Journal of Chromatography, 86 (1973) 263–268

CHROM. 6935

Note

Quantitative thin-layer chromatography of histamine and its metabolites*

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(Received July 10th, 1973)

A primary interest in the demonstration of differences in the metabolism of histamine in atopic and normal dogs led to improvement of the methodology relative to analysis of histamine and its metabolites from the urine. This paper reports on a thin-layer chromatographic (TLC) method for the separation and quantitation of histamine (H), methylhistamine (MH), acetylhistamine (AH), methylimidazoleacetic acid (MIAA) and imidazoleacetic acid (IAA).

A number of solvent systems have been recommended for the separation of histaminic amines: Butanol–glacial acetic acid–water, butanol–pyridine–water, isopropanol–ammonia–water[1], acetone–absolute ethanol–water[2], diisobutyl ketone–acetic acid–water, ethanol–acetic acid[3], and methanol–chloroform–water[4]. Optimum results were achieved in our laboratory with a modification of the butanol–glacial acetic acid–water system.

Visualization of separated histaminic amines traditionally is obtained by exposing the developed plates to iodine vapors. With this procedure, MH and MIAA are stained weakly and are often difficult to visualize. Furthermore, iodine staining is only temporary and does not allow for quantitation of the amines. These two difficulties were resolved by producing fluorescent compounds of the five amines which could be quantitated by absorption or fluorescence detection.

MATERIALS

Equipment

TLC plates. Analtech (Newark, Del., U.S.A.), silica gel G, 250 μm, 20 × 20 cm.

Tanks. Corning (New York, N.Y., U.S.A.), rectangular chromatography jar, $5\frac{3}{8} \times 6\frac{3}{8} \times 10\frac{1}{2}$ in.

Chromoscan densitometer. Joyce, Loebl & Co. (Burlington, Mass., U.S.A.), Model CSC with thin-layer attachment, tungsten halogen light source.

Schoeffel spectrodensitometer. Schoeffel Instruments (Westwood, N.J., U.S.A.), Model SD 3000.

Solvents and reagents

Glacial acetic acid. J. T. Baker (Phillipsburg, N.J., U.S.A.) No. 9507.

* Supported by National Science Foundation Grant GB 24701 Amend No. 1.

Methanol. J. T. Baker, No. 9076.
n-Butanol. J. T. Baker, No. 9054.
Iodine. E. Merck (Rahway, N.J. U.S.A.), U.S.P. resublimed.
Histamine dihydrochloride. J. T. Baker, No. N330.
1,4-Methylhistamine, dihydrochloride. Calbiochem (San Diego, Calif., U.S.A.), A grade.
1-Methyl-4-imidazoleacetic acid, hydrochloride. Calbiochem, A grade.
Imidazoleacetic acid, hydrochloride. Calbiochem, A grade.
N-Acetylhistamine. Calbiochem, A grade.

METHODS

TLC plates, 5 cm wide, were scored to produce 10-mm lanes. The plates were not activated nor treated prior to use. Standards of each amine and a combination of all five amines were made in methanol (containing a few drops of 0.1 *N* hydrochloric acid) to a final concentration of 1 μg/μl. Varying dilutions of these stock solutions were made so that 10 μl contained 1–10 μg of each amine or a combination of the five amines. In all cases, 10 μl was spotted at the origin, 2 cm from the bottom of the plate, in the central 8 mm of the lane.

The plates were placed in a non-lined tank and developed in butanol–glacial acetic acid–water (60:22:23). During a 4–5 h development period, the tank was kept in an incubator room at an ambient temperature of 37° and relative humidity below 20%. The solvent system was allowed to rise 17 mm from the origin, at which time the plate was removed from the tank and positioned vertically for 10 min in a ventilated hood, and then horizontally in an oven for an additional 10 min at 70°. The plate then was placed in a tank with iodine vapor for 30 min; and upon removal it was placed horizontally in a ventilated hood for 5 min and subsequently in an oven for 30 min at 300°.

The plate was scanned in the densitometers in the absorption (Joyce, Loebl & Co.) or fluorescent (Schoeffel) modes. In the former method, the primary filter employed was 300–400 nm and the secondary filter 340 nm (narrow band). In the latter method, the primary filter was 340 nm (narrow band) and the secondary filter 490 nm (narrow band). Peak areas were calculated by triangulation.

RESULTS AND DISCUSSION

Following exposure to iodine vapors and heat, the amines could be visualized as brown bands with ordinary light (MH and MIAA to a lesser degree) and all elicited a readily discernible yellow fluorescence with UV irradiation.

