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High-performance liquid chromatography of histamine and 1-methylhistamine with on-column fluorescence derivatization

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ABSTRACT

An on-column fluorometric derivatization method was developed for the determination of histamine and 1-methylhistamine (HMs) by high-performance liquid chromatography. The system for the derivatization consisted only of a commercially available single-plunger pump and a reversed-phase C_{18} column supported on synthetic polymer with a mobile phase of acetonitrile and alkaline borate buffer solution containing *o*-phthalaldehyde as a derivatization reagent. It required no additional reaction system as for a post-column derivatization method. Injected HMs might be derivatized to a fluorophore on the inlet site of the high-performance liquid chromatographic column, followed by chromatography on the same column. Optimization of the on-column reaction conditions resulted in a simple and sensitive analytical method for the determination of HMs with excellent reproducibility and linearity of 0.05–5 μg/ml of both HMs. Application of this method to the determination of HMs in food samples resulted in a limit of quantification of 0.05 mg/100 g and in a greater than 95% overall mean recovery at a fortification of 0.1 mg/g of both HMs. This method was furthermore applicable to the determination of histamine released from rat peritoneal mast cells.

INTRODUCTION

Histamine, an inflammatory substance released from mast cells, is regarded as one of the chemical mediators which are generally known as autacoids. Each mediator plays an important role in living systems, and there is a need in clinical medicine to monitor procedures involving them and their metabolites, such as 1-methylhistamine.

However, it is known that allergic intoxication sometimes occurs with the intake of histamine-contaminated food, such as spoiled fish or fish products. The amount of histamine formed depends on the species of fish, the composition of the bacterial population, temperature, and the handling and storage of the fish. Shimidu [1] suggested that the critical concentration for toxic reactions is 60 mg/100 g. Therefore, there has also been a need to develop a simple routine analysis method to determine histamine in order to control the quality and evaluate the safety of various kinds of foods.

A number of analytical methods such as bioassay [2], fluorocolorimetry [3], enzyme isotope assay [4], gas chromatography (GC) [5] and high-performance liquid chromatography (HPLC) [6–11] have so far been used for the determination of histamine in

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a variety of substances. Most reports on the HPLC method which has been widely used in many fields have employed the pre-column [6–8] or post-column [9–11] derivatization technique using *o*-phthalaldehyde (OPA) [7–11] or dansyl chloride [6] as a fluorescence labelling reagent. However the pre-column procedure suffers from certain disadvantages, such as the requirement for carefully controlled reaction conditions and the time involved. Furthermore, the reaction product is inferior in terms of reproducibility because of its very limited stability.

On the other hand, a post-column procedure is claimed to be less troublesome than a pre-column procedure and can eleminate its drawbacks, but it requires expensive additional equipment for the chromatographic system. Further, peak broadening and dilution of samples are caused by the addition of post-column reagent.

The present paper proposes an on-line and on-column derivatization method employing a polymer C_{18} column with the mobile phase containing OPA reagents. This method was designed to overcome some of the drawbacks of the current derivatization method. The on-column method discussed in this paper is applicable to the quantitative analysis of histamine in various foods and in rat peritoneal mast cells.

EXPERIMENTAL

Chemicals and reagents

Standard solutions of histamine and 1-methylhistamine (HMs) were prepared by dissolving histamine dihydrochloride (Tokyo Kasei Kogyo, Tokyo, Japan) and 1-methylhistamine dihydrochloride (Sigma, St. Loius, MO, USA) in 0.05 *M* hydrochloric acid. Acetonitrile used was of HPLC grade (Wako Pure Chemical Industries, Osaka, Japan), water was glass-distilled and deionized. N-Acetyl-L-cysteine (NAC) was of biochemical grade (Merck, Darmstadt, Germany) and OPA (Tokyo Kasei Kogyo) and all other chemicals were of analytical-reagent grade and used without further purification.

Amberlite CG-50 (type I, 100–200 mesh; Rohm and Haas, Philadelphia, PA, USA) for the clean-up column was conditioned with 0.2 *M* sodium acetate buffer (pH 4.6) according to a conventional method, and the wet resin was then packed in a glass column (135 mm × 9 mm I.D.) at a height of 1 cm.

