

# CAPSULES

preliminary notes and applications from Bioanalytical Systems, Inc.

## Separation Of Catecholamine Enantiomers By HPLC

### Purpose

Separation of enantiomers of norepinephrine