CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

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

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.

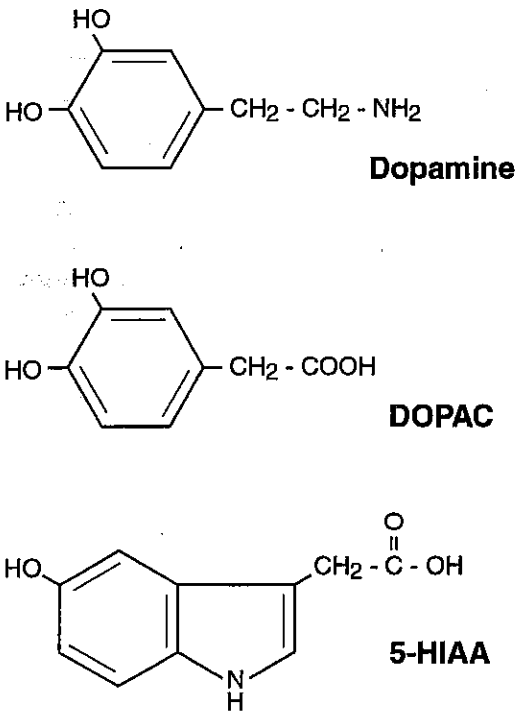

Figure 1. Structure of dopamine, DOPAC, and 5-HIAA

Existing methods

LCEC 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: BAS PM-80 Liquid Chromatograph with BAS LC-26 degasser.

Column: UniJet SepStik column kit (BAS P/N MF-8949). The stationary phase was 3 µm 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 µL/min, using the microbore capabilities of the BAS PM-80 pump. For conventional pumps, use the BAS flow splitter kit (P/N MF-8947).

Injector and connections: BAS Sample Sentinel Autosampler. 5µL was injected. A 20 cm long 0.005 inch i.d. stainless steel tubing was connected between the autosampler and the Rheodyne 9125 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: BAS LC-4C Amperometric Detector.

Electrode: UniJet 3 mm Glassy carbon (BAS P/N MF-1003)

Potential: +550 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.

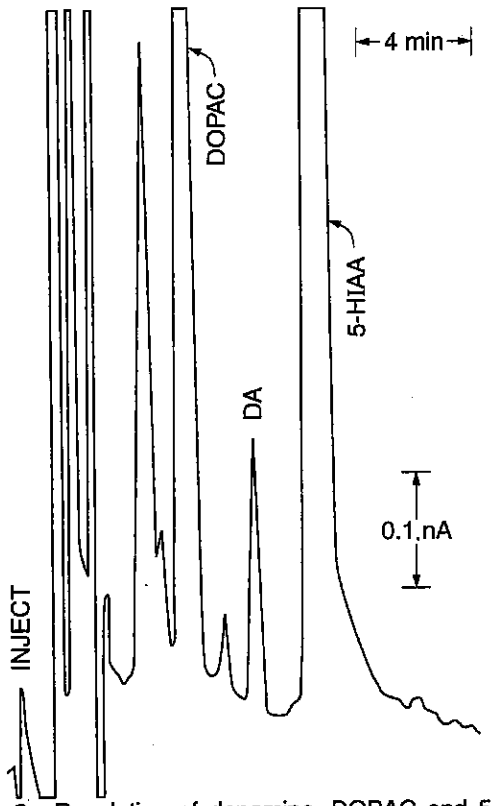

Figure 2. Resolution of dopamine, DOPAC and 5-HIAA. 5 µL of Ringer's solution containing DA (1 pg/µL), DOPAC (100 pg/µL) and 5-HIAA (100 pg/µL) was injected.