min. The method, however, needs to be evaluated on actual food samples where its final feasibility will depend on the presence and/or removal of interfering substances in the food.

4. ACKNOWLEDGEMENT

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