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Note

Determination of vanillylmandelic acid in urine by pre-column dansylation using micro high-performance liquid chromatography with fluorescence detection

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Vanillylmandelic acid (VMA) has been known as the final metabolite of catecholamines. Various analytical procedures for urinary VMA determination such as thin-layer chromatography [1], gas chromatography—mass spectrometry [2—4] and high-performance liquid chromatography [5, 6] have been reported. Recent investigations in our laboratory suggest a correlation between the content of VMA in urine and catecholamine metabolism. The importance of VMA determination compelled us to develop a simple and sensitive procedure for measuring the content of the metabolite in urine.

In our previous papers, benzoylation of urinary estrogen [7] and tosylation of noradrenaline in rat brain [8] were utilized for micro determination. In the present work, we applied the dansylation procedure for determination of urinary VMA. Dansyl derivatives have been very widely used in clinical analysis [9, 10]. In this paper, the phenolic hydroxyl group of VMA which was extracted from urine was dansylated as the fluorescent derivative and then separated and determined using micro high-performance liquid chromatography (MHPLC) [11] equipment with a fluorimeter.

MATERIALS AND METHOD

Standard VMA was obtained from Tokyo Kasei Kogyo (Tokyo, Japan).

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All other chemicals used were of reagent grade purity. A Model Familic 100 (Jasco, Tokyo, Japan), a micro high-performance liquid chromatograph, equipped with a Model FP 110 fluorescence spectrophotometer was used for analysis. An SS-10 silica gel (particle size 10 μm) column 17 cm × 0.1 cm I.D. (Jasco) was used. The mobile phase was ligroin (boiling range 80—100°C)—chloroform—ethyl acetate (1:4:8). The flow-rate was 8 μl/min and the chromatographic separation was monitored with the fluorescence spectrophotometer set at 360 nm was the excitation wavelength and at 505 nm as the fluorescence wavelength. Chromatograms were recorded with a single-pen recorder (Rikadenki Kogyo, Tokyo, Japan). The chart speed was 12 cm/h.

According to the method of Felice and Kissinger [12], 1 ml of urine was transferred into a 10-ml glass tube containing 200 μg of *p*-hydroxybenzoic acid (PHBA) as an internal standard. The mixture was adjusted to pH 2.0 with 1 *N* hydrochloric acid and 0.3 g of sodium chloride was added to the solution from which VMA was extracted twice, each time with 3 ml of ethyl acetate and then back-extracted with 1 ml of 1 *M* potassium carbonate. An aliquot of 0.5 ml of the carbonate layer was transferred into a 25-ml glass-stoppered tube, and 5 ml of 0.01% dansyl chloride in acetone solution was added. After incubation at 40°C for 45 min, 2 ml of water were added, and the dansylated VMA was extracted twice, each time with 3 ml of chloroform. The combined extracts were evaporated to dryness under a stream of air and the residue was redissolved in 5 μl of ethyl acetate. A 0.01-μl sample of the solution was applied to the chromatograph. VMA was determined by the peak height ratio of VMA to PHBA. The standard curve of the peak height ratios showed good linearity within a range of injected sample amounts of 5—20 μg/ml.

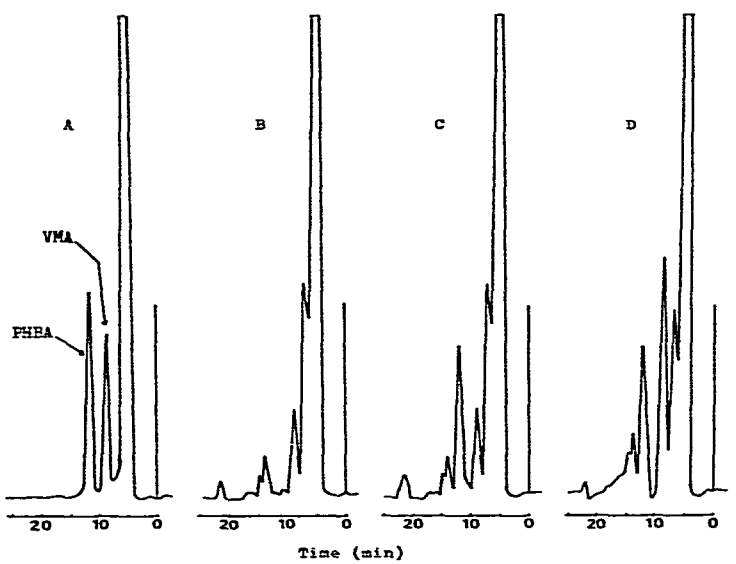

Fig. 1. Chromatograms of labeled samples: (A) VMA (10 μg/ml) and PHBA (200 μg/ml) (internal standard); (B) human urine blank; (C) human urine containing 200 μg/ml of PHBA; (D) human urine containing 200 μg/ml of PHBA and 20 μg/ml of VMA.

RESULTS AND DISCUSSION

MHPLC using micro-columns has been known to be a useful micro technique [11]. The combination of MHPLC and fluorescence detection is suitable for sensitive micro-determination of urinary VMA. Examination of the various procedures for the extraction, derivatization, and separation of VMA in biological samples suggested the optimized procedure described in the Experimental section. The results are shown in Fig. 1.

VMA content of urine can be measured down to 1 μg/ml. Under the present conditions, VMA and PHBA were eluted at 8.5 and 12.1 min, respectively, and were well separated from the other peaks of normal urine constituents. The method was reproducible with a standard deviation of less than ± 4% using 10 μg/ml samples of VMA. This technique seems to be promising for research concerning the influence of drugs on catecholamine metabolism and diagnostic analysis of related problems in both animals and humans. Human urinary VMA values obtained by this method were 1.3—19.5 μg/ml, with an average value of 7.6 ± 2.09 μg/ml (men, n = 10, age 25—37 years), which was in agreement with reports from other laboratories [1]. Application of this method for the investigation of the correlation between the VMA content and phaeochromocytoma will be reported in future papers.

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