Selective Determination of Histamine in Urine by Solvent Extraction with Tetrabromophenolphthalein Ethyl Ester and Atomic Absorption Spectrophotometry

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A simple determination method of histamine in urine by solvent extraction and atomic absorption spectrophotometry has been established. The method is based on the formation of histamine–Cu(II) chelate cation, followed by the extraction of ion-associates with tetrabromophenolphthalein ethyl ester into 1,2-dichloroethane. Histamine in urine can be successfully determined without separation and purification from other histamine derivatives and amines. A linearity is obtained in the concentration range $(0.4—4)\times10^{-5}$ M (1 M = 1 mol dm^{-3}) histamine in aqueous solutions. The relative deviation is *ca.* 1.8%.

It is well known that histamine is produced from histidine in the presence of histidine decarboxylase and stored in the mast cells. The activity is considerably noted because histamine in mammalian tissues shows an important physiological action in the central nervous system. Therefore, the measurements of histamine in urine, tissues and brain are of great importance in clinical chemistry.

Fluorimetry with column chromatography,[1] high-performance liquid chromatography,[2] high-performance liquid chromatography with fluoresence detection[3] and radioisotope technique using ^{14}C-histamine for the assay and activity measurements of histamine and its derivatives in urine and tissues were reported. However, these procedures were somewhat complicated for the separation and purification.

The present paper describes a simple and selective atomic absorption spectrophotometric method without separation and purification of other biogenic amines and histamine derivatives for the assay of histamine in urine. The method is based on the formation of chelate compounds between Cu(II) and histamine and solvent extraction with hydrophobic tetrabromophenolphthalein ethyl ester (TBPE) into 1,2-dichloroethane. Acetylcholine and serotonine at the concentration of $(3—6)\times10^{-4}$ M do not cause errors. Catecholamines such as adrenaline and noradrenaline at the concentrations less than 6×10^{-5} M do not interfere, either.

Experimental

Reagents. Reagent solutions were prepared from reagent grade chemicals and distilled water, and they are stored in polyethylene bottles. Histamine standard solution of 1×10^{-2} M was prepared by dissolving 1.8407 g of histamine dihydrochloride (M.W. 184.07) to make the solution 1 L with distilled water. A 5×10^{-3} M copper(II) chloride solution was prepared by dissolving 0.8524 g of copper(II) chloride dihydrate in distilled water to make it 1 L. A 4×10^{-3} M TBPE ethanol solution was prepared by dissolving 0.2800 g of tetrabromophenolphthalein ethyl ester potassium salt in hot ethanol to make it 100 ml. A borate-phosphate buffer solution(pH 9.0) was prepared by adding 1 M NaOH to a 0.3 M potassium dihydrogenphosphate solution containing 0.1 M sodium borate. 1,2-Dichloroethane was used as an extractant.

Apparatus. A Nippon Jarrell-Ash, Model AA-780, atomic absorption spectrophotometer equipped with 10-cm slit burner and a cupric hollow-cathode lamp(Hamamatsu TV Co., Ltd.) was used and peak hights were recorded with a Yanako Model YR-110 chart recorder. Hitachi-Horiba, Model F-7II, pH meter was used for pH measurements. An Iwaki shaker, Model KM, was used for the solvent extraction.

Recommended Procedure. Take 2 ml of sample solutions containing about 90 μg/ml of histamine, 1.5 ml of 5×10^{-3} M copper(II) chloride solution, 5 ml of a buffer solution (pH 9.0) and 2 ml of a TBPE solution into a 50 ml calibrated flask, and dilute the mixture to 50 ml with water. Transfer the aqueous solution into a 100 ml separatory funnel and shake it for 5 min with 10 ml of 1,2-dichloroethane. Keep the funnel standing for 15 min and then separate the organic layer. Run off the extract into a glass tube through a filter paper to remove water droplets. Add 4 ml of ethanol to 1 ml of the extracts. Record the absorption signal at 3247 Å after mixing the solution. Take the peak height as the analytical signal. Run a reagent blank at the same instrumental settings and subtract it from the analytical values. Linear relationship between the absorbance of the extract and the concentration of histamine was held for the concentration range of $(0.4—4)\times10^{-5}$ M histamine in aqueous solutions.

