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Note

Determination of vanilmandelic acid and homovanillic acid in urine by high-speed liquid chromatography

AKIRA YOSHIDA*, MASANORI YOSHIOKA, TAKENORI TANIMURA and ZENZO TAMURA

Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113 (Japan)

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Homovanillic acid (HVA) and vanilmandelic acid (VMA) are the main metabolic end-products of dopamine and norepinephrine or epinephrine, respectively. Levels of VMA and HVA in urine have often been of importance in the diagnosis of neuroblastoma and phaeochromocytoma, and in the course of treatment of these diseases and Parkinson's disease.

A number of methods for separating and identifying VMA and HVA in urine have been described such as paper partition chromatography[1–3], thin-layer chromatography[4,5] and electrophoresis[6,7], which are relatively time consuming and not quantitative. Gas chromatography[8,9] requires sample clean up from crude materials and conversion into volatile derivatives. Another method applicable for the analysis of body fluids is liquid chromatography. Mrochek *et al.*[10] determined many constituents of urine, including VMA and HVA, by high-resolution anion-exchange chromatography; twenty hours were required for a complete analysis. Änggård *et al.*[11] also separated methyl esters of VMA and HVA using a column of Sephadex LH-20. Lange *et al.*[12] separated a standard mixture of catecholamine metabolites by cation-exchange chromatography. Recently, Thomas *et al.*[13] separated VMA and 3-hydroxy-4-methoxymandelic acid (iso-VMA) by cation-exchange chromatography, but the method was not applied to biological materials.

In this paper we describe a rapid and simple quantitative method for the determination of VMA and HVA in urine by high-speed adsorption liquid chromatography.

EXPERIMENTAL

Materials

VMA and HVA were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). All of the other chemicals used were of reagent grade purity.

Instrument

A high-speed liquid chromatograph was assembled from a micro-pump of the

* Present address: Central Research Laboratories, Mitsubishi Petrochemical Co., Ltd., Ami-machi, Inashiki-gun Ibaragi-ken 300-03, Japan.

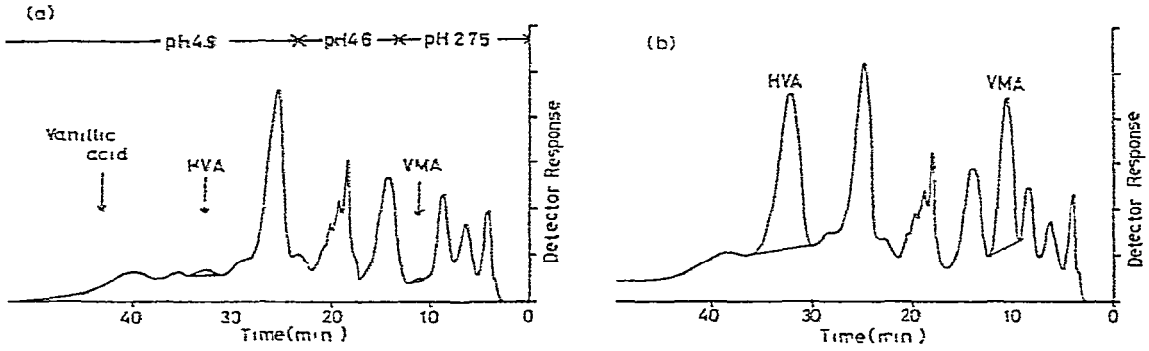

Fig. 1. Liquid chromatograms of 4-ml samples of (a) normal urine and (b) normal urine containing 0.43 mg VMA and 0.41 mg HVA.

double-plunger type (KWU-H, Kyowa Seimitsu, Tokyo, Japan), glass tubing (500 × 3 mm I.D.) packed with Hitachi Gel No. 3010 (spherical porous particles of styrene–divinylbenzene; particle diameter, *ca.* 25 μm), a UV monitor (LDC Model 1280; wavelength, 280 nm) and a recorder (Model EPR-2TC, Toa Electronics, Tokyo, Japan). The column temperature was maintained at 30°, and the flow-rate of the mobile phase was 1.3 ml/min. The meter ranges of the UV monitor and recorder were set at 0.02 absorbance units and 2.5 mV full scale, respectively.

