

Quantitative Determination of 1,4-Methyl-Imidazoleacetic Acid in Urine by High Performance Liquid Chromatography

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A reversed phase ion-pair high performance liquid chromatographic (HPLC) method for quantitative determination of the major histamine metabolite 1,4-methyl-imidazoleacetic acid (1,4-MIAA) in urine is described. The sample handling is minimal as the sample only has to be centrifuged and diluted before analysis, in contrast to earlier gas chromatographic and thin-layer chromatographic methods. The method correlates well with a thin-layer chromatographic determination ($r = 0.79$, $n = 15$), and results from analysis of urine from different patients suffering from mastocytosis, chronic urticaria, atopic dermatitis and asthma are presented together with samples from normal individuals. Most of the patients analysed showed increased excretion of 1,4-MIAA compared with the controls. Future applications of the method, including analysis on patients with food allergy and patients with abnormalities in their histamine metabolism, are discussed.

Key words: 1,4-methyl-imidazoleacetic acid, HPLC.

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The study of histamine metabolism and the possible role of histamine as a potent mediator in various conditions has received great interest for many years. The role of histamine in asthma has been long controversial (1, 3, 12, 14) but it has been shown recently that there is an increased histamine turnover in acute attacks of intrinsic bronchial asthma (10) and in allergen-induced asthma (11).

In 1956 Schayer & Cooper studied the metabolism of C^{14} -histamine in man and identified 1,4-methyl-imidazoleacetic acid (1,4-MIAA) as one of the metabolites (13). This metabolite was detected for the first time in human urine (not following ingestion of C^{14} -histamine) from patients with mastocytosis in 1962 by Demis (2), and in 1963 Ham & Schayer (7) isolated the compound from the urine of a patient with dermatitis medicamentosa. Later 1,4-MIAA was

found in healthy humans by Kerr (8) using paper-chromatography.

It has been shown (9) that 1,4-MIAA is the major metabolite of histamine as about 60% of a 10 μ g dose of C^{14} -histamine given subcutaneously is excreted as 1,4-MIAA in the first 12 h. 1,4-MIAA is excreted non-conjugated as there is no increase in 1,4-MIAA after rigorous hydrolysis (13). Thus the determination of 1,4-MIAA should be a good indicator of the overall histamine turnover in humans. Another metabolite of histamine, imidazoleacetic acid (IAA), is not a good indicator of histamine metabolism (4, 9) since large amounts of this compound originate from histidine. The isomer of 1,4-MIAA (1,5-MIAA) is not a normal metabolite of histamine in man (9, 13) and it has been suggested that it is of dietary origin (17, 18). Since no other precursor of 1,4-MIAA than

histamine has been found it seems that determination of 1,4-MIAA in urine could be of great value in conditions where histamine is known or suspected to play a pathophysiological role. The only requirement is that certain dietary restrictions are followed in order to minimize the intake of histamine (6).

Measurement of 1,4-MIAA has been done by the use of thin-layer chromatography (TLC) (5) and gas-chromatography (GC) (16) and in both methods it is necessary to make a derivative of 1,4-MIAA. This increased sample handling can now be overcome by the use of reversed phase ion-pair high performance liquid chromatography (HPLC) where the samples have only to be cleared of precipitate and diluted before injection on to the column. The chromatography of polar compounds like 1,4-MIAA can be done in a reversed phase system by the use of a suitable ion-pairing agent such as sodium dodecylsulphate (SDS) or by the use of ion exchange chromatography, although this has lower efficiency.

The drawback of earlier methods for measuring 1,4-MIAA is the time-consuming derivatization and extraction steps which mean that only a small number of samples can be processed daily. This can be overcome by the present semi-automatic method by which the full potential of measuring 1,4-MIAA in urine under different conditions can be elucidated.