Results and Discussion

Analytical Conditions for Copper Determination. The operating conditions used for copper determination were as follows: lamp current, 9 mA; wavelength, 3247 Å; flow-rate of acetylene, 1.75 l/min; flow-rate of air, 8.5 l/min; burner height, 23 mm; absorption-sens, 0.1 and burner length, 10 cm.

Effect of pH on Extraction. It was reported[5] that histamine formed the complexes with transition metal ions such as copper, zinc, cobalt, nickel, and iron. Of these metals, however, it was found that the height of the absorption peak given by the extract was considerably increased when copper(II) was present in the aqueous solution. Therefore, the effect of pH on the extraction in the presence of copper(II) was studied by extracting Cu(II)–histamine chelate cations with TBPE anion from a series of aqueous buffer solution of various pH. Figure 1 shows that the atomic absorption of copper(II) in the extracts was maximum and constant over the pH range of 8.5—10, whereas in the absence of histamine, copper(II) was not ex-

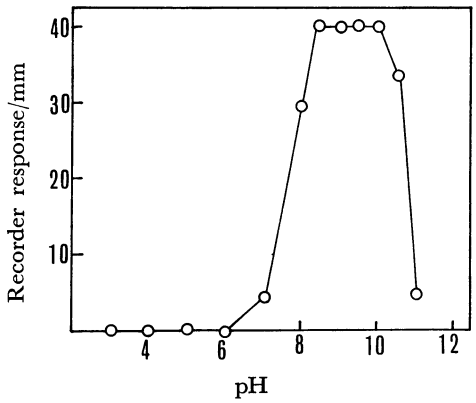


Fig. 1. Effect of pH on extraction.
Histamine: 3×10^{-5} M, Cu(II): 1.5×10^{-4} M, TBPE: 1.6×10^{-4} M.

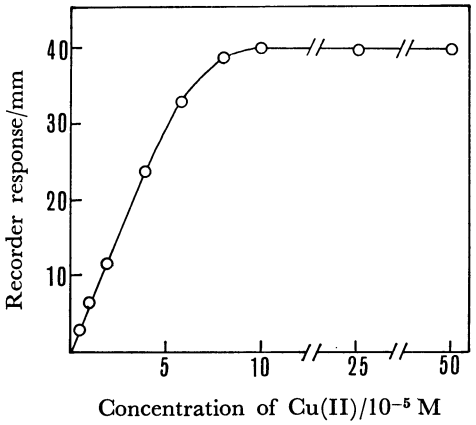


Fig. 2. Effect of copper(II) concentration.
Histamine: 3×10^{-5} M, TBPE: 1.6×10^{-4} M, pH: 9.0.

tracted with TBPE. In both acidic and alkaline media, the atomic absorption of copper(II) was markedly decreased. This may be caused by the decrease of Cu(II)–histamine complex formation in the acidic medium (pK_a for histamine, 5.1[6]), as well as copper(II) hydroxide precipitation in the alkaline medium. Extraction is recommended to carry out at pH 9.0.

Effect of Copper(II) Concentration. Various amounts of copper(II) were added to aqueous solutions of histamine and the extraction was carried out as described above. Figure 2 shows that 3-fold molar ratio of Cu(II) to histamine is enough to obtain maximum and constant atomic absorption. A copper(II) concentration of 1.5×10^{-4} M is recommended when histamine concentration is 3×10^{-5} M.

Effect of Counter Ions for the Extraction. Hydrophobic anions such as perchlorate, tetraphenylborate (TPB), picrate and TBPE were studied as a counter ion for the extraction of hydrophilic Cu(II)–histamine cations. The ion-associates with perchlorate and picrate anions were not extractable into nitrobenzene, though it has a higher dielectric constant. On the other hand, the addition of TPB showed a high atomic absorption, but the peak height was remarkably decreased when TPB concentration was maintained more than 1×10^{-4} M, because the concentrated TPB and histamine produce white precipitates in the aqueous