Apparatus

The HPLC system consisted of an LC-6A pump (Shimadzu, Kyoto, Japan), a Model 7125 injector (Rheodyne, Berkeley, CA, USA) with a 20-μl volume loop and a Model RF-535 fluorescence detector (Shimadzu) set at excitation and emission wavelengths of 340 and 450 nm, respectively. The recording and integrating device was a Chromatopac C-R6A (Shimadzu). The analytical column was an Asahipak ODP-50 (150 mm × 4.6 mm I.D.; Asahi Chemical Industry, Kanagawa, Japan).

The HPLC mobile phase used was acetonitrile–50 m*M* sodium tetraborate (borax) aqueous solution (18:82, v/v) containing 1 m*M* OPA and 1 m*M* NAC. The prepared mobile phase was transferred to an amber glass bottle and shielded from exposure to sunlight. The mobile phase was prepared daily and run isocratically at ambient temperature at a flow-rate of 0.5 ml/min.

Food sample preparation

A sample of 1 g of food was homogenized in a 10-ml volumetric test tube with 4 ml of 5% trichloroacetic acid for 1 min, then brought to a total volume of 10 ml with water. After centrifugation at 1800 *g* for 5 min, the supernatant was filtered with Toyo Roshi No. 5B filter paper. A 1-ml aliquot of the filtrate was neutralized to pH 6–7 with 1 *M* sodium hydroxide, followed by addition of 1 ml of 1 *M* acetate buffer (pH 4.6) and dilution with water to a total volume of 5 ml. The solution was then placed on an Amberlite CG-50 column and eluted at a flow-rate of less than 1 ml/min. After washing the column with 15 ml of 0.2 *M* acetate buffer (pH 4.6) and subsequently with 5 ml of water, HMs were eluted with 3 ml of 0.05 *M* hydrochloric acid. The first 1 ml was discarded, and the subsequent 2-ml eluates were taken as the HMs fraction. The fraction was evaporated to dryness *in vacuo*, and the residue was dissolved with 1 ml of 0.05 *M* hydrochloric acid. A portion of the 10-μl volume of this final solution was injected for HPLC analysis.

Stability of OPA-labelled HMs

The stability of the OPA-labelled histamine obtained by the proposed method and by a batchwise operation, *i.e.* the pre-column method, was examined as follows. For the on-column method, the delivery of mobile phase was stopped exactly 2 min

after injection of histamine (10 ng), and after certain periods had elapsed (10, 20, 30 and 60 min) the mobile phase was made to flow again. The HPLC column was thermostated at 25 ± 0.5°C throughout the experiment. For the pre-column method, 1 μg of histamine was incubated in 1 ml of the solution of the HPLC mobile phase used for the on-column method at 25 ± 0.5°C. After an elapsed period (10, 20, 30 and 60 min), 10-μl aliquots were injected into the HPLC system. The mobile phase used was a mixture of acetonitrile–50 m*M* borax (18:82, v/v) without OPA–NAC, and other conditions were similar to those for the on-column method.

Measurement of histamine release from rat peritoneal mast cells

Mast cells were separated from the peritoneal cavity fluid of Wistar rats using the method described by Nakagomi *et al.* [12]. Each test material (compound 48/80, mastoparan and somatostatin) was added to the mast cell suspension (20 μl) and the mixture was incubated at 37°C for 10 min. The reaction was terminated by cooling the mixture in an ice bath. After centrifugation at 1500 *g* for 5 min, 30 μl of the supernatant were taken, and the same volume of 0.1 *M* hydrochloric acid was added. A portion of the 10-μl volume of this final solution was injected for HPLC analysis without further purification. In this case, HPLC conditions were slightly modified: the HPLC column size used was 250 mm × 4.6 mm I.D., and the column was thermostated at 40 ± 0.5°C throughout the experiment. Other conditions were similar to those already described.