MATERIAL AND METHODS

Apparatus

The chromatographic system consisted of a model 6000 A pump, a WISP 710 B automatic sample processor and a model 440 variable wavelength detector (Waters Associates, Milford, Mass.) and a dual channel recorder (Houston Instruments, Austin, Texas). The column was a Hibar® RT 250-4 lichrosorb RP-18 5 µm particles prepacked column (E. Merck, Darmstadt, G.F.R.) heated to 45°C in a water bath (Heto, Birkerød, Denmark) which maintained the temperature within 1/10 of a degree.

A ultrasonic bath was used to degas the solvents (Elgasonic, Bienne, Switzerland).

Chemicals

The following chemicals were used: Acetonitrile of lichrosolv quality, and orthophosphoric acid, concentrated hydrochloric acid, sodium dihydrogenphosphate, sodium hydroxide, and methanol were analytical grade from E. Merck (Darmstadt, G.F.R.) and sodium dodecylsulphate specially purified (BDH, Poole, England). 1,4-methyl-imidazoleacetic acid was from Calbiochem (Lucerne, Switzerland) and 1,5-methyl-imidazoleacetic acid was a gift from Göran Granerus. Imidazoleacetic acid, 1-methyl-histamine, L-1-methyl-histidine, L-3-methyl-histidine, creatine, creatinine, taurine, glycine and 21 L-amino acids were from Sigma (Saint Louis, Missouri, U.S.A.).

The water used was distilled and filtered through a 0.45 µm membrane filter (Millipore, Bedford, Mass.).

Procedures

The aqueous part of the mobile phase consisted of 1 ml/l of 85% phosphoric acid in water with the addition of 0.69 g/l $\text{Na}_2\text{H}_2\text{PO}_4$, 10 g/l sodium dodecylsulphate (SDS) and the pH adjusted to 2.80 with 1 N NaOH. The final mobile phase consisted of the aqueous part mixed with acetonitrile 180/70 (v/v) degassed in an ultrasonic bath for 15 min and constantly stirred during the separation. The column was equilibrated for 30 min at a flow of 1.5 ml/min at 45°C before the first sample injection. Before storage the column was washed with methanol for 15 min at the same flow rate.

The urine samples were acidified by addition of 5 ml 6 N HCl to the collection bottles and kept frozen until use. The only preparation of the urine samples prior to injection was centrifugation for 5 min at 4000 g to remove precipitates, and dilution 20 times with the aqueous part of the mobile phase.

For analysis of actual urine samples 900 µl of the aqueous part of the mobile phase was mixed with 50 µl urine and 50 µl of pure water

or water with a known concentration of 1,4-MIAA, usually 5 µg/ml. Two hundred µl of this mixture was injected and the concentration calculated using the formula:

$$h_s / \frac{h_s' - h_s}{S}$$

where h_s is the peak height for 1,4-MIAA in the sample, h_s' is the peak height in the sample with added 1,4-MIAA and S is the concentration of 1,4-MIAA in the added standard in µg/ml.

The accuracy of the dilution in the two cases could be controlled by comparing the height of other peaks in the chromatogram.

RESULTS

A typical chromatogram is shown in Fig. 1. In order to identify the 1,4-MIAA peak in the chromatogram and to calculate the amount of

the compound in the samples the samples were run in duplicate with one sample spiked with water and one sample spiked with a known amount of 1,4-MIAA. No interference with the 1,4-MIAA determination was observed from the isomere 1,5-MIAA, imidazoleacetic acid, 1-methyl-histamine, L-1-methyl-histidine, L-3-methyl-histidine creatinine, creatine, glycine, taurine and 21 L-amino acids as they were well separated from 1,4-MIAA. Only one urine sample showed interference with the measurement of 1,4-MIAA but as this particular sample was submitted in a soft plastic bag and not in an ordinary collection bottle this problem was not considered significant. The urine samples were acidified and kept frozen at -20°C until analysed. Repeated thawing and freezing did not seem to affect the analytical results. Furthermore, urine samples containing toluene to minimize bacterial growth posed no problems.

