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SIMULTANEOUS DETERMINATION OF VANILLYL MANDELIC ACID (VMA), 5 HYDROXY INDOLE ACETIC ACID (5-HIAA), HOMOVANILLIC ACID (HVA), 6ß-HYDROXY CORTISOL USING RP HPLC.

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ABSTRACT

In present research work, a simultaneous estimation method of Vanillyl mandelic acid (VMA), 5 Hydroxy indole acetic acid (5-HIAA), Homovanillic acid (HVA), 6ß-Hydroxy cortisol using reverse phase HPLC method was developed and validated. Rat 24 hr urine samples were collected and injected directly without extraction, detection was carried out at 235 nm using UV detector. The developed method is précised, accurate and sensitive.

Key words: VMA, HVA, metabolic disorders, RP HPLC method.

INTRODUCTION:

Measurement of urinary 3-methoxy-4-hydroxymandelic acid (vanillylmandelic acid, VMA) a metabolite of noradrenaline (NA),Adrenaline and 3-methoxy-4- hydroxyl phenylacetic acid (homovanillic acid, HVA (DA) metabolite of dopamine considered useful for detection and monitoring of patients with pheochromocytoma, neuroblastoma, or other catecholamine releasing tumors[1,2].Urinary excretion of 5-hydroxy-3-indoleacetic acid (5-HIAA), a main metabolite of serotonin, is increased in patients with carcinoid syndrome. 6ß-Hydroxy cortisol is

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the metabolite of cortisone and is the indicative of time course of enzyme induction in metabolism of drugs, hyper -cortisolemic states[3-5] The urinary excretion rates of these analytes are useful for study of neuropsychiatric, metabolic and related disorders characterizing the effects of many drugs. Simultaneous measurement of these metabolites is often advantageous in increased diagnostic specificity[6].

A wide variety of methods reported for urinary estimation of VMA**,** 5-HIAA, HVA[7-9] In present study, we describe a simple, accurate and Précised method for the simultaneous estimation of VMA, 5-HIAA, HVA and 6ß-Hydroxy cortisol by HPLC from the urine[10-13].

MATERIALS AND METHODS

Apparatus

Gradient high-pressure liquid chromatography (Shimadzu HPLC Class VP series Version 5.03) with LC-10AT VP pumps was used in the experiment. A variable wavelength programmable UV/VISIBLE Detector, SPD-10A VP, CTO-10AS VP column oven (Shimadzu) and reverse phase (RP)-C18 column (250mm x4.6mm I.D; Gemini 5µ 110A) was used. The samples were injected through a 20µl loop injector (Rheodyne Inc., Berkeley, CA).

The mobile phase consisted of potassium dihydrogen phosphate buffer (0.1M, pH 3.0 adjusted with orthophosphoric acid) combined with acetonitrile (HPLC grade) by isocratic-elution technique in the ratio of 85:15 %v/v at a flow rate of 0.9ml/min was used , as the separation of VMA and iso-VMA requires a mobile phase with low content of organic solvent. The mobile phase was filtered through 0.13 µm membrane filter. The column temperature was maintained at 40^0C and the detection was carried at 235nm. Iso-vanillylmandelic acid **(**iso-VMA.) was used as an internal standard.

Reagents and standards

VMA, iso-VMA, HVA, and 5- HIAA were purchased from Sigma Chemical Co., St. Louis, MO. Stock standard solutions of each (1mg/ml), prepared in dilute HC1 (10 mmol/L).Standard stock solution of 6ß-Hydroxy cortisol (1mg/ml) was prepared in HPLC grade methanol. They were stored at 4^0C which showed the stability for six months. Working standards were prepared by diluting these with mobile phase (Acetonitrile: Buffer 15:85, pH - 3).

Procedure

Urine specimens (24 h) were collected in polyethylene bottles containing 0.5 mL of 6 mol/L HCL from female rat (SD) and then stored frozen if not assayed within 24 h. Urine samples were centrifuged at 2000 rpm for 10 min, then 200µl of each sample was combined with 0.1ml of iso-

VMA standard (250 ng/ml), and made volume to 1 ml. The preparation was then centrifuged; filtered through 0.45µm membrane filter, and 20µL aliquot of this was injected into the chromatographic system.

RESULT AND DISCUSSION

Chromatographic profiles for urine samples are presented in Figure 1.The retention times of VMA, iso VMA, 5-HIAA, HVA and 6-Hydroxy cortisol were observed at 5.008, 5.708, 9.742, 12.408 and 13.208 min respectively. The results of interday and intraday precision for determinations of VMA, 5-HIAA, HVA, and 6ß-Hydroxy cortisol is summarized in (Table 1)

The ratio of VMA, HVA, 5-HIAA and 6ß-Hydroxy cortisol to the internal standard (y) was linearly related to the concentrations of the standards (x) over a range of 25 to2500 ng/ml. The linear regression equations observed are as follows y = 0.007x + 0.100, r = 0.991for VMA; y = 0.003x + 0.132, r = 0.989 for HVA, y = 0.007x + 0.268, r = 0.996 for5-HIAA; and y = 0.004x + 0.27, r = 0.996; for 6ß-Hydroxy cortisol.

