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Note

Determination of tetramethylammonium ion in shellfish by ion chromatography

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Neptunea intersculpta and *N. arthritica* are shellfish living in fairly deep areas of the Sea of Japan and the Pacific Ocean. They belong to Buccinidae and are a human foodstuff. But they can cause food poisoning[1,2] accompanied by such symptoms as nausea, dizziness and headache.

It is considered that the food poisoning is caused by tetramethylammonium ion, $(CH_3)_4N^+$ (tetramine), which occurs only in the salivary glands of shellfish. Tetramine has been analysed by thin-layer chromatography (TLC)[3], but high sensitivity and accuracy were difficult to achieve.

For several years, the authors have been trying to introduce ion chromatography (IC) into food chemistry[4–6]. The present paper reports the use of IC to analyse tetramine with a high sensitivity and accuracy, as well as choline chloride.

EXPERIMENTAL

Apparatus

A Dionex Model 2010i ion chromatograph was used, equipped with cation separator column and suppressor system, and a Perkin-Elmer Sigma 10 data processor.

Reagents

All reagents used were of analytical grade.

Sample preparation

Tetramine was extracted from the shellfish by the method shown Fig. 1; *ca.* 10 g of salivary glands were collected. After being homogenized with 50 ml of methanol, this was digested at 80°C for 30 min and cooled. The residual methanol was removed, and the pH of the solution adjusted to 5 with 1 *N* hydrochloric acid. Ether was added to remove fats, and the solution was concentrated, diluted with 10 ml of water (1 g/ml) and analysed by IC.

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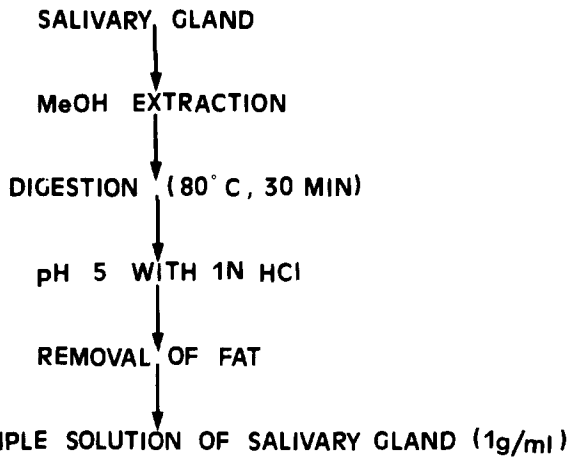

Fig. 1. Flow sheet for preparation of sample solution.

IC conditions

Table I shows the conditions for determination of tetramine by IC. Fig. 2 shows a typical chromatogram of a standard solution.

RESULTS AND DISCUSSION

Eluent

For the analysis of Na^+, NH_4^+, K^+, and some other cations, hydrochloric acid or nitric acid are generally used as the eluent. For tetramine, hydrochloric acid elution produced good separation, but nitric acid elution did not, so hydrochloric acid was used.

It was shown that choline exists in the salivary glands. In IC, choline elutes in close proximity to tetramine, so the elution times of these ions were examined. Fig. 3 shows the relationship between HCl concentration and the elution times of tetramine and choline. It was observed that as the HCl concentration decreased, the elution times of tetramine and choline tended to become longer. However, when the hydrochloric acid concentration was too low, the analysis took a long time and the peaks were broad. Therefore, 10 m*M* hydrochloric acid was used.

TABLE I

CHROMATOGRAPHIC CONDITIONS FOR TETRAMINE ANALYSIS

Separator column	:	Dionex CS-2 (50 × 4 mm I.D. and 250 × 4 mm I.D.), filled with low-capacity cation-exchange resin
Suppressor system	:	Packed hollow fiber suppressor (regenerant 0.025 *M* K_2CO_3)
Eluent	:	10 m*M* HCl
Sample volume	:	50 μl
Flow-rate	:	1.5 ml/min
Detection	:	Conductivity