To determine the reproducibility of the

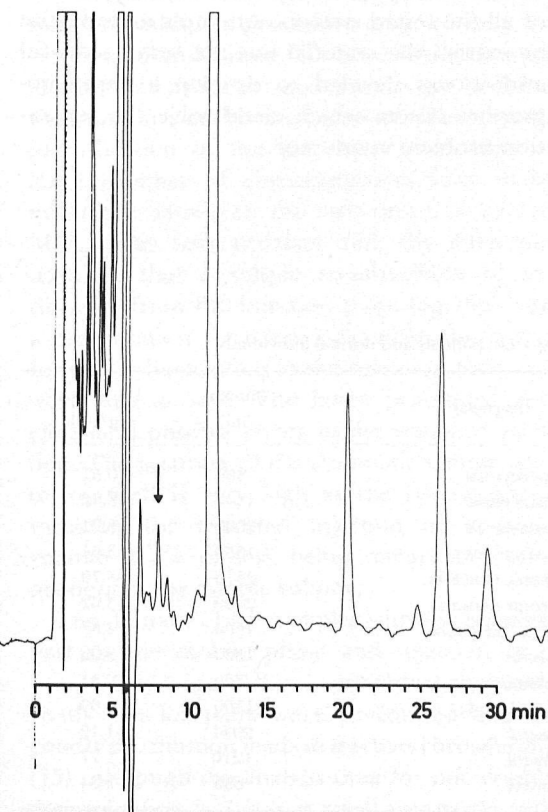


Fig. 1. Chromatogram showing the separation of 1,4-MIAA from other urinary constituents.

Sample: 200 µl urine diluted 20 times with the aqueous part of the mobile phase. Mobile phase: 0.1% phosphoric acid in water with 0.69 g/l NaH_2PO_4 and 10 g/l SDS pH 2.80 mixed with acetonitrile 180:70. Flow: 1.5 ml/min. Column: Merck Hibar® RP-18 5 µm controlled at 45°C. Detector: 220 nm, 0.04 AUFS.

The actual sample contained 5.82 µg/ml of 1,4-MIAA.

Table 1

Reproducibility of the analysis at different 1,4-MIAA concentrations

Mean µg/ml	4.74	10.72	15.90
Standard deviation	0.236	0.329	0.600
Coefficient of variation %	4.98	3.07	3.77
Number of samples	6	7	10

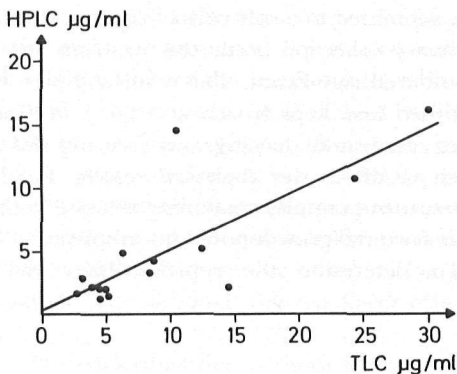


Fig. 2. Comparison between a thin-layer chromatographic method for semiquantitative determination of 1,4-MIAA (5) and the present HPLC method. The correlation coefficient $r = 0.79$, the slope of the regression line is 0.47 and the intercept is 0.44.

method patient samples with different amounts of 1,4-MIAA were analysed several times. The results are shown in Table 1.

The results from 15 samples analysed both by the present method and by the thin-layer chromatographic method described by Granerus & Magnusson (5) are shown in Fig. 2. Furthermore, the method was applied to samples from normal healthy individuals and a number of patients suffering from urticaria, rheumatoid arthritis, mastocytosis and atopic dermatitis, and results are presented in Table 2.

DISCUSSION

The separation of 1,4-MIAA from other urinary constituents was attempted on a variety of different stationary phases such as $-NH_2$, C_8 and phenyl bonded materials. The ion-pair chromatography on a C_{18} bonded material at elevated temperature gave the best separation of all the tested systems. Attempts to derivatize or extract the material was not very successful and it was decided to develop a chromatographic system which could solve the separation problem in one step.