The amines ascended in the following order: MH, H, MIAA, AH, IAA. In Table I are the distances from the origin each amine migrated and their R_F values in the butanol–glacial acetic acid–water solvent system (60:22:23) along with separation with other solvent mixtures. Figs. 1 and 2 are representative of tracings of combined (5 amines) sample after scanning in absorption and fluorescence. Fig. 3 illustrates the linear relationship of each amine when concentration is plotted against peak area.

The heating of the histaminic amines after exposure to iodine vapors had the effect of producing stable fluorescent compounds which may be quantitated in the μg

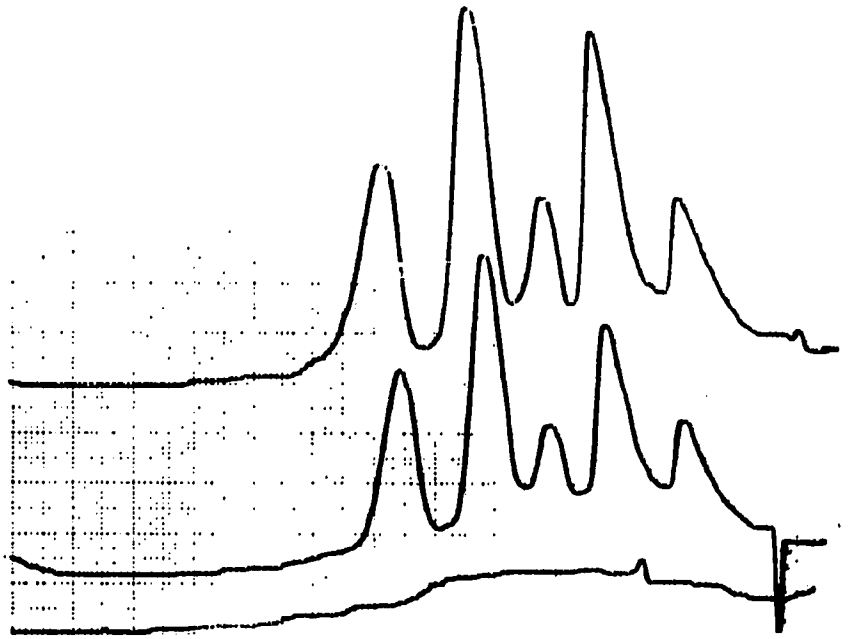

Fig. 1. Separation of histaminic amines by TLC on silica gel G plates. Solvent, butanol–glacial acetic acid–water (60:22:23). Absorption mode with Joyce-Loebl Chromoscan, C cam and 2 O.D. wedge, primary filter 300–400 nm and secondary filter 340 nm. Ten μl of combined standards containing 5 μg (middle tracing) and 10 μg (upper tracing) each of H, MH, MIAA, AH and IAA applied at origin. Peaks from right to left are: H, MH, MIAA, AH and IAA. The bottom tracing represents a solvent blank.

range. Over a period of one year, plates stored have shown no change in tracings and peak areas. It should be noted however, that peak areas will vary with plates from different batches and with ambient environmental conditions.

Activation of the plates prior to development did not improve the results of the procedure, and at times lessened the intensity of the fluorescence. The latter also was noted when a liner was used in the tank. Equilibration of the tank had no beneficial effect. The time necessary for development was related to ambient temperature and relative humidity. Developing at 37° significantly increased solvent flow, as did increasing relative humidity. The gain in shortening the development time by increasing the relative humidity to 40–50% was negated, however, by the tendency of higher relative humidities to result in smudging and tailing of the bands.

Heating the plate for 10 min at 70° had the effect of increasing the intensity of fluorescence of the histaminic amines. Care in removing the plate from the 300° oven must be taken to avoid cracking of the glass. Placing the plate immediately after removal on a pre-heated smooth and flat $^1/_4$ in. thick sheet of steel resolved this problem.

Optimum absorption and fluorescence emission was found to be 340 nm and 490 nm, respectively, for each of the amines.