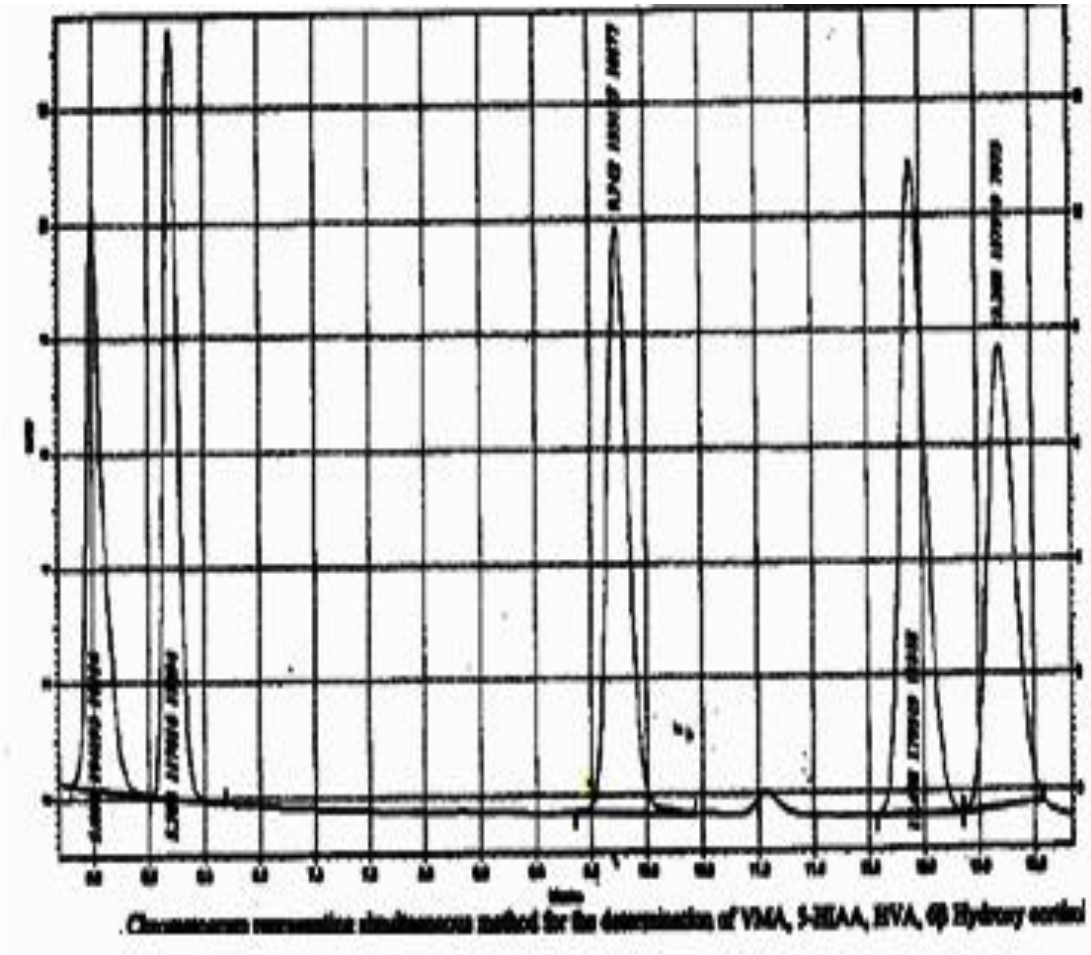

Figure I chromatogram representing simultaneous method for determination of VMA, 5HIAA, HVA and 6 ß Hydroxy cortisol

Table 1: Precision of the assay

Concentration (50 ng/ml)	Intraday precision (n=6)		Interday precision(n=6)	
	Observed Mean concentration (ng/ml)	%RSD	Observed Mean concentration (ng/ml)	%RSD
VMA	54.493	2.177	54.541	2.213
5-HIAA	55.021	2.284	55.515	1.034
HVA	43.815	0.698	43.328	0.1581
6ß-hydroxy cortisol	55.559	0.341	56.163	1.854

Table 2:Analytical recovery of VMA, HVA, 5HIAA and 6β-hydroxycortisol added to pooled urine

n=3	Endogenous Concentration(ng/ml)	Added Concentration(ng/ml)	Recovery (%)
VMA	20.5	100	102
	20.1	250	100
	20.2	500	102
HVA	289.5	100	98
	300.1	250	98
	298.2	500	99
5HIAA	10.25	100	100
	10.82	250	101
	10.24	500	103
6β-Hydroxycortisol	51.05	100	100
	50.80	250	105
	50.01	500	100

The lowest concentrations of VMA, HVA, 5-HIAAand 6ß-Hydroxy cortisol distinguishable from base line noise with 95% confidence were 16.5, 13.75, 15.82 and 8.81ng/ml. Analytical recovery of four substances was determined by adding known amounts of VMA, 5HIAA, HVA and 6ß-Hydroxy cortisol to a pooled sample of urine and assaying it. (Table 2)

The HPLC method we described has several advantages. It allows the simultaneous quantitative determination of VMA, 5-HIAA, HVA and 6ß-Hydroxy cortisol and is rapid (17-18 min for each chromatographic run).

CONCLUSION:

The developed method is accurate, sensitive and precise for the simultaneous analysis of Vanillyl mandelic acid (VMA), 5 Hydroxy indole acetic acid (5-HIAA), Homovanillic acid (HVA), 6ß-Hydroxy cortisol by HPLC from the urine collected from the rats. So it can be used routine clinical analysis in pharmaceutical company.Nonstandard abbreviations: VMA, vanillyl mandelic acid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid.

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