RESULTS AND DISCUSSION

The histamine introduced from an injector was presumed to be derivatized spontaneously to its OPA derivative at the inlet site of the HPLC column in the presence of the OPA reagent in the mobile phase, and the derivative was subsequently chromatographed on the same column. Therefore, the parameters examined, which were varied in order to effect the required reactivity and separation, were the concentration of the OPA, NAC, borax and acetonitrile in the mobile phase, its pH and the column temperature. The column employed for the on-column derivatization method was an Asahipak ODP-50, which is a polymer-supported C_{18} column advertised as being tolerant towards alkaline eluent. For the active thiol compound which is indispensable for the OPA-labelling reaction, NAC was used, because it has only a faint odour, whereas β-mercaptoethanol and ethanethiol, which have been used until now, are stinking liquids and difficult to handle.

The optimum concentrations of OPA–NAC in the mobile phase were examined with a mixture of acetonitrile–50 m*M* borax (18:82, v/v) containing 0.1–5 m*M* OPA–NAC. The molar ratio of OPA and NAC concentration was always set at equivalence. Fig. 1A shows that each peak area increased with increasing concentration (0.1–1 m*M*) of OPA–NAC and reached a plateau in the range 1–2 m*M*. As for the peak shape, symmetrical peaks were observed above 0.5 m*M* OPA–NAC, while slightly leading and tailing shapes were observed below 0.2 m*M*. On the other hand, the constant capacity factor (k') values in Fig. 1A suggested that the separation of OPA-labelled HMs was not influenced by the OPA or NAC concentration. Since the lower concentration of OPA–NAC was desirable for the HPLC background chromatogram, 1 m*M* OPA–NAC was chosen for subsequent work.

The derivatization of histamine with OPA is generally best carried out at alkaline pH, approximately pH 10. As might be expected, there was a significant drop in the peak area as the pH was lowered below 9, while a plateau was observed above pH 9.5 (see Fig. 1B). Symmetrical peaks were observed above pH 9, while leading or tailing peaks were observed below pH 8.5. The k' values of the HMs showed immutability regardless of the pH variation. Since the pH of the mixed solution of acetonitrile and 50 m*M* borax aqueous solution (18:82, v/v) containing 1 m*M* OPA–NAC was in the range 9.5–10, no additional pH adjustment was employed for subsequent work.

Fig. 1C shows the effect of the borax concentration. The peak area of both HMs increased with increasing borax concentration in the range 5–40 m*M* and reached a plateau over the range 40–70 m*M*. The variation in k' values showed a pattern similar to that of the peak area.

The variation in the acetonitrile concentration hardly influenced the peak area of the HMs, while

