Journal of Chromatography, 311 (1984) 167—169
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2242

Note

Ascorbic acid oxidase speeds up analysis for catecholamines, indoleamines and their metabolites in brain tissue using high-performance liquid chromatography with electrochemical detection

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(First received March 19th, 1984; revised manuscript received June 21st, 1984)

We report a simple procedure to speed up high-performance liquid chromatographic—electrochemical detection (HPLC—ED) of catecholamines, indoleamines and their metabolites in brain samples using commonly available HPLC equipment and minimal sample preparation. We analyze for norepinephrine (NE), epinephrine (E), dopamine (DA), 5-hydroxytryptamine (5-HT) and the metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindole-3-acetic acid (5-HIAA), and homovanillic acid (HVA) in 30 min per sample by direct injection of supernatant from sonicated brain tissue [1]. The first component eluted, NE, is frequently overlapped by a large solvent front. Alternative mobile phases using increased ion-pairing agent or decreased organic solvent in order to resolve NE result in untimely appearance of 5-HT. There is a trade-off necessary between using a method that allows short enough sample time for routine processing of large numbers of samples and resolution of the NE peak. Processing samples in 0.1 *M* perchloric acid, which is expected to lower protein concentration of the supernatant, did not decrease the solvent front. The primary cause of this large solvent front in brain samples is ascorbic acid. A shorter sample time can be obtained by a simple enzyme addition (ascorbic acid oxidase, AAO) which reduces the solvent front by 80%. The small quantity of AAO is added during sample preparation, and NE, the first peak of interest, is readily detected. The alternative way to increase the rate at which samples are processed is to change to 3-μm particle columns with the added equipment expense and more stringent sample handling procedures [2].

0378-4347/84/$03.00

MATERIALS AND METHODS

Chromatographic analysis was carried out using an Altex Ultrasphere ODS reversed-phase separation column (5 μm particle size, 250 × 4.6 mm) attached to a glassy carbon electrode (Bioanalytical Systems, TL5) and a BAS-LC4A amperometric controller. A Milton-Roy single piston pump (No. 396-57) and a Universal Scientific repackable guard column (C_{18}, 10 μm particle size; 46 × 2.0 mm) were used.

A methanol—water (10:90) eluent, pH 4.0, containing 8.2 g sodium acetate (0.1 *M*), 14.0 g citric acid (0.07 *M*), 100 mg octylsulfonic acid (sodium salt) ($5 \cdot 10^{-4}$ *M*), and 25 mg EDTA flowed at a rate of 1 ml/min. E_{app} was 0.61 V. Temperature was controlled via a water-jacketed column at 32° C. (A shorter sample time of 18 min on a similar column was possible using 15% methanol and running at room temperature.)

Ascorbic acid oxidase solution was made from 1.0 mg of ascorbic acid oxidase (E.C. 1.10.3.3) from Boehringer-Mannheim into 1.0 ml of acetate buffer, pH 5. Refrigerated enzyme solution stays active for at least seven days. The pH 5 buffer used for sample homogenization is composed of 0.1 *M* Ac^-, $3.5 \cdot 10^{-2}$ *M* in glacial acetic acid; the pH was adjusted to 5.0 with sodium hydroxide.

Samples of human brain tissue (weight ≈ 30 mg) are sonicated on ice in 450 μl of pH 5 buffer, 50 μl of $1 \cdot 10^{-6}$ *M* dihydroxybenzylamine (DHBA), internal standard solution. Of the ascorbic acid oxidase solution 10 μl are added to each sample, which is then centrifuged at 0° C at 40,000 *g* for 20 min. The action of the AAO is immediate. The supernatant is injected directly into the chromatographic system.

RESULTS

Fig. 1 shows the comparison of human brain sample chromatograms

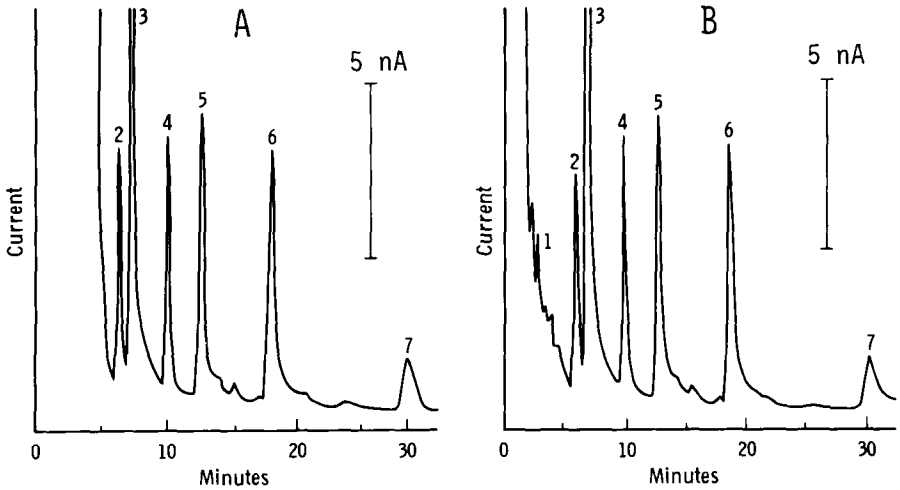

Fig. 1. HPLC—ED chromatograms of human brain tissue (septal region) without (A) and with (B) the addition of ascorbic acid oxidase to brain tissue supernatant. Peaks: 1 = norepinephrine; 2 = DHBA; 3 = DOPAC; 4 = dopamine; 5 = 5-HIAA; 6 = HVA; 7 = 5-HT.

without and with the addition of the ascorbic acid oxidase. With this method we are able to detect many biogenic amines oxidizable at 0.61 V in 30—35 min sample time with minimal sample preparation. AAO-treated human brain supernatant spiked with known quantities of NE, DA and 5-HT gave linear calibration curves. Catechol and indole stability was not dependent on the presence of ascorbic acid. We have found after injection of 100 samples (700 mg of tissue supernatant) the precolumn packing must be changed, but the separation column has resolved more than 1800 samples and retained its separation ability after a year of use.

ACKNOWLEDGEMENT

The support of the National Institutes of Health via Grant 5RO1 NS08740 is gratefully acknowledged.

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