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Note

Simultaneous determination of biogenic amines by reversed-phase high-performance liquid chromatography

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Biogenic amines including polyamines are known to be present in many biological materials. Prokaryotic cells produce biogenic amines[1] which are also found in foodstuffs contaminated by putrefactive organisms[2]. Among the biogenic amines, histamine is well known as a causative agent of allergy-like food poisoning[3], in which cadaverine or putrescine acts as a stimulator of the allergy-like symptom[4]. On the other hand, some of polyamines are useful for the quality assessment of foodstuffs, especially fresh marine food[2,5–7]. As has been well recognized, the extent of allergy-like symptoms caused by amines is dose dependent[3], and amines that participate in marine food quality are also closely related to the metabolism of some common amino acids, those amines which are related to allergy-like food poisoning or quality of food being derived from common or closely related amino acids. Whether particular foodstuffs benefit from polyamines or may cause allergy-like food poisoning seems to be delicately balanced.

In view of the above, there is a need for a rapid and economical method for determining histamine and other polyamines simultaneously. For sea food, the detection of agmatine is also important, because it is an index of freshness for the assessment of squid[6]. The greatest but most difficult requirement is to determine histamine and other polyamines including agmatine simultaneously.

Several methods have been proposed for the determination of histamine and other polyamines. Seiler and Knödgen[8] reported the reversed-phase high-performance liquid chromatography (RP-HPLC) of polyamines using gradient elution. Subsequently, several improved methods were developed[9–13]. However, to separate histamine, agmatine and polyamines, generally more than 40 min is required for one analysis. Also, the gradient programmes are sometimes complicated and the separation of each component is not necessarily satisfactory.

Gamoh and Fujita[14] reported the rapid and simultaneous determination of biogenic polyamines by RP-HPLC using an ion-pair method. Although their method seems to be very useful for clinical analysis, they did not give information on the separation of histamine and agmatine.

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We also have attempted to develop a highly sensitive, economic, simple, rapid and reproducible method for the simultaneous determination of histamine and major biogenic polyamines. We found that using sodium hexanesulphonate in place of octanesulphonate achieved a sufficient separation of histamine and other major biogenic polyamines within 35 min.

The present improved method is based on the post-column HPLC method of Gamoh and Fujita[14], *i.e.*, hexanesulphonate was used for ion pairing and the *o*-phthalaldehyde (OPA) method was used for post-column derivatization. By modifying their method, we established improved conditions for the determination biogenic amines such as histamine agmatine and other polyamines. This method should be useful not only in food science but also in clinical medicine.

EXPERIMENTAL

Chemicals

Standard amines were obtained from Sigma (St. Louis, MO, U.S.A.) and sodium hexanesulphonate from Tokyo Kasei Kogyo (Tokyo, Japan); all other reagents were of guaranteed-reagent grade from Wako (Osaka, Japan). All reagents and buffers were filtered through a 0.2-μm membrane filter (Millipore, Bedford, MA, U.S.A.) before use. Standard amines (hydrochloride form) were dissolved in distilled water.

Apparatus

Hitachi Model L-6200 high-performance liquid chromatograph equipped with a loop injector (Rheodyne, Cotati, CA, U.S.A.) and a fluorescence detector (Hitachi 650-10M) was used. Elution peaks were detected fluorimetrically using the excitation wavelength at 345 nm and emission wavelength at 455 nm. The separation of the amine mixture was carried out by reversed-phase chromatography on a Shim-Pak CLC-ODS column (15 cm × 6.0 mm I.D.) (Shimadzu, Kyoto, Japan) kept at 50°C.

The post-column labelling reaction of amines with *o*-phthalaldehyde (OPA) reagent was effected at 20 ± 5°C through a PTFE coil (70 cm × 0.25 mm I.D.) by mixing the eluates with OPA reagent. The OPA reagent was purified with a Hitachi Model L-6000 pump. A Hitachi Model D-2500 data processor was used.

Solvents and gradient system

The elution system consisted of the gradient system shown in Table I, which was prepared from two buffer systems: (A) 0.1 *M* sodium perchlorate (pH 4.0) containing 0.01 *M* sodium hexanesulphonate and (B) a mixture of buffer A and methanol (1:3, v/v) maintained at pH 3.0. The flow-rate of the elution buffer was 1.1 ml/min and that of the OPA reagent was 0.5 ml/min.