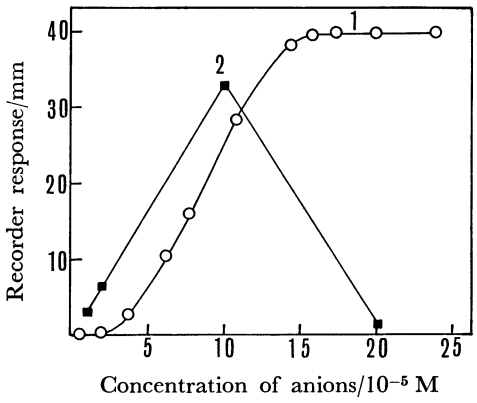


Fig. 3. Effect of TBPE and TPB concentrations on extraction.
1: TBPE, 2: TPB, histamine: 3×10^{-5} M, Cu(II): 1.5×10^{-4} M, pH: 9.0.

phase. Figure 3 shows that the extraction of Cu(II)–histamine–TBPE ion-associates was maximum and constant, when TBPE used was more than 1.5×10^{-4} M (5-fold molar ratio to histamine). Excess amounts of phosphate buffer did not give any influence on the atomic absorption, but separation of two layers became incomplete when the addition of the buffer solution was less than 1 ml. The previous papers[7] described that ion-associates with TBPE was the most extractable species into 1,2-dichloroethane.

Effect of Shaking Time and Stability of the Extract. Extraction was completed quantitatively within 30 s, and shaking for up to 15 min did not produced any further change in absorption signal, and which was remained almost constant for at least 5 h after extraction.

Calibration Curve and Precision. Under the optimum conditions, the calibration curve was made with a histamine standard solution containing 1.5×10^{-4} M copper(II) chloride solution. It shows a good linearity over the range of $(0.4—4)\times10^{-5}$ M (0.736—7.36 μg/ml) histamine with an aqueous/organic solvent volume ratio of 5. The relative standard deviation (ten determinations) was estimated to be 1.8% for 3×10^{-5} M histamine.

Interferences of Diverse Substances. To the solution containing 3×10^{-5} M histamine and 1.5×10^{-4} M copper(II) chloride solutions, various amounts of foreign compounds were added and their influences were examined. The results are summarized in Table 1. Inorganic substances, such as sodium chloride, potassium chloride, sodium nitrate, sodium carbonate, and sodium sulfate did not show any interference. Furthermore, calcium, magnesium, cobalt, lead, nickel, zinc, iron, and iodide ions of 5-fold molar excess over 3×10^{-5} M histamine did neither show any influence. Though citrate ion interfered considerably with the determination, it was minimized by addition of calcium ions at a concentration almost equal to that of citrate ion.

Histidine, serotonine, acetylcholine and imidazole-4-acetic acid, being analogous to histamine and giving a strong influence on other measurements,[1,3] did not interfere with histamine in 10—20 fold ratio. Of bio-

Table 1. Effect of various substances on determination of histamine

Substances	Mole ratio [Ion]/[HA]	Mole ratio [Ion]/[Cu(II)]	Recovery of histamine %
Sodium chloride	500	100	100
Potassium chloride	500	100	100
Sodium nitrate	50	10	100
Sodium carbonate	50	10	102
Sodium sulfate	50	10	101
Calcium chloride	5	1	103
Magnesium chloride	5	1	105
Cobalt(III) chloride	5	1	105
Lead(II) chloride	5	1	103
Nickel sulfate	5	1	100
Zinc acetate	5	1	101
Iron(III) nitrate	5	1	102
Ammonium chloride	5	1	102
Potassium chromate	5	1	100
Potassium iodide	5	1	100
Histidine	10	2	100
Acetylcholine	20	4	98
Serotonine	10	2	102
Imidazole-4-acetic acid	10	2	99
Adrenaline	2	0.4	95
	1	0.2	100
Noradrenaline	2	0.4	97
	1	0.2	99
Dopa	1	0.2	100
(Dihydroxyphenyl-alanine)	2	0.4	100
Dopamine	2	0.4	98
	1	0.2	100

Histamine taken: 3×10^{-5} M, Cu(II) taken: 1.5×10^{-4} M, TBPE: 1.6×10^{-4} M, pH on extraction: 9.0, solvent: 1,2-dichloroethane

genic amines, catecholamines such as adrenaline, noradrenaline and dopamine which coexist with histamine in biological materials did not interfere less than 1—2 fold ratios to 3×10^{-5} M histamine. Histamine is a major component in biological materials and the content ratio for [Adrenaline]/[Histamine(HA)] is 0.03; for [Noradrenaline]/[HA], 0.2; [Dopamine]/[HA], 0.02 in heart and the corresponding ratios are 0.06, 0.4, 0.02 in spleen.[8] Consequently, about 5 μg/ml levels of histamine in tissues and urine can be selectively determined by this method.

