

Determination of Dopamine in Microdialysates Without Interference From Excessive DOPAC and 5-HIAA Concentrations

1008

Purpose

Measurement of trace concentration of dopamine (DA) in microdialysates, to the exclusion of oxidative metabolites such as DOPAC (3,4-dihydroxyphenylacetic acid) and 5-HIAA (5-hydroxyindole-3-acetic acid) at 100-fold higher concentrations (F1). With its 1-mm internal diameter, the UniJet SepStik column increases the concentration of the eluting dopamine up to 21-fold compared to standard LC columns. DOPAC and 5-HIAA, which often interfere with dopamine determination, are well separated from the dopamine peak with this mobile phase.

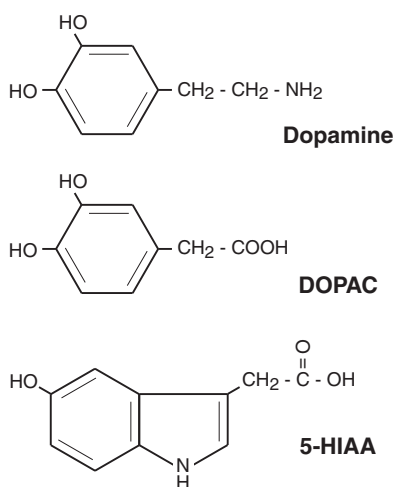


Figure 1. Structure of dopamine, DOPAC, and 5-HIAA

Existing Methods

HPLC Electrochemical Detection with conventional or microbore columns. Large concentrations of DOPAC and 5-HIAA often interfere with the resolution of trace amounts of dopamine in rat brain microdialysates.

Conditions

System: Liquid Chromatograph with degasser.

Column: UniJet SepStik column kit (BASi P/N MF-8949). The stationary phase was 3 μ m ODS, packed in a 100 x 1 mm bed.

Mobile phase: The buffer contained 0.1 M monochloroacetic acid, 0.5 mM EDTA, 0.15 g/L sodium octyl sulfate, and 10 mM sodium chloride. Adjust the buffer pH to 3.1 and mix the buffer with solvents in the ratio of: buffer: acetonitrile: tetrahydrofuran = 94.3:5:0.7

Flow Rate: 90 μ L/min.

Injector and connections: Using an autosampler, 5 μ L was injected. A 20 cm long 0.005 inch i.d. stainless steel tubing was connected between the autosampler and the Rheodyne customized BASi microbore injector valve, so that both auto and manual injections could be used. The UniJet SepStik column was

directly connected between the manual injector valve and the detector cell. When using the autosampler, the manual injector was in the LOAD position.

Detector: BASi LC-4C Electrochemical Detector.

Electrode: 3 mm Glassy carbon (BASi P/N MF-1095)

Potential: +60 mV vs. Ag/AgCl

Detector Gain (range): 1.0 nA full scale

Filter: 0.1 Hz

Detection Limit: 0.5 pg injected (S/N = 3)

Sample Preparation

Dialysates can be directly injected.

Notes

1. Separation of DA in the presence of DOPAC and 5-HIAA is shown in F2.
2. Mobile phase reservoir should be well capped. The evaporation of organic solvents in the mobile phase will prolong the retention. THF is extremely volatile.
3. This column and the mobile phase also can be used for simultaneous determination of DOPAC, DA, 5-HIAA, HVA and serotonin. Serotonin will elute within 15 minutes. Norepinephrine and epinephrine may elute with the void peaks, depending on the sample nature.

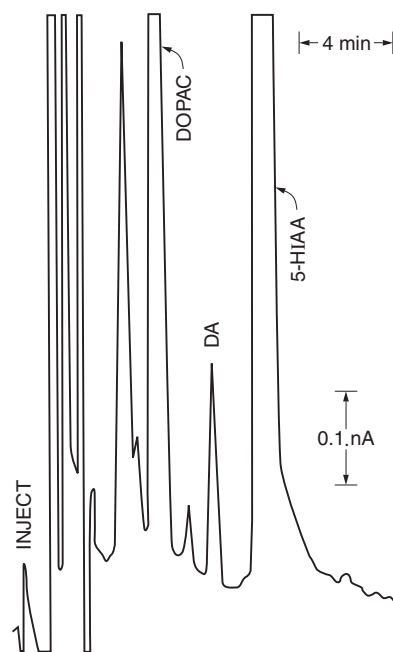


Figure 2. Resolution of dopamine, DOPAC and 5-HIAA. 5 μ L of Ringer's solution containing DA (1 pg/ μ L), DOPAC (100 pg/ μ L) and 5-HIAA (100 pg/ μ L) was injected.