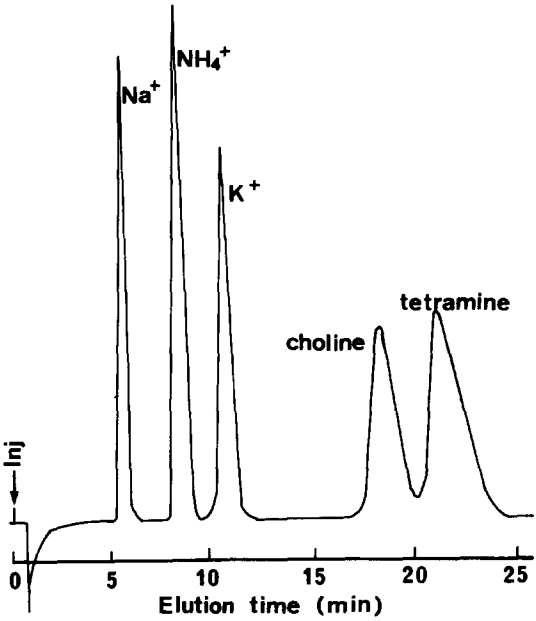

Fig. 2. Typical chromatogram of standard solution.

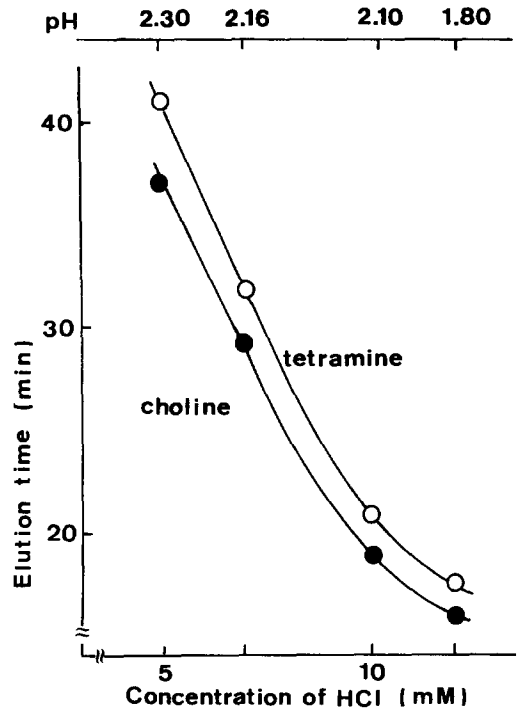

Fig. 3. Relationship between eluent concentration and elution time.

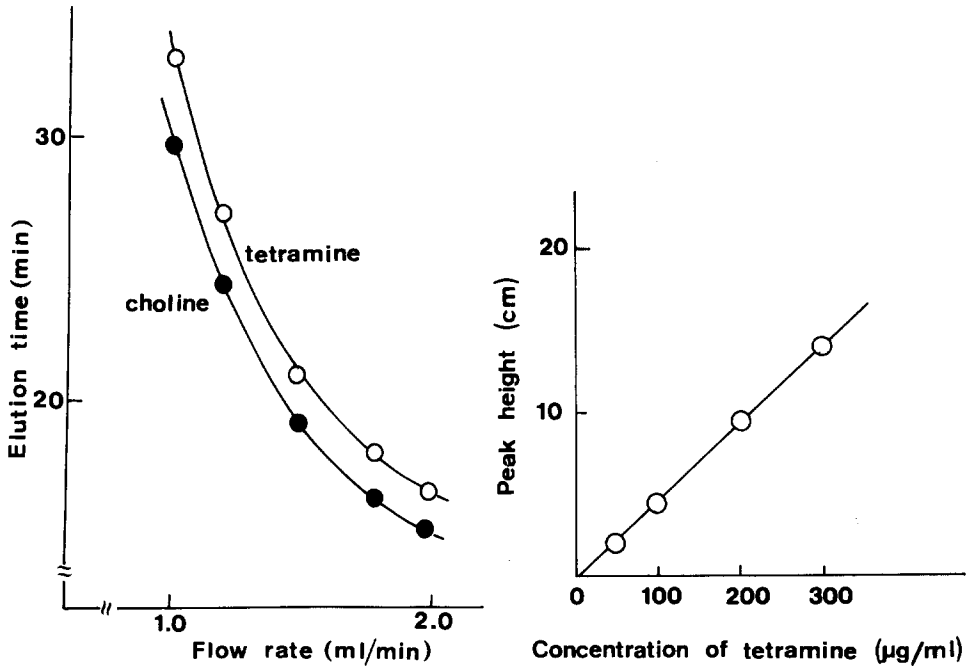

Fig. 4. Relationship between flow-rate and elution time.
Fig. 5. The calibration curve for tetramine.

