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Tech Notes

Keywords:
Dopamine Analysis, Seratonin Analysis, Microbore, Microdialysate Analysis, Electrochemical Detection

Microbore Options for Dopamine Analysis with Serotonin

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Purpose

Determination of dopamine in small volume samples such as those obtained from microdialysis.

In vivo sampling methods, such as microdialysis and ultrafiltration, generate low-volume, low-concentration samples, typically in the 1-10 µL range. Separation of these samples is best handled by microbore columns, which result in the least on-column dilution.

Dopamine [F1] is frequently determined by those studying brain function and dysfunction. In a typical separation of catecholamines and their metabolites, however, dopamine elutes late and may be obscured by the more-abundant monoamine metabolites DOPAC and 5-HIAA. Here we develop separations on BASi UniJet SepStik microbore columns that bring dopamine out early and free of interferences.

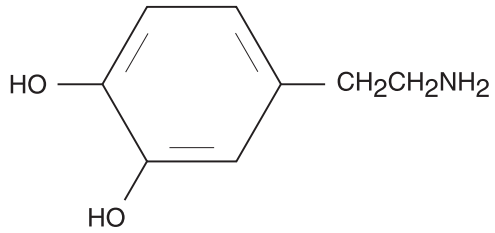

Figure 1. *Dopamine*

Existing Methods

Gas chromatography-mass spectrometry, radioimmunoassay, and liquid chromatography with fluorescence or electrochemical detection.

Conditions

System: BASi Electrochemical Detector Package with a HPLC pump configured for microbore chromatography.
Electrochemical Detector Electrode: Cross-flow 3 mm glassy carbon (PN MF-1000) or Radial-flow electrode (PN MF-1095).
Potential: +750 mV vs. Ag/AgCl
Temperature: 35 °C
Flow Rate: 70 µL/min
Column 1: 5 µm, C_{18}, 150 x 1 mm UniJet SepStik (PN MF-8912)
Column 2: 3 µm, C_{18}, 100 x 1 mm UniJet SepStik (PN MF-8949)
Mobile Phase 1: 100 parts buffer (27 µM disodium-EDTA, 100 mM monochloroacetic acid, 2 mM 1-decanesulfonic acid, pH to 3.2 with NaOH); 20 parts acetonitrile
Mobile Phase 2: 100 parts buffer (same as above); 14 parts acetonitrile.
Detection limits: 150 fg (1 x 10^{-15} moles) with electrochemical detection radial-flow electrode, 230 fg (1.5 x 10^{-15} moles) with cross-flow (S/N = 3).

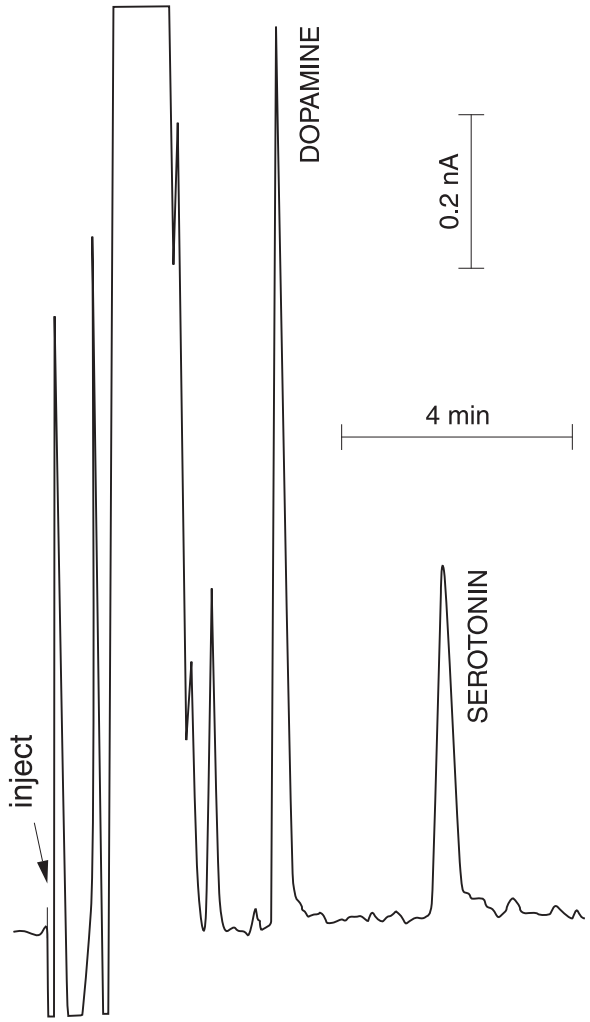

Figure 2. *Separation of Dopamine (20 pg)and Serotonin (10 pg injected) on column 1 with mobile phase 1. Cross-flow cell.*

