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Note

Cation-exchange chromatography of histamine in the presence of ethylammonium chloride

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The potent vasopressor substance histamine has been characterized by a number of methods including thin-layer chromatography[1], ion-exchange chromatography using a fluorescent derivative[2], and several reversed-phase high-performance liquid chromatographic (HPLC) methods[3,4] using derivatization after extraction. Since intravenous injection of small quantities of histamine can cause undesirable physiological responses in humans[5], it is desirable to have an easy, fast and accurate means of analysis for histamine.

In this paper we describe a fast, accurate method of analyzing histamine which requires no derivatization or extraction and is applicable to commercial amino acid solutions, whole blood and other biological fluids. The use of small amounts of ethylammonium ions to decrease retention and the effects of pH of the mobile phase on separation are discussed.

EXPERIMENTAL

Cation-exchange HPLC was carried out on a 25 cm × 4.6 mm I.D. Partisil 10 SCX column (Whatman, Clifton, NJ, U.S.A.) using a Waters liquid chromatographic system equipped with a Model 6000 A pump, a Model U6K sample injector and a Schoeffel Model SF-770 variable-wavelength detector operated at 210 nm. All measurements and chromatography were at room temperature. The mobile phase consisted of 0.03 *M* K_2HPO_4 in distilled water at pH 4.5. Ethylamine hydrochloride at 0.2%, 0.25% and 0.5% was added to the mobile phase to decrease retention of histamine. The mobile phase was filtered through a 0.45-μm Millipore Type HA filter before use.

Histamine dihydrochloride (Eastman Kodak, Rochester, NY, U.S.A.) and tryptamine hydrochloride (Aldrich, Milwaukee, WI, U.S.A.) were purchased as analytical reagent grade and used without further purification.

Nephramine® and FreAmine® (American McGaw, Irvine, CA, U.S.A.) are amino acid solutions containing ten (5.4%, w/v) and fifteen (8.5%, w/v) individual amino acids respectively.

Whole blood was lysed by an ultrasonic device and centrifuged to obtain a clear supernate. Saliva was centrifuged and the supernate used for analysis.

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RESULTS AND DISCUSSION

Table I contains the effects of ethylammonium chloride concentration on k' for histidine, histamine and tryptamine. As can be seen, increasing the ethylammonium ion concentration substantially alters the k' value for the three components. The addition of ethylammonium ions also greatly improves peak symmetry of histamine and tryptamine. The best separation and a reasonable time of analysis were accomplished using 0.35% ethylammonium chloride. Fig. 1 shows typical chromatograms of histamine in water, an amino acid solution, and plasma. Tryp-

TABLE I

EFFECTS OF ETHYLAMMONIUM CHLORIDE CONCENTRATION ON k' FOR HISTIDINE, HISTAMINE AND TRYPTAMINE IN WATER

Ethylammonium chloride (%, w/v)	k'		
	Histidine	*Tryptamine*	*Histamine*
0	2.92	4.80	6.85
0.20	2.58	4.25	5.45
0.25	2.33	4.15	5.14
0.50	2.16	3.09	3.48

