

CAPSULES

preliminary notes and applications from Bioanalytical Systems, Inc.

Determination Of Catecholamines In Rat Brain Microdialysates Without Interference From Excessive Metabolite Concentrations

Purpose

Measurement of trace concentrations of norepinephrine, epinephrine and dopamine in microdialysates, to the exclusion of oxidative metabolites present at much higher concentrations.

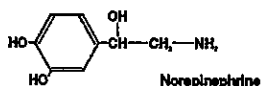
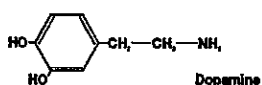
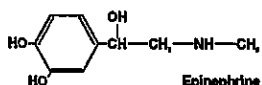


Figure 1. Structure of norepinephrine, epinephrine and dopamine.

With its 1 mm internal diameter, a SepStik column increases the concentration of the eluting catecholamines up to 21-fold compared to standard LC columns. Present in large excess, metabolites may be eluted near the void volume peaks, well separated from the catecholamines.

Existing Methods

LCEC with conventional or microbore columns. Large concentrations of metabolites often interfere with the resolution of very small catecholamine peaks.

Conditions

System: BAS 481 Liquid Chromatograph with BAS LC-26 degasser.

Column: SepStik kit (BAS P/N MF-8901) The packing was C₈, 5 µm silica in a 100 x 1.0 µm bed.

Mobile Phase: The buffer contained 60 mM sodium acetate, 0.5 mM EDTA and 0.5 g/L 1-decanesulfonic acid, sodium salt. Adjust the buffer to pH 6.0 and mix it with acetonitrile in the ratio of 100:15.

Flow Rate: 95 µL/min actual flow rate. For conventional pumps, use BAS P/N MF-8947

Flow Splitter Accessory kit and maintain about 2 mL/min at the pump. Adjust splitter ratio to give 90-100 µL/min through the SepStik column by adjusting the restrictor length and/or pump flow rate.

Detector: BAS LC-4C, Amperometric Detector.
Electrode: Glassy carbon (BAS P/N MF-1000)
+ 650 mV vs Ag/AgCl.

Range: 1.0 nAfs

Filter: 0.1 Hz

Detection Limit: 0.5 pg injected yields a S/N of 3.

The injection volume was 5 µL.

Sample Preparation

Dialysate was directly injected into the system.

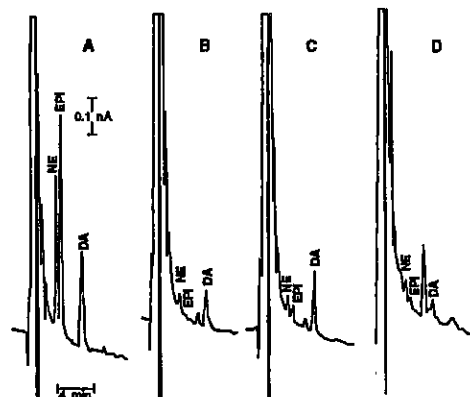


Figure 2. Chromatograms of catecholamine standards and various rat brain microdialysate samples.

A: Catecholamine and metabolite standards. 5 µL injection of a 10nM solution of norepinephrine, epinephrine and dopamine and 100 nM of 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindole-3-acetic acid (5-HIAA) and homovanillic acid (HVA). B, C & D: Various rat brain microdialysates.

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