Flow-rate

As the eluent flow-rate decreases, the elution times generally become longer and the distance between the peaks of the two ions increases. Fig. 4 shows the relationship between the eluent flow-rate and the elution times of tetramine and choline. As the eluent flow-rate decreased, the elution times become longer, and no significant effect on the peak interval was observed. Therefore, 1.5 ml/min was chosen as the eluent flow-rate.

Reproducibility and detection limit

Under the conditions shown in Table I, the coefficient of variation (CV) in nine measurements of tetramine in the standard solution and in the sample solution was 2.3% and 4.4%, respectively.

Fig. 5 shows a calibration curve of tetramine by peak height: the curve is essentially a straight line from the origin to 500 μg/ml. As calculated from the calibration curve and S/N = 2, the detection limit of tetramine was 5 μg/ml.

Tetramine in shellfish

Sample solutions of tetramine in shellfish were prepared as shown in Fig. 1 and analyzed by the standard addition method. Fig. 6 shows a chromatogram of a sample from *N. intersculpta*. No choline was detected by the chromatogram. Table II lists the results for tetramine from the salivary gland in a raw condition and heat-

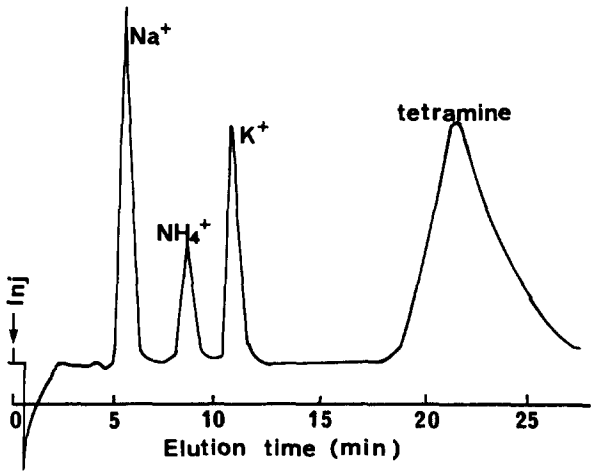

Fig. 6. Typical chromatogram of sample solution (*N. intersculpta*).

TABLE II

ANALYTICAL RESULTS OF TETRAMINE

	Analytical value by IC (μg/g)	*Dose–lethal time curve (μg/g)*	*Literature (μg/g)*
N. arthritica			
Raw	7490	3400	4000–7500
Heated	5040	2180	
N. intersculpta			
Raw	3920	3030	5500–9000
Heated	1800	1790	

treated (100°C for 5 min). The concentrations of tetramine as read from a prepared dose–lethal time curve* are also shown.

For *N. arthritica,* the measured IC values were two to three times larger than those read from the dose–lethal curve, but were close to the values found in literature. For *N. intersculpta,* the measured IC values agreed with both the values from the dose–lethal time curve and the values from the literature.

If it is assumed that 40 mg is a lethal dose for humans, the measured IC values indicate that five pieces of raw *N. arthritica* or ten pieces of raw *N. intersculpta* can cause food poisoning. This agrees with the literature values.

It was also found that the tetramine value of heated shellfish was 50 to 60% that of raw shellfish.

* One dose each of tetramine solutions of several different dilutions was injected into the peritoneals of three mice and the lethal time measured. The results were plotted on logarithmic graph paper, with the reciprocal of the average lethal time as the abscissa and the dose (μg/g) per unit weight (g) of the mice as the ordinate. This gave a dose–lethal time curve. Analysis of tetramine in the solution was performed by using the straight part of this curve.

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