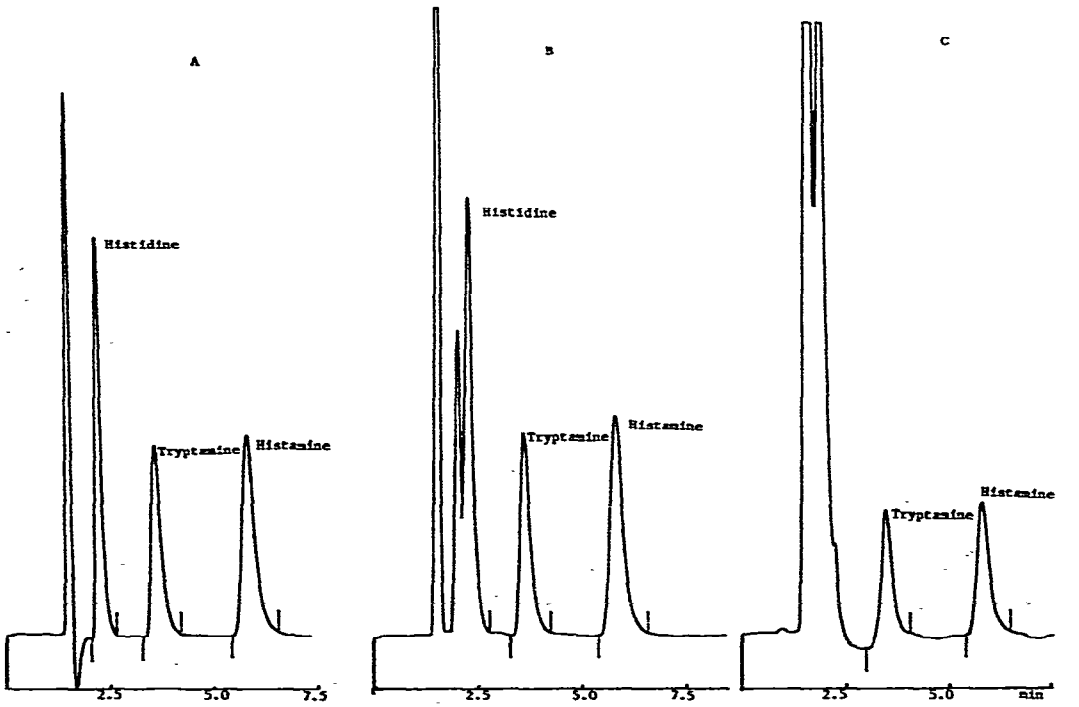

Fig. 1. Chromatograms for the separation of histamine spiked at 25 μg/ml using 2.5 μg/ml of tryptamine hydrochloride as an internal standard and 0.25% ethylammonium chloride in (A) water, (B) FreAmine III and (C) plasma.

tamine was chosen as an internal standard due to its retention lying between that of histidine and histamine.

The common interferences in the analysis of histamine using derivatization methods such as *o*-phthaldehyde are amino acids, proteins and primary and secondary amines[6]. By choosing the mobile phase at pH 4.5, the amino acids, proteins, amines and ethylammonium chloride are completely ionized. The interferences from amino acids and most proteins are eliminated due to their lack of retention in their completely ionized state. A lower pH (2.9) gave longer retention and a higher pH (7.0) gave shorter retention for histamine, but in both cases increased interferences from amino acids and proteins.

Table II contains the limits of detection and actual quantities found for histamine in water, two amino acid solutions, whole blood and saliva. The amino acid solutions showed no detectable quantities of histamine as supplied by the manufacturer.

TABLE II

LIMITS OF DETECTION OF HISTAMINE IN WATER, AMINO ACID SOLUTIONS, PLASMA AND SALIVA USING 0.25% ETHYLAMMONIUM CHLORIDE

Solution	*Limit of detection (μg/ml)*	*Actual quantity found (μg/ml)**
Water	0.020	0
FreAmine	0.020	0
Nephramine	0.020	0
Whole blood	0.050	0.090 ± 0.006
Saliva	0.042	0.150 ± 0.010

* Mean ($n = 5$) ± S.D.

The results of this investigation indicate that this technique can successfully and conveniently determine histamine in a variety of solutions without extraction and/or derivatization. It is also anticipated that the use of ethylammonium ions can decrease retention and improve peak shape of selected amines when using cation-exchange chromatography under isocratic conditions.

REFERENCES

1 W. Diemair and S. Diemair, *Deut. Wein Ztg.*, 102 (1966) 1146.
2 G. deSaint-Blanquat and R. Derache, *Trav. Soc. Pher. Montpellier*, 28 (1968) 23.
3 R. E. Subden, R. G. Brown and A. C. Noble, *J. Chromatogr.*, 166 (1978) 310.
4 Y. Tsuruta, K. Kohashi and Y. Ohkura, *J. Chromatogr.*, 146 (1978) 490.
5 J. Schneyder, *Mitt. Rebe Wein*, 22 (5) (1972) 313.
6 F. Engbaek and I. Magnussen, *Clin. Chem.*, 24 (1978) 376.