References


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Assay of 4-Hydroxy-3-methoxyphenylacetic (Homovanillic) Acid by Liquid Chromatography with Electrochemical Detection

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We describe a method for the assay of urinary 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid), based on its separation on a microparticulate reversed-phase column and direct electrochemical detection. Patients with neuroblastoma, pheochromocytoma, and Parkinson’s disease have increased amounts of this compound in their urine.

Additional Keyphrases: neuroblastoma · pheochromocytoma · Parkinson’s disease · normal values

Homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid, HVA) represents a major end-product of dopamine metabolism. The concentration of this compound in human urine equals that of vanillylmandelic acid (4-hydroxy-3-methoxymandelic acid, VMA). Increased amounts of HVA are detected in the urine of patients with neuroblastoma and pheochromocytoma. HVA is more frequently increased than VMA in neuroblastoma (1).

Methods for HVA assay are rather laborious for the routine clinical laboratory. They are based on gas chromatography and require extraction and derivatization (1, 2). A colorimetric method for HVA determination that requires solvent extraction has been recently described (3). Felice and Kissinger (4) have shown that HVA can be detected electrochemically after preliminary isolation on thin-layer chromatography followed by separation on a microparticulate resin.

In this work, we illustrate that HVA can be assayed by injecting diluted urine directly onto a microparticulate reversed-phase column and monitoring with an electrochemical detector. The method is applied to the assay of urine from patients with neuroblastoma, pheochromocytoma, and Parkinson’s disease. The main advantages of this method are its simplicity and specificity compared with other methods.

Materials and Methods

Instrumentation

A Model 110 A pump (Altex Scientific Inc., Berkeley, CA 94710) was used to deliver the solvent through a 250 x 4.6 mm (i.d.) column of Lichrosorb RP 18 of 10-μm average particle size (E. Merck, Cincinnati, OH 45212) at a flow rate of 1.5 mL/min. The samples were introduced through a 20-μL loop injector, Model 7120 (Rheodyne Inc., Berkeley, CA 94710). The effluent was monitored with a glassy carbon electrode cell, TL 8A (Bioanalytical Systems, West Lafayette, IN 47960). The amplifier was assembled in the laboratory according to the schematic of Keller et al. (5). The oxidation potential was maintained in the cell at 1.2 V. Amplifier output was adjusted to allow samples containing 50 mg/L to be recorded with a full-scale deflection on the recorder. The column was kept at ambient temperature.

Reagents

Homovanillic acid standard (stock): 100 mg diluted to 1 L with distilled water and stored refrigerated, stable at least one month.

Homovanillic acid standard (working): freshly prepared daily by diluting the stock standard 10-fold with water.

Mobile phase: 280 mL of methanol added to 770 mL of phosphate buffer (60 mmol/L, pH 3.0).

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**Results and Discussion**

Recorder response is plotted in Figure 1 as a function of the oxidation potential of HVA with a glassy carbon electrode. We found that two different glassy carbon electrode cells showed different maxima for their oxidation potential; therefore, the voltage at which the response is maximized should be determined for each cell.

**Representative chromatograms of homovanillic acid (HVA)**

Fig. 2. Representative chromatograms of homovanillic acid (HVA) from A, a normal person; B, a patient with neuroblastoma; and C, a 10 mg/L standard.

**HVA retention time, 10 min**

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<th>Table 1. Reproducibility of HVA Assay</th>
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Representative chromatograms of a normal specimen, an above-normal specimen, and a standard are illustrated in Figure 2. The retention time for HVA is 10 min with the routine mobile phase of methanol/phosphate buffer. The method is linear between 1 and 100 mg/L. The average analytical recovery of HVA added (20 mg/L) to five different urine pools was 98%. The reproducibility of the method is summarized in Table 1.