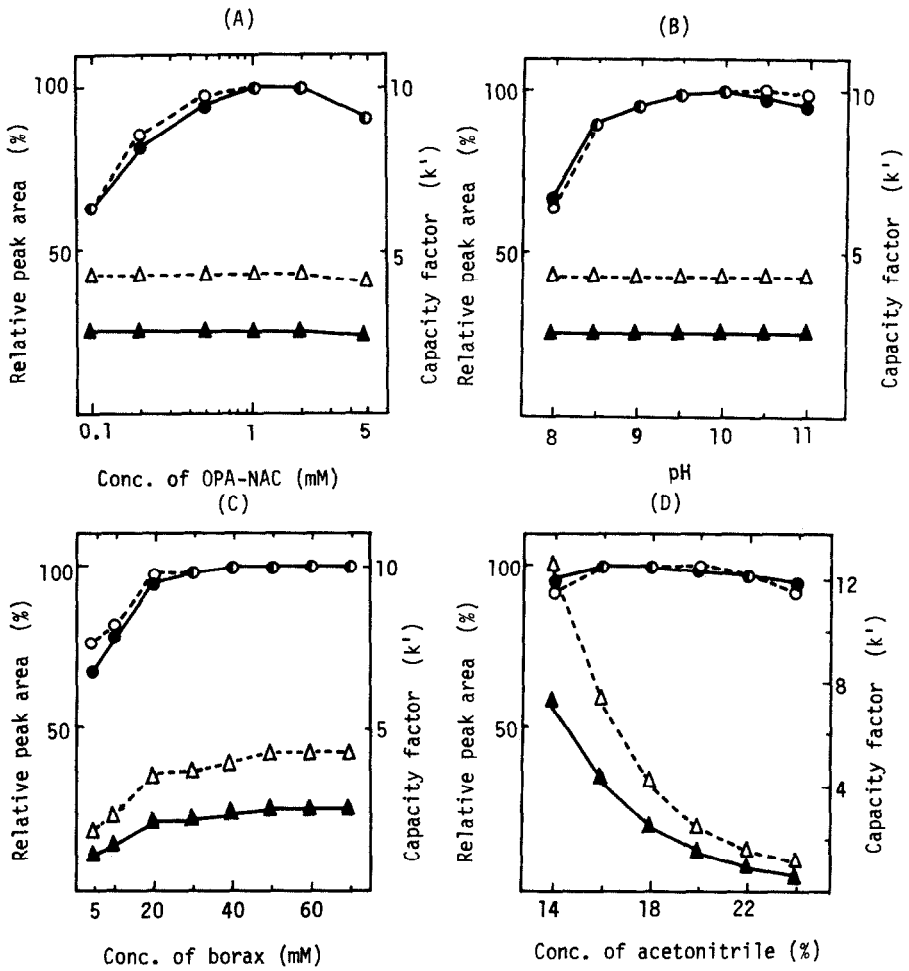

Fig. 1. Effect of (A) OPA–NAC concentration, (B) pH, (C) borax concentration and (D) acetonitrile concentration of the mobile phase on relative peak area (● = histamine; ○ = 1-methylhistamine) and capacity factor (▲ = histamine; △ = 1-methylhistamine). The mobile phase conditions were as follows: (A) 18% acetonitrile in 50 m*M* borax containing various concentrations of OPA–NAC; (B) 18% acetonitrile in 50 m*M* borax (adjusted to various pH values) containing 1 m*M* OPA–NAC; (C) 18% acetonitrile in various concentrations of borax containing 1 m*M* OPA–NAC; (D) various concentrations of acetonitrile in 50 m*M* borax containing 1 m*M* OPA–NAC. Flow-rate: 0.5 ml/min. Injection: 10 ng for both histamine and 1-methylhistamine. HPLC column: Asahipak ODP-50 (150 mm × 4.6 mm I.D.); column temperature: 25°C.

the k' values decreased with increasing acetonitrile concentration, as shown in Fig. 1D. When the acetonitrile concentration was *ca.* 18%, the k' values of both HMs were in the range 2–5, and the HMs showed good baseline separation. On the basis of these results, the mobile phase of acetonitrile–50 m*M* borax (18:82, v/v) containing 1 m*M* OPA–NAC was determined to be optimum for the proposed methodology.

As for the column temperature, relatively constant values for the HMs peak areas were obtained in the range 25–50°C, and a slightly declining tendency was observed above 50°C. The k' values of both HMs decreased with increasing temperature. Therefore, we usually performed experiments at ambient temperature.

Reproducibility and linearity

The precision and linearity of the on-column derivatization method were examined. The repetitive analyses ($n = 5$) of the HMs standard solution (0.05, 0.5 and 5 μg/ml) gave a corresponding relative stan-

dard deviation (R.S.D.) of the peak area for each concentration: 1.2, 0.5 and 0.8% for histamine; 1.3, 1.2 and 0.9% for 1-methylhistamine. The R.S.D. (n = 15) of the retention times was 0.5% for histamine and 0.3% for 1-methylhistamine.

The calibration graphs of peak area *versus* HMs concentration showed excellent linearity over the range 0.05–5 μg/ml for both HMs. The detection limit was 0.5 ng (signal-to-noise ratio = 3) for both HMs.