Table 2

Results from analysis of 1,4-MIAA in urine from patients and normal individuals

Patient	Sex	Age (years)	Diagnosis	Diuresis (ml)	1,4-MIAA mg/24 h
O.K.	M	9	mastocytosis	360	0.57
E.M.	M	60	mastocytosis	1540	48.82
P.E.	M	37	mastocytosis	2000	8.22
U.L.	F	37	mastocytosis	1550	3.61
C.O.	M	19	chronic urticaria	2570	8.79
A.T.	F	62	chronic urticaria	2800	3.02
K.A.	M	36	atopic dermatitis	1500	4.67
J.K.	M	47	asthma	1200	8.92
C.S.	M	25	asthma/atopic dermatitis	600	2.41
B.R.	F	34	sclerodermia	1500	0.69
S.N.	M		control	2051	1.19
K.N.	F		control	1210	1.77
H.P.	M		control	686	2.34

The factors that influence the separation of 1,4-MIAA from other constituents of urine are the concentration of the ion-pairing agent, the concentration of the organic modifier, pH, ionic strength and temperature. In order to optimize the separation the concentration of SDS and acetonitrile was kept constant and the retention time was adjusted by adding NaH_2PO_4 . The pH was then varied between 2.30 and 2.90 and the temperature varied between 20°C and 80°C in order to finally adjust the separation. The temperature effect on k' of 1,4-MIAA itself is small while the k' of other urinary constituents varies widely, giving interference or very long analysis times.

The chromatographic conditions were optimized using the prepacked Merck Hibar RP-18 columns with 5 μm particles and it is likely that small adjustments have to be made when changing to other column packings because different column packings have different amounts of bonded phase.

The method has been used both with the standard addition procedure and by using external standardization. The two methods work equally well as far as results are concerned, but the standard addition procedure allows easier identification of the 1,4-MIAA peak when a large number of chromatograms have to be evaluated. However, the retention time of 1,4-MIAA was very constant with the apparatus used so that a simple measurement of the distance from the injection point together with a recalibration for every 10 samples, to correct for minor fluctuations in the retention time, can work just as well. The latter procedure also eliminates possible errors in the standard addition. The accuracy of the automatic sample processor itself is very high as the coefficient of variation for repeated injection of a given volume is 1% or less, being remarkably independent of the sample volume.

The dilution of the samples with the aqueous part of the mobile phase and injection of a comparably larger volume served two purposes: firstly the ion-pairs were preformed and secondly the dilution leads to less band broadening (15). Although the analysis time for one double determination is 1 h it is small compared with

the thin-layer chromatographic method as this method can analyse eight samples a week using 150 ml of urine while the present method can analyse about 120 samples a week using the automatic sample processor, allowing time for sample preparation, calibration and calculations.

As shown in Fig. 2 the correlation between the TLC method (5) and the present HPLC method is good, taking into account that the TLC method is presented as a semiquantitative method. However, the correlation is not absolute as seen from the slope of the regression line.

Two samples show large discrepancies from the general picture. The cause of the discrepancies might be that the TLC plates were developed in iodine vapour, giving rise to background staining with some urine samples. The analysis of urine samples from patients suffering from mastocytosis, chronic urticaria, atopic dermatitis and asthma show that increased amounts of 1,4-MIAA are excreted as compared with normal individuals. The excretion of 1,4-MIAA by normal individuals has been determined by Granerus (6) to be 2.7 mg/24h on a standard diet and by Tham (17) to be 2.65 mg/24 h with a range of 0.76–4.99 mg/24 h.

Future applications of 1,4-MIAA analysis include studies on patients with food allergy where the allergen is not known, detection and significance of abnormalities in histamine metabolism, i.e. block in histamine metabolism leading to low clearance of histamine, measurement of 1,4-MIAA after provocation tests to further consolidate the diagnosis, and finally, studies on patients with rheumatoid arthritis. In this context it is interesting that some patients with symptoms that could be related to increased histamine liberation have not shown increased excretion of 1,4-MIAA. This, of course, would be the case if the symptoms were due to another mediator but it could just as well indicate that the metabolism of histamine was blocked somewhere along its metabolic pathway. In these patients the ratio of histamine to 1,4-MIAA in the urine would be increased. Studies along these lines are in progress in our laboratory.

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