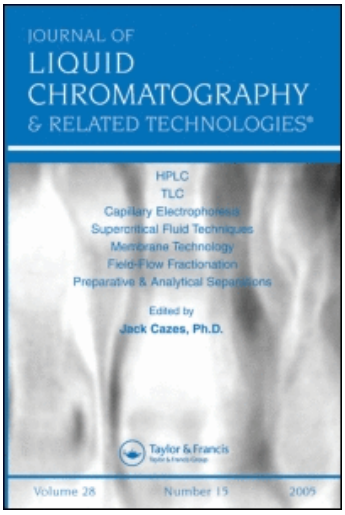

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Determination of the Stability of Dopamine in Aqueous Solutions by High Performance Liquid Chromatography

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DETERMINATION OF THE STABILITY OF DOPAMINE IN AQUEOUS SOLUTIONS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Methods for the ananlysis of dopamine and its degradation products in aqueous solutions is described. The technique of reverse phase chromatography with electrochemical detection is used to investigate the stability of dopamine in various aqueous solutions. In neutral and basic solutions, dopamine is rapidly oxidized by dissolved oxygen to form degradation products. The results demonstrate that dopamine is stable in 0.1 N HCl solution, pH < 1. The study indicates that EDTA can slow down the oxidation process. The detection limit for the analysis of dopamine is 0.1 μM with 100 μl injection.

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INTRODUCTION

Dopamine is a well-established neurotransmitter in the mammalian central nervous system. [1], [2], [3], [4] Dopamine may be the inhibitory neurotransmitter released from interneurone in autonomic nervous system ganglia. [1] The major metabolites of dopamine include dihydroxyphenylacetic acid, homovanillic acid (HVA) and 3-methoxytyramine. Within the terminal, a free dopamine is first deaminated and oxidized, and then it is methylated to form HVA after leaving the terminal.

In neutral and basic conditions, as it was found in hydroquinone previously, [5], [6] dopamine can be rapidly oxidized by oxygen dissolved in aqueous solutions. The main products are hydrogen peroxide and dopamine orthoquione, with superoxide serving as a chain-propagating radical in the autoxidation of dopamine. [7] Because the number of samples in biological experiments is usually very large, and the analysis can take a whole day and sometimes the extracted samples may be stored for days before analysis, it is vital to preserve the analyte during the sample extraction and analysis. It is considerably interest to determine the stability of dopamine in various aqueous solutions. The investigation was carried out to determine the optimal conditions for (1) the protection of dopamine against degradation and (2) the separation of dopamine and its degradation products. The method developed for the analysis of dopamine and degradation products is reverse phase chromatography with electrochemical detection. The detection limit for

dopamine is 0.1 μM with 100 μl injection. This study indicates that the initial oxidation products of dopamine can undergo further oxidation or reduction.

EXPERIMENTAL

The instrumentation used included a Waters 6000-F pump, a Rheodyne 7125 injection valve with a 20 μl loop, an LC-4B/17(D) amperometric detector for liquid chromatography, and a Fisher recordall Series 5000 chart recorder. Separations were accomplished using a stainless steel column (3 mm x 100 mm) prepacked with 5 μm Biophase octadecyl silica and a guard column (4.5 mm x 20 mm) packed with Waters 40 μm C18/Corasil. The detector consisted of a thin-layer glassy-carbon electrode, a Ag/AgCl reference electrode mounted downstream from the glassy-carbon electrode, and a LC-4B amperometric controller for electrochemical detection. The flow rate was 0.8 ml/minute and the injection volume was 100 μl. The detection potential was 800 mV and the current was 2 nA for the detection of dopamine at the concentration below 0.4 μM and 5 nA at the concentration above 0.4 μM.

The mobile phase was 16.67 mM sodium phosphate, 0.41 mM 1-heptanesulfonic acid, 0.08 mM ethylenediaminetetraacetate disodium salt dihydrate (EDTA) and 3% methanol. The pH value of this mobile phase was 6.5. The mobile phase was filtered with 0.45 μm filter and then sparged by helium at 10 psi for 20 minutes before use. The solution pH was determined with a Corning 610A pH meter calibrated with VWR buffers. A 5 μM dopamine

stock solution was prepared in 0.1 N HCl. Three dopamine solutions were prepared by diluting the stock solution with 0.1 N HCl, mobile phase, or mobile phase without EDTA.

Sodium phosphate (monobasic), 1-heptanesulfonic acid (sodium salt) and EDTA were purchased from Aldrich, methanol from Baxter Healthcare Corporation, 3-hydroxytyramine hydrochloride (dopamine hydrochloride) from Sigma Chemical Company. All solutions were prepared with triply distilled water purified by a Millipore Milli-Q reagent system.