Stability of the OPA-labelled histamine

According to the report by Simmons and Johnson [13], an isoindole form of an OPA-labelled primary amino compound exhibites fluorescence but gradually convertes to a non-fluorescent substance because of its instability. Therefore, the stability of the OPA-labelled histamine in the HPLC column was compared with that in a batchwise solution. As a result, the decreasing fluorescence of the fluorophore, *i.e.* the decomposition reaction, was revealed to be a pseudo-first-order reaction for both methods. The rate constant obtained was $1.8 \cdot 10^{-3}$ min^{-1} for the on-column method and $3.6 \cdot 10^{-3}$ min^{-1} for the batchwise operation. Based on these results, the OPA-labelled histamine was more stable in the HPLC column than in a batchwise solution. Owing to a mutual interaction with the octadecyl group and/or the polymer support, the fluorophore was supposed to be more rigid in the HPLC column than in the batchwise solution. Further, though a steric effect of the octadecyl group was supposed to play an important role in the derivatization and decomposition reaction, the exact role is unclear at this point in time. However, there is no problem with pratical application of the on-column method because of the reproducibility of retention time and peak area and the linearity of wide-range calibration.

Extraction and clean-up

Sample extraction methods currently used for HMs detection in various foods require a clean-up step before quantitative analysis. A procedure described by Kawabata *et al.* [14] was slightly modified: the resin volume of the clean-up column was reduced on a scale of *ca.* one fifth. This shortened the total analytical time considerably and made this part of the procedure more convenient. The HMs adsorbed on the Amberlite CG-50 column were found in the 3 ml of 0.05 *M* hydrochloric acid eluate after washing with 15 ml of 0.2 *M* acetate buffer followed by 5 ml of water. No HMs were found in the 1.5 ml of the first eluate, where other impurities, such as amino acids when present in foods, were found. Therefore, the 1-ml of the first eluate was discarded with the subsequent 2-ml eluates taken as the HMs fraction. Fig. 2 shows typical chromatogram of HMs standard (A) and soy sauce (B) after removal of impurities.

Recovery study and analysis of commercial samples

Table I summarizes the recoveries of the HMs from commercial samples of dried sardine, soy sauce and soybeen paste fortified with 10 mg/100 g of each HM. Overall mean recoveries greater than 95% and standard deviations of 3% were obtained with every sample.

The histamine content found in fish and some kinds of commercial bean products varied from 0.07 to 20.0 μg/g, as shown in Table II. On the other hand, 1-methylhistamine was found not to be present in these samples.

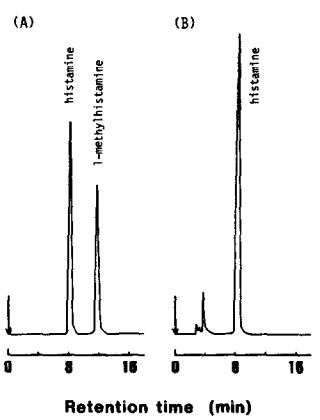

Fig. 2. Typical chromatograms of histamine and 1-methylhistamine from (A) a standard (10 ng for both histamine and 1-methylhistamine) and (B) soy sauce (14 ng of histamine) after clean-up. The ordinate shows relative fluorescence intensity. HPLC column: Asahipak ODP-50 (150 mm × 4.6 mm I.D.); mobile phase: acetonitrile–50 m*M* borax (18:82, v/v) containing 1 m*M* OPA–NAC; flow-rate: 0.5 ml/min; column temperature: 25°C.

TABLE I

RECOVERIES OF HISTAMINE AND 1-METHYLHISTAMINE ADDED TO FISH AND SOY PRODUCTS

Sample[a]	Recovery (mean ± S.D., n = 5)	
	Histamine	1-Methylhistamine
Sardine	96.5 ± 2.0	97.0 ± 2.7
Soy sauce	99.3 ± 1.5	98.1 ± 0.6
Bean paste	99.1 ± 1.2	99.7 ± 0.8

[a] Histamine and 1-methylhistamine were added to each sample at the level of 10 mg/100 g.

Analysis of histamine released from rat peritoneal mast cells

The applicability of this proposed method to the measurement of histamine-releasing activities of drugs, such as compound 48/80, mastoparan and somatostatin, which are well known to induce histamine release from mast cells, was examined. In this application, the purification procedure of the test solution was omitted, since the coexistent substance in the matrix was less than in the food samples. As shown in Fig. 3, the chromatograms were found not to contain any peaks that interfered with the measurement of the histamine peak. Histamine added at a level of 1 μg/ml to each test solution was quantitatively recovered (98.3–100.3%). Therefore, the assay developed here for the measurement of histamine released from rat peritoneal mast cells was sufficiently practical for routine analysis.