Results from direct urine injection were compared with those from solvent extraction with dichloromethane. Solvent extraction was quite selective and yielded a cleaner chromatogram—most of the extraneous peaks eluting close to HVA were absent. However, the patients' values for HVA were similar by the two methods (Table 2). Compared with results for VMA assay by liquid chromatography (6), in the HVA assay very few peaks elute after the compound of interest, so samples can be injected every 12 min. HVA peaks for samples from about 100 patients with different disorders were symmetrical, indicating homogeneity of the peak and absence of interferences. Furthermore, the identity of the HVA peak and the absence of interferences were confirmed by comparing the retention times of HVA in solvents of different composition. For example, the retention time of HVA is considerably reduced when acetonitrile is used instead of methanol, or when acetate buffer (50 mmol/L, pH 3.0) is used instead of the phosphate buffer. We have checked the following compounds and drugs and have found they do not interfere in the test: VMA, epinephrine, norepinephrine, metamphetamine, tyramine, octopamine, vanillin, 5-hydroxyindolacetic acid, L-3-(3,4-dihydroxyphenyl)-2-methylalanine (a-methyldopa), 3-(3,4-dihydroxyphenyl)-L-alanine (DOPA), and 4-(2-aminoethyl)pyrocatechol (dopamine). Thus, for routine work, we found direct urine injection for HVA assay to be adequate and reliable.

The normal range for urinal HVA, based on values for 65 adults, is 0-7 mg/L, 0-9 mg/24-h urine, or 0-8 mg/g of creatinine. Seven patients with neuroblastoma, three with pheochromocytoma, and seven with Parkinson's disease showed greatly increased concentrations of HVA by the present method.
method, especially the last group, who had been treated with L-dopa.

The effects of direct urine injection on column performance have also been described (6). The method we propose, compared with other methods for HVA assay, is quite simple, specific, free from interferences, and suitable for automation.

References


Blood Thyroxine Concentration Is Lower in Low-Birth-Weight Infants

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From our data bank, we analyzed results for thyroxine concentration in blood samples spotted on filter paper from different age groups (one to nine days postpartum) of our low-birth-weight and normal population. It was significantly lower in the low-birth-weight infants in almost all the age groups, and in both groups it significantly declined after postnatal day 5. The influence of weight is more important in the low-birth-weight population, but does not completely explain the smaller values. The results indicate that blood samples used in screening for congenital hypothyroidism in the neonate should be taken on postnatal days 3 to 5, and if a recall specimen is collected on days 5 to 9, one should expect the thyroxine concentration in the second specimen to be lower than in the first.

Additional Keyphrases: age-and weight-related effects · neonates · normal values · thyroid disease · congenital hypothyroidism · screening

Since 1974, several regional programs for detecting neonatal hypothyroidism have been instituted in North America (1) in which the thyroxine (T4) concentration is measured in the eluate from spots of blood on filter paper. If a low T4 concentration is found, thyrotropin is measured in the same eluate. Subnormal concentrations of both may reflect the congenital absence of thyroxine-binding globulin (2) or secondary or hypothalamic hypothyroidism (3). Much more frequently, however, such cases represent the usual values for T4 and thyrotropin concentrations of low-birth-weight (LBW) infants. Although we have previously published our analysis of the influence of birth weight on the T4 concentration and the correction factor to be applied, we now have analyzed this problem further with respect to time (4).

Methods
We pooled data collected during three years of such screening of T4 in neonates. Our previous study (4) showed that the mean and variance varied significantly between assays; thus the results were standardized so that the specimen population had the same distribution from day to day. From our data and the form accompanying the specimen (5) we took the following three variables: T4, weight, and age of child. An analysis of covariance was made, in which T4 was related to birth weight, and groups were formed from infants having the same age. We separated infants that weighed less than 2.5 kg at birth from those that weighed more and classified the former as infants with LBW.

Results
As shown in Table 1 and Figure 1, between two and nine days after birth, LBW newborns show T4 concentrations that clearly are statistically (p < 0.0001) lower than those for infants with normal birth weights. Changes in these concentrations in the two groups parallel one another, attaining their lowest point at nine days of age (1.4 ng per 40 μL of eluted blood, compared with 1.6 ng for the normal infants). The correlation between birth weight and T4 concentration in the normal-weight infants is greater in the first two days of life; thereafter it stabilizes at around r = 0.1. In the LBW infants the correlation is greatest at about the sixth day of postnatal life and is more significant: the regression coefficient in the LBW group was almost fivefold that of normal weight infants.