RESULTS AND DISCUSSIONS

Dopamine solutions preserved with 0.1 N hydrochloric acid solution and mobile phase with EDTA were analyzed at various times. In comparison, dopamine solution at neutral pH without preservatives was also analyzed at various times. Figures 1A and 1B show the chromatograms of 1 μM dopamine in 0.1 N HCl solution (pH < 1.0). A freshly prepared sample was injected into the HPLC (Figure 1A). The sample was stored in a refrigerator (4 °C) for 10 days and injected into the HPLC (Figure 1B). The only peak found in the chromatograms (Figures 1A and 1B) was dopamine, which indicated that dopamine was stable and no oxidation occurred in the strong acidic solution. Figures 2 show the chromatograms of 1 μM dopamine, which was made with mobile phase containing 0.2 M EDTA at pH 6.5. The sample was injected into HPLC as fresh (2A), after 5 days (stored at 4 °C, 2B), 8 days (4 °C, 2C) and 12

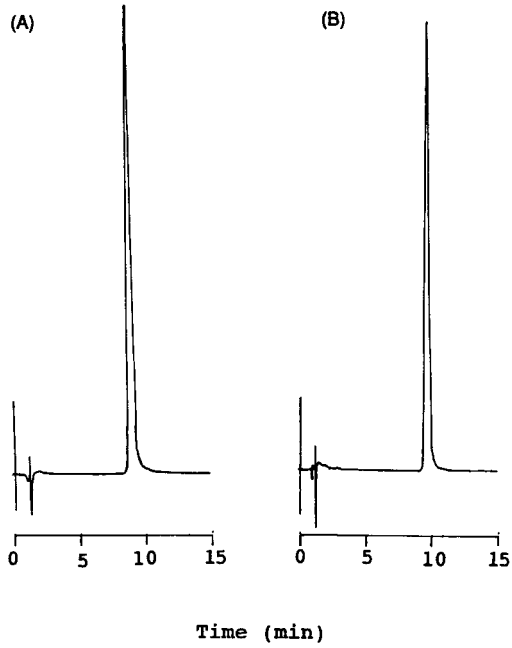


Figure 1 Chromatograms of 0.01 μM dopamine in 0.1 N HCl. Injected after a few minutes (A); after 10 days (B).

days (4 °C, 2D). The chromatograms show that the signal of dopamine (peak a) decreases and the signal of the degradation product (peak b) increases as the time increases. The indication is that dopamine is oxidized in a neutral solution. The oxidation is even more significant in 1 μM dopamine solution made with mobile phase without EDTA at pH 6.5, stored at 4 °C (Figures 3). As shown in Figure 3C, the dopamine peak was completely disappeared after 8 days while in Figure 2D dopamine was still found after 12 days, which suggested that EDTA somewhat protected dopamine from oxidation. It was suggested by Poirier *et al* [7] that a trace amount of transitional metal must

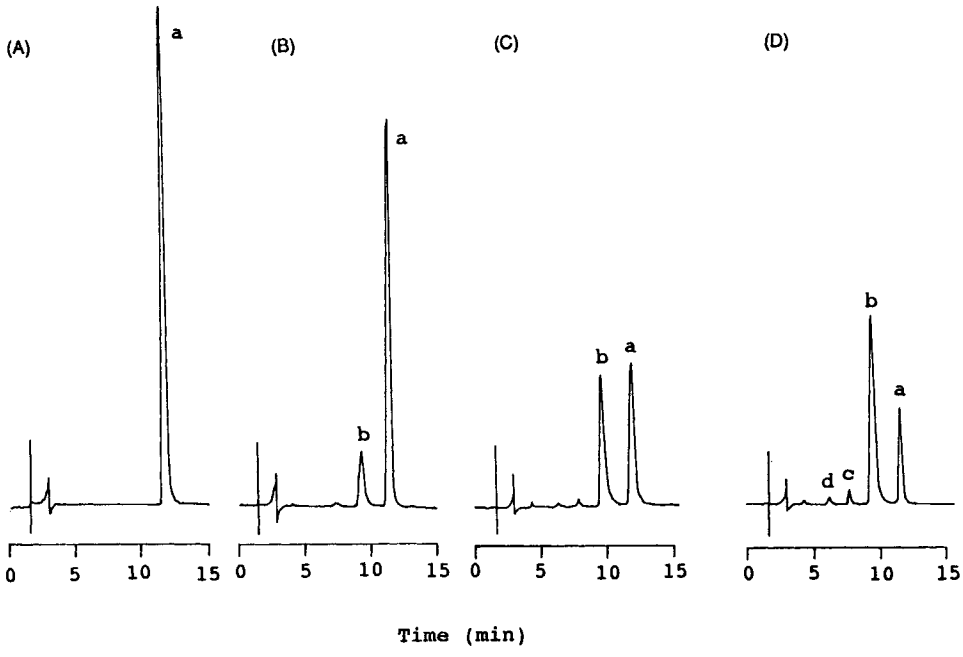


Figure 2 Chromatograms of 0.01 μM dopamine in mobile phase (with 0.2 M EDTA) at pH 6.5, injected after a few minutes (A); after 5 days (B); after 8 days (C); after 12 days (D).