The utilization of the described procedure, after suitable extraction of the urine for histamine and its metabolites, has provided a method for quantitation of these

TABLE I
R_F VALUES FOR HISTAMINIC AMINES IN VARIOUS BUTANOL–GLACIAL ACETIC ACID–WATER SOLVENT SYSTEMS

Ratio			*R_F values*					*Migration from origin (mm) (SF= 170 mm)*				
n-Butanol	*Glacial acetic acid*	*Distilled water*	*MH*	*H*	*MIAA*	*AH*	*IAA*	*MH*	*H*	*MIAA*	*AH*	*IAA*
60	22	23	0.17	0.28	0.34	0.43	0.49	29	47	57	73	84
60	15	15	0.10	0.19	0.24	0.34	0.41	17	32	40	58	69
50	32	23	0.24	0.36	0.44	0.49	0.58	48	61	74	83	98
57	25	23	0.18	0.28	0.35	0.41	0.48	30	47	59	70	82

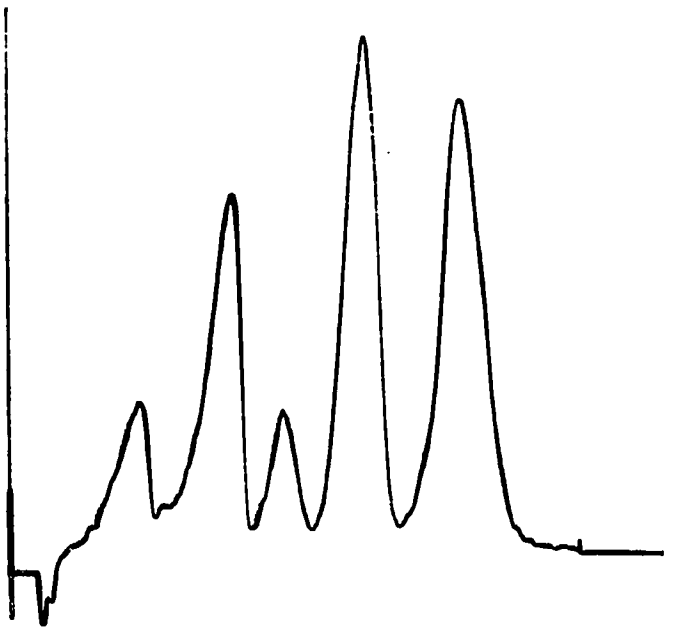

Fig. 2. Separation of histaminic amines by TLC on silica gel G plates. Solvent, butanol–glacial acetic acid–water (60:22:23). Fluorescent mode with Schoeffel spectrodensitometer, primary filter 340 nm and secondary filter 490 nm, ×1.0. Ten μl of combined standards containing 5 μg each of H, MH, MIAA, AH and IAA applied at origin. Peaks from left to right are: H, MH MIAA, AH and IAA.

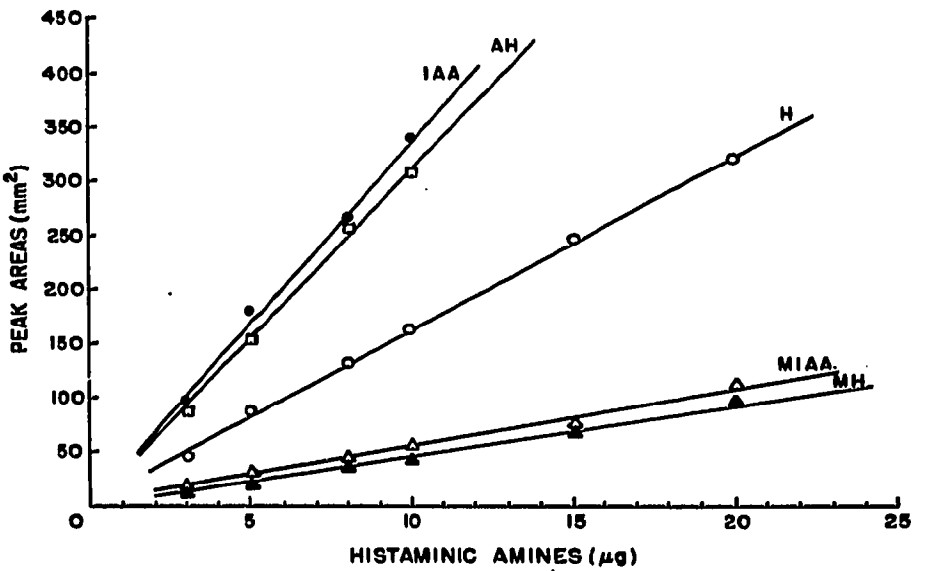

Fig. 3. Relationship between μg of histaminic amines and peak areas.

amines. The extraction quantitation of urinary histaminic amines will be reported elsewhere. Further studies into the applicability of this procedure to the quantitation of other unsaturated compounds were not performed, but it is hoped that other investigators will undertake this and report their results accordingly.

ACKNOWLEDGEMENT

The author wishes to thank Mrs. D. Schneider and Dr. J. C. Touchstone whose technical advise and assistance were invaluable.

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