Composition of the Extracted Species. In order to clarify the composition of the extracted species, continuous variation plots were made. The results shown in Figs. 4 and 5 reveal that a 1:1 chelate cation is formed between Cu(II) and histamine, and that in the organic phase a 1:3 ion-associate is formed between Cu(II)–histamine cations and TBPE anions. Moreover, an ion-associate was dried at reduced pressure for 48 h and the powdery precipitates obtained was analyzed. Found: C, 39.7; H, 2.4; N, 2.0; O, 8.6; Br, 46.6; Cu, 1.0%. Calcd for Cu(II)–histamine–TBPE associate: C, 39.7; H, 2.2; N, 1.3; O, 9.1; Br, 45.8; Cu, 1.9%. Thus the results obtained by the

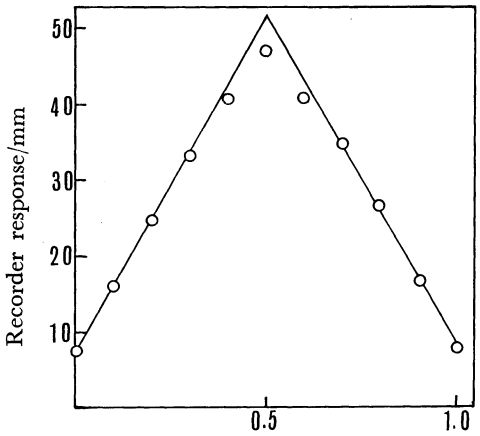


Fig. 4. Continuous variation plots.
[HA]+[Cu(II)]=1×10^{-4} M, TBPE: 1.6×10^{-4} M, pH: 9.0.

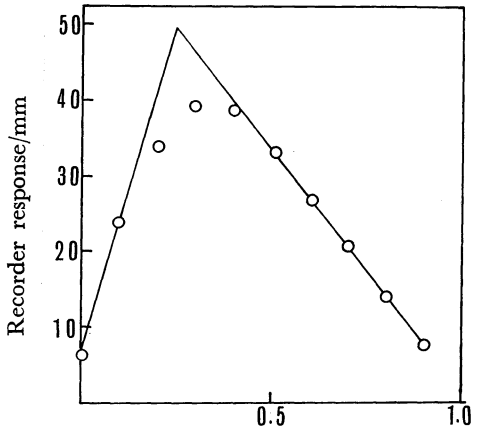
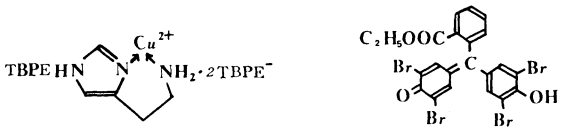


Fig. 5. Continuous variation plots.
[HA]+[TBPE]=2×10^{-4} M, Cu(II): 2×10^{-4} M, pH: 9.0.



Ion-associate with TBPE TBPE molecule

Chart 1.

two methods suggest that the chemical formula of the extracted species may be formulated as [Cu(II)–histamine]·$(TBPE)_3$. The ion-associate produced is assumed to show its structure in Chart 1 because the absorption spectrum of the associate with TBPE showed the charge transfer complex type in our previous paper,[7] being a maximum wavelength at 515 nm.

Application to Histamine Determination. Known amounts of histamine standard solution were added in human urine. Sample solutions containing 92 μg of histamine were analyzed by the proposed method. The results obtained were 81 μg and 79 μg histamine for two samples (3 determinations) and the recoveries were 88 and 86%, respectively. On the other hand,

5 mg/ml of histidine dihydrochloride was injected intravenously to two rats (about 150 g, ♂). Urine was withdrawn after 24 h and histamine in urine was determined by this method. 58 μg histamine could consequently be found in 50 ml of the recovered urine.

The recovery of histamine by the proposed method is not high enough as we expected. However, the procedure is very simple and this is available for the practical determination of histamine in biogenic amines, as the selective method.

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