Methods

VMA and HVA were extracted from urine according to the method of Dziedzic *et al.*[9]. 4 ml of urine in a 10-ml glass-stoppered centrifuge tube were acidified with 0.3 ml of 6 *N* hydrochloric acid, 1 g of sodium chloride was added and the resulting mixture was extracted three times with 5 ml of ethyl acetate by shaking for 2 min. The ethyl acetate in the collected upper phase was evaporated under reduced pressure and the residue was stored in a freezer until the chromatographic analysis. The residue was dissolved in 0.2 ml of methanol and 10 μl of the solution was injected on to the column. VMA and HVA were eluted with 0.05 *M* tartrate buffer–methanol (4:1). The pH of the buffer was varied as shown in Fig. 1.

RESULTS AND DISCUSSION

Under the present conditions, VMA and HVA were eluted at 10.5 and 31.1 min, respectively, and were well separated from the other constituents in normal urine. Calibration graphs of peak height against concentration of VMA or HVA in urine were linear as shown in Fig. 2. Additions of known amounts of VMA and HVA to samples of normal urine resulted in increased peak heights. The peak heights of VMA and HVA were directly proportional to the amounts added (Fig. 1). Vanillic acid, a metabolite of dietary vanillin, which resulted in too high a value for HVA in the colorimetric assay of Ruthven and Sandler[14], did not interfere with the present liquid chromatographic method. No attempt was made to measure iso-VMA and 3-hydroxy-4-methoxyphenylacetic acid (iso-HVA), because the excretion of these isomers in urine is said to be within a few percent of those of VMA and HVA[15,16].

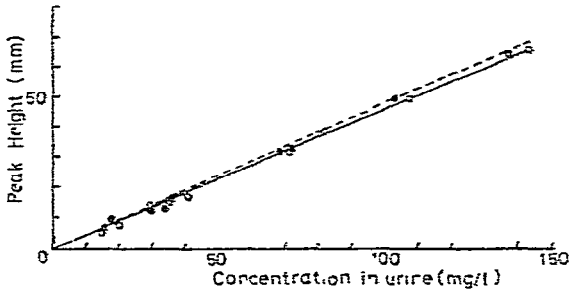

Fig. 2 *Calibration graphs of peak height against concentration for* VMA (○—○) and HVA (●—●) in urine.

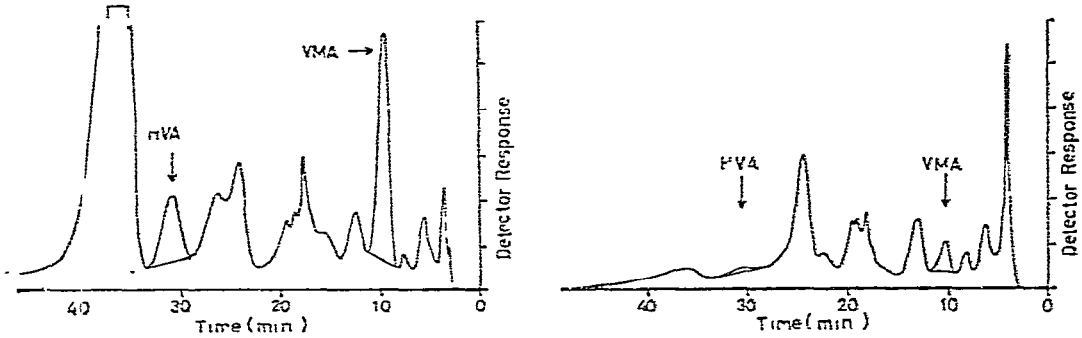

Fig. 3. Liquid chromatogram of urine from a patient with neuroblastoma.

Fig. 4. Liquid chromatogram of urine from a patient with phaeochromocytoma.

Our method was reproducible and the peak heights deviated by less than ± 5% when a solution containing 100 mg/l of the acids was repeatedly injected. The minimum detectable concentration for both acids was *ca.* 4 mg/l.

We applied this chromatographic technique to the urine of a patient with neuroblastoma. The results are shown in Fig. 3. The concentrations of VMA and HVA in urine were 125 mg/l (or 77.5 mg per day) and 43.5 mg/l (or 27 mg per day), respectively. The chromatogram of urine from a patient with phaeochromocytoma is shown in Fig. 4. The concentrations of VMA and HVA were 16 mg/l (or 15 mg per day) and 4 mg/l (or 3.7 mg per day), respectively.

The method seems to be promising for routine analysis of VMA and HVA in urine. All of the operations could be carried out within 2 h. After chromatographic separation, if necessary, the individual peaks may be analyzed by other methods such as gas chromatography or mass spectrometry.

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