have been present in a quantity sufficient to potentiate the autoxidation of dopamine through the generation of superoxide, hydroxyl radicals, hydrogen peroxide and reactive semiquinones. It is well known that EDTA can scavenge metal by forming an EDTA-metal complex. The catalysis of the degradation of dopamine by cadmium is presently investigated in our laboratory and the result will be published in the next paper. The experimental results suggest that in neutral solution dopamine is rapidly oxidized by dissolved oxygen as in the case of hydroquinone, catechol and resorcinol. [5], [6]

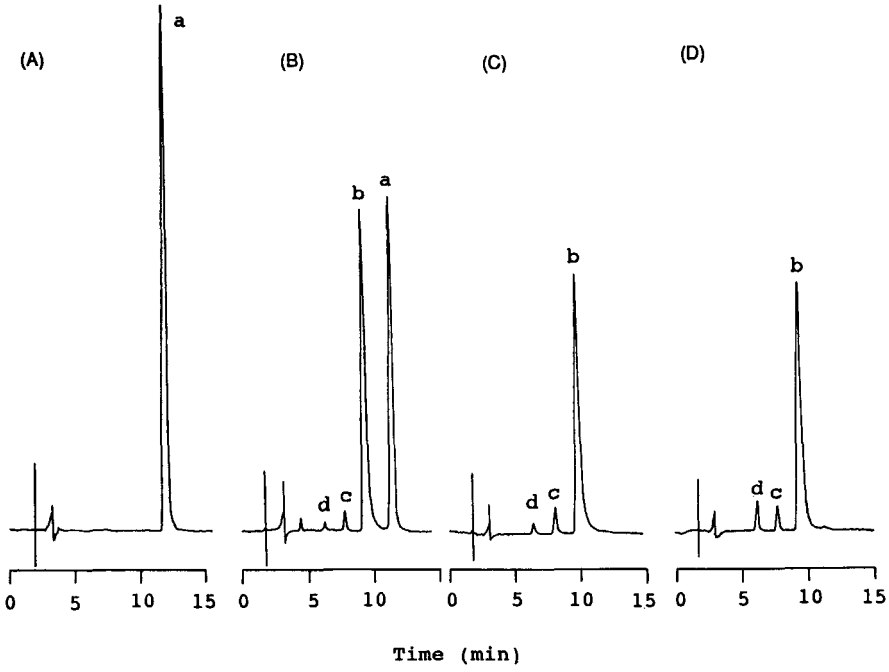
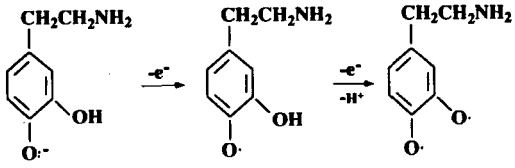
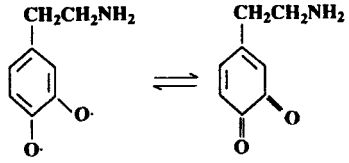


Figure 3 Chromatograms of 0.01 μM dopamine in mobile phase without EDTA at pH 6.5, injected after a few minutes (A); after 5 days (B); after 8 days (C); after 12 days (D).



(1)



(2)

Several other products are also observed (peaks c and d in Figure 2D, peaks c and d in Figures 3B, 3C and 3D), which can be attributed to the further oxidation or reduction of the initial oxidation product. The possible candidates of these oxidation products could be 5, 6-dihydroxyindoline, dopaminochrome and 5, 6-dihydroxyindole, as suggested previously. [8], [9] Since the standards of these degradation products are not available commercially, to the authors' knowledge, it is not possible to identify them.

An electrochemical detector has very high sensitivity for the detection of dopamine and some of its degradation products. Using an electric potential of 800 mV and a current of 2 nA, the detection limit was determined to be 0.1 μM (3 x baseline noise). Although dopamine has stronger signal at higher electric potential, because many molecules are oxidized at potential over 1,000 mV, measurements performed at such high potential will result noisy background and poor signal separation.

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