Notes

Either column could be used for separation, with the appropriate mobile phase (F2-F4). The column that was not being used was placed in the flow stream before the injector, to increase system pressure and maintain its equilibration with the mobile phase.

At microbore flow rates, the radial-flow cell provides a larger signal with less noise than the cross-flow cell. In these separations detection limits were 1/3 lower for the UniJet electrode.

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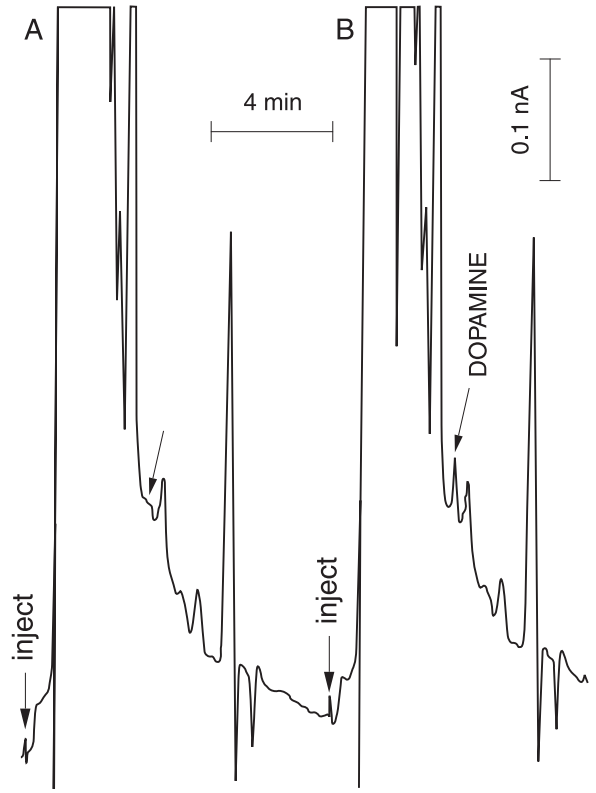

Figure 3. *Rat brain dialysate separated on column 1 with mobile phase 1 and radial-flow cell. A = 5 µL dialysate. B = 5 µL dialysate spiked with 1 pg dopamine. Arrow indicates elution time of dopamine.*

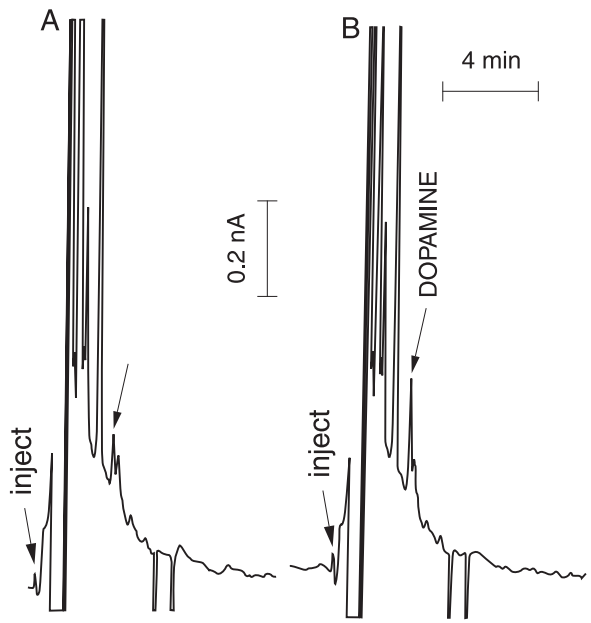

Figure 4. *Rat brain dialysate separated on column 2 with mobile phase 2 and radial-flow cell. A = 5 µL dialysate. B = 5 µL dialysate spiked with 1 pg dopamine. Arrow indicates elution time of dopamine.*

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