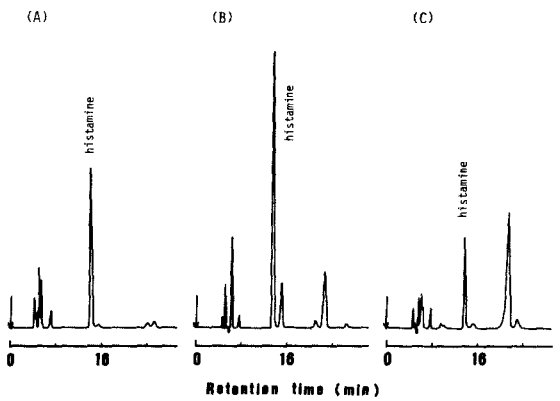

Fig. 3. Typical chromatograms of histamine released from rat peritoneal mast cells induced by (A) 1 μg/ml compound 48/80, (B) 10 μ*M* mastoparan and (C) 10 μ*M* somatostatin. The ordinate shows relative fluorescence intensity. HPLC column: Asahipak ODP-50 (250 mm × 4.6 mm I.D.); mobile phase: acetonitrile–50 m*M* borax (18:82, v/v) containing 1 m*M* OPA–NAC; flow-rate: 0.5 ml/min; column temperature: 40°C.

TABLE II

CONTENTS OF HISTAMINE AND 1-METHYLHISTAMINE IN VARIOUS FOODS

Determination limit: 0.05 mg/100 g; N.D. = not detected.

Sample	Histamine (mg/100 g)	1-Methylhistamine (mg/100 g)
Sardine	0.09	N.D.
Pacific saury	0.2	N.D.
Dry tuna	1.44	N.D.
Shelfish (n = 10)	0.29–2.73	N.D.
Soy sauce (n = 7)	0.08–20.0	N.D.
Bean paste (n = 10)	0.07–7.71	N.D.
Fermented soybeans	N.D.	N.D.

In conclusion, the on-column derivatization method described here is simple, highly sensitive, specific and routinely useful for the quantitative analysis of HMs in various foods and in rat peritoneal mast cells. This method resolves the problem associated with the stability of the fluorophore produced in the reaction of OPA with HMs and, moreover, it is convenient and can be readily incorporated into existing laboratory HPLC system as a new derivatization method.

REFERENCES

1 W. Shimidu, *Bull. Jpn. Soc. Sci. Fish*, 21 (1955) 365.
2 J. R. Vane, *Br. J. Pharmacol.*, 20 (1964) 360.
3 P. A. Shore, A. Burkhalter and V. H. Cohn, Jr., *J. Pharmacol. Exp. Ther.*, 127 (1959) 182.
4 R. E. Shaff and M. A. Beaven, *Anal. Biochem.*, 94 (1979) 425.
5 H. Mita, H. Yasueda and T. Shida, *J. Chromatogr.*, 221 (1980) 1.
6 A. Yamatodani, T. Seki, M. Taneda and H. Wada, *J. Chromatogr.*, 144 (1977) 141.
7 Y. Tsuruta, K. Kohashi and Y. Ohkura, *J. Chromatogr.*, 224 (1981) 105.
8 E. Kasziba, L. Flancbaum and J. C. Fitzpatrick, *J. Chromatogr.*, 432 (1988) 407.
9 F. Perini, J. B. Sadow and C. V. Hixon, *Anal. Biochem.*, 94 (1979) 431.
10 A. Yoshida and A. Nakamura, *J. Food Hyg. Soc. Japan*, 23 (1982) 339.
11 Y. Arakawa and S. Yachibana, *Anal. Biochem.*, 158 (1986) 20.
12 K. Nakagomi, M. Takeuchi, H. Tanaka, N. Tomizuka and T. Nakajima, *J. Antibiotics*, 43 (1990) 462.
13 S. S. Simmons, Jr. and D. Johnson, *J. Org. Chem.*, 43 (1978) 2886.
14 T. Kawabata, Y. Uchida and T. Akano, *Bull. Jpn. Soc. Sci. Fish.*, 26 (1960) 1183.