Keywords:
Microbore, Catecholamine Analysis,
Microdialysate Analysis,
Electrochemical Detection

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

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Purpose

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

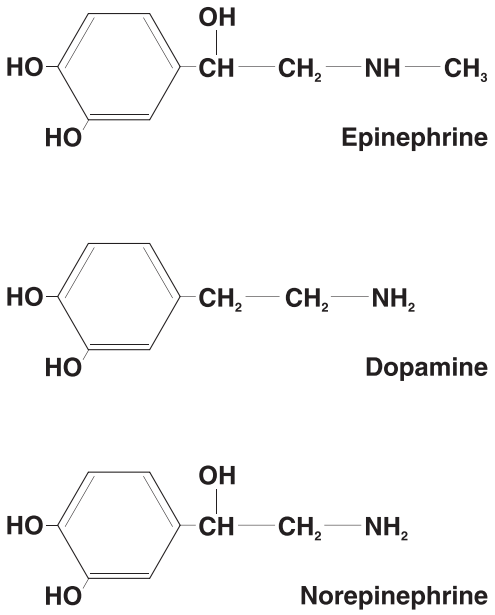

Figure 1. *Structure of norepinephrine, epinephrine and dopamine.*

With its 1 mm internal diameter, a UniJet SepStik microbore 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: Microbore capable Liquid Chromatograph with a degasser.
Column: UniJet SepStik kit (BAS P/N MF-8901) The packing was C_8, 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.
Detector: BASi LC-4C Electrochemical Detector.
Electrochemical 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.

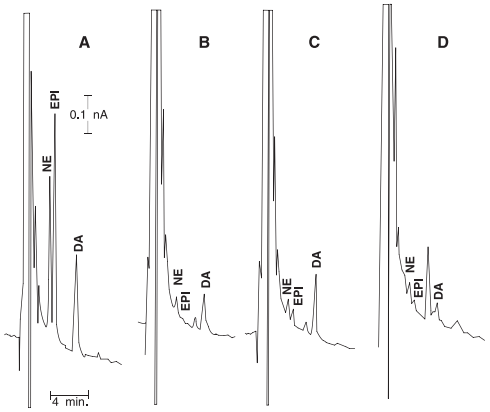

Figure 2. *Chromatograms of catecholamine standards and various rat brain microdialysate samples.*

A: Catecholamine and metabolite standards. 5 µL injection of a 10 nM 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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