Semiautomated Method for Measurement of Dopa in Plasma

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A semiautomated fluorometric method for analysis of dopa [3-(3,4-dihydroxyphenyl)L-alanine] is described, with ferricyanide used as an oxidant. To separate dopa from interfering catecholamines it is adsorbed on alumina, eluted with 0.1N HCl, then adsorbed on and eluted from a cation-exchange column. The method is suitable for use with a large volume of samples. It provides a simple, sensitive, accurate, and reproducible analytical technique for routine use.

Additional Keyphrases fluorometry • dihydroxyphenylalanine • cation-exchange chromatography • AutoAnalyzer • ferricyanide oxidant

L-DOPA [3-(3,4-dihydroxyphenyl) L-alanine] is being used as a therapeutic agent in the treatment of Parkinsonism. For this reason new emphasis has been placed on dopa analysis in body fluids (1). Several methods for the analysis of dopa have been published. Among these are the methods of Sourkes and Murphy (2), Takahashi and Fitzpatrick (3), and Anton and Sayre (4). These methods use iodine, potassium ferricyanide, or sodium periodate to oxidize dopa to a fluorophore in the presence of alkali; the fluorophore is stabilized by the addition of ascorbic acid. These methods present difficulties when many analyses are required because they involve numerous reagent pipettings and precise timing.

We have developed a semiautomated method for dopa that gives the same analytical results as a manual method with which it was compared (2).

Materials and Methods

Determinations were made with an AutoAnalyzer (Technicon Corp., Tarrytown, N.Y.) equipped with Sampler II, Aminco (American Instrument Co., Silver Spring, Md.) fluoromicrophotometer, and Technicon recorder. A GE lamp H8543 was used as the source of ultraviolet light.

Reagents

(a) Phosphate buffer, 0.1 mol/liter, pH 6.5, containing 0.1 g of ethylenediamine tetraacetic acid (disodium salt) per 100 ml. Adjust 0.1 molar sodium phosphate, monobasic (NaH2PO4·7H2O, Mallinckrodt no. 7892) containing 1.0 g of EDTA (Baker no. 8993) per liter to pH 6.5 with 0.1 molar sodium phosphate dibasic (Na2HPO4·7H2O, Mallinckrodt no. 7914), also containing 1.0 g of EDTA per liter.

(b) Potassium ferricyanide, 2.5 g/liter, containing 5 g of Brij-35 per liter. Dissolve 250 mg of K3Fe(CN)6 (Baker no. 3104) and 0.5 ml of Brij-35 (Technicon AR, no. 110-62) and dilute to 100 ml with water. Stable for one day.

(c) Ascorbic acid, 10 g/liter. Dissolve 1.0 g of ascorbic acid (Nutritional Biochemicals Corp.) and dilute to 100 ml with water. Stable for one day.

(d) Sodium hydroxide, 2.5 mol/liter. Dissolve 100 g of NaOH (Baker no. 3722) and dilute to 1 liter with water.

(e) Standards. Prepare a stock standard of dopa by dissolving 100 mg of L-dopa (Hoffmann-La Roche Inc.) in 100 ml of 0.01N HCl. This standard is stable for at least two weeks when refrigerated. Working standards ranging from 0.001 μg/ml are

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prettiert in phosphate buffer (reagent a). Keep for one day only.

Procedure

The catecholamines are adsorbed on alumina according to the method of Anton and Sayre (5), but eluted from alumina with 0.1N HCl.

The dopa in the eluate is separated on columns of AG 50W-X4 (Bio-Rad Labs) in the Na* form and eluted with 20 ml of phosphate buffer (reagent a) (6-8; Spiegel, H. E., and Christian, R. P., in preparation; Weise, V. K., personal communication). Eluates were assayed for dopa by our semiautomated technique.

Instrumental Considerations and Operating Procedure

The manifold and flow diagram are shown in Figure 1. The sampler is fitted with a 40-specimen per-hour (2:1) sampling cam. The sample is intro-

duced into the air-segmented buffer solution and mixed with potassium ferricyanide solution. After the reaction is allowed to proceed in a double mixing coil, alkaline ascorbate is added to form and stabilize the fluorophore. The fluorophore is measured in the fluoromicrophotometer, with Corning filter 7-60 (360 nm) in the primary position and a Wratten 8 (485 nm) filter in the secondary position. The aperture was set at 1. A standard flow cell was used.

Use of air-segmented sodium hydroxide before mixing with ascorbic acid was described by Vik-torra et al. (7). The coil with an A2 fitting, used for mixing sodium hydroxide, obviated the need to frequently change the mixing coil or rinse it with water.

Results

Precision

The precision of the automated method was estimated from 10 measurements of a 0.05 μg/ml standard, which averaged 0.0511 μg/ml (SD, ± 0.0031), with a cv of 6%.

Sensitivity

The sensitivity of this method was as low as 1 ng/ml with the detection equipment used. There remains the additional means of increasing the sensitivity through the use of various scale and aperture combinations on the fluorometer.

Automated and Manual Method Comparison

A correlation coefficient was calculated for results of the automated method compared with those of the manual method by using the results obtained in 173 analyses of eluates from the cation-exchange columns, the samples being extracts from plasma (Table 1). It was 0.9916, which indicates that the automated method described gives results that are virtually identical with those obtained by the manual method of Sourkes and Murphy (2) in extracts derived from biological sources.

Discussion

Semiautomated procedures for norepinephrine and epinephrine analysis have been reported (7-9) in which catecholamines are isolated by chromatographic procedures and the acid eluates are analyzed with an AutoAnalyzer equipped with a continuous-flow fluorometer. Potassium ferricyanide is the oxidant in these procedures, and produces a fluorophor that is stabilized in alkali-ascorbate. In the course of developing the analytical tech-
niques reported for norepinephrine and epinephrine, dopa was also studied for its ability to form a fluorophore by use of ferricyanide (8, 10). It was shown that dopa can form a fluorophor having a low fluorescence compared with norepinephrine and epinephrine. Dopamine also fluoresces weakly.

Since norepinephrine, epinephrine, and dopamine can potentially interfere with the fluorescence of dopa, an additional ion-exchange separation step was added to separate dopa from these other compounds. We used a modification of the procedure of Sedvall et al. (8) for this purpose, and effectively separated dopa from all possible interfering catecholamines. We considered this a particularly desirable feature in the case of urine from patients on L-dopa, in which both dopa and dopamine are increased (Spiegel, H. E., and Christian, R. P., in preparation). Therefore, the only way to get an accurate analysis for dopa was to completely separate it by ion-exchange.

In the manual technique that we used (8), iodine is the oxidizing agent. Our early attempts to analyze increasing numbers of samples by directly automating this technique were not successful. Drifting baselines, nonreproducible peaks, and symmetrical peaks were among the problems encountered; these problems were not observed when ferricyanide was used.

Speed, sensitivity, reproducibility, and accuracy of the semiautomated method reported here for measuring dopa are important advantages in favor of considering this method of analysis.

References