OPA reagent

The buffer for OPA reagent consisted of 24.7 g of boric acid, 10 g of sodium hydroxide and 1 g of Brij-35 in 1 l of distilled water. A 5-ml volume of OPA solution (250 mg in 5 ml of ethanol) and 1 ml of 2-mercaptoethanol were mixed with 494 ml of the buffer solution for OPA reagent prepared as mentioned above. The OPA reagent was prepared immediately before use.

Preparation of sample

Herring muscle that had been dried in a flow of air for 3 days at 20°C was used as the material for amine analysis. A 5-g amount of the dry herring muscle was homogenized with 20 ml of 10% trichloroacetic acid (TCA) in a mortar. After centrifugation at 550 *g* for 20 min, the precipitate was used for re-extraction of amines by the same procedure as above. The supernatants were pooled and diluted to 50 ml. The TCA extract thus prepared was then filtered through a 0.22-μm membrane filter (Millipore) and 5 μl of the sample solution were injected directly into the HPLC system.

RESULTS AND DISCUSSION

A good separation of the major biogenic amines was obtained using the gradient elution programme shown in Table I. Fig. 1 shows a representative elution profile of authentic amines. The calibration graphs for putrescine, cadaverine, histamine, agmatine and spermidine were linear in the range 5–100 pmol for the each amine, but for spermine good linearity was obtained in the range 20–100 pmol (data not shown). Acetylpolyamines, 1,3-diaminopropane and 1,8-diaminooctane were also separated from the amines tested here.

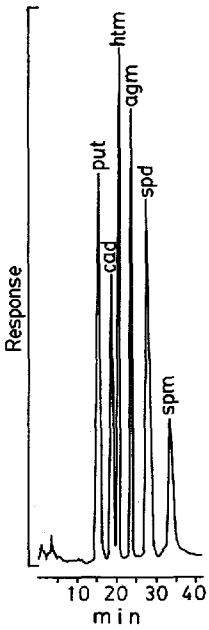
Under the given analytical conditions, the data were highly reproducible and sensitive enough for microscale analysis. The combination of hexanesulphonate for ion pairing and sodium perchlorate as the separation buffer in the elution solvent gave a satisfactory separation of polyamines in a relatively short elution time compared with previously reported systems[7,12].

As mentioned the main task was to accomplish the simultaneous micro-determination of major biogenic amines on a HPLC column. The system developed here permits the simultaneous micro-determination of major food-borne polyamines. Fig. 2 shows an example of the practical application of the system to the determination of biogenic amines in the dried herring. The sample solution containing TCA-extractable materials was directly injected into the HPLC system without any pretreatment except filtration. In this instance, histamine was not detected, which means that the sample was safe for consumption (Fig. 2A). We added 80 pmol of histamine to the sample solution to test whether this method can be used in practice. The exogenously added histamine was separated with other endogenous amines (Fig. 2B), and the

TABLE I

ELUTION PROGRAMME FOR POLYAMINE ANALYSIS

Time (min)	*Buffer A (%)*	*Buffer B (%)*	*Gradient mode*
0	96	4	–
7	96	4	Isocratic
10	85	15	Linear
22.5	65	35	Linear
30	65	35	Isocratic
30.5	96	4	Linear
35	96	4	Isocratic



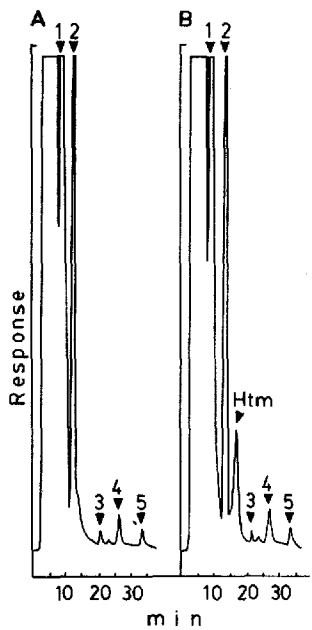


Fig. 1. Chromatogram of authentic biogenic amines. A 100-pmol amount of each amine was injected. Put = putrescine; cad = cadaverine; htm = histamine; agm = agmatine; spd = spermidine; spm = spermine.

Fig. 2. Representative chromatogram of amines extracted from dried herring: (A) dried herring extract; (B) A + histamine (80 pmol). Peaks 1 = put; 2 = cad; 3 = agm; 4 = spd; 5 = spm (abbreviations as in Fig. 1).

amounts of all the amines could be calculated reproducibly from the calibration graph.

Using this method, the time courses of the change in content of biogenic amines in dried herring and polyamine species in many foodstuffs have been determined and details will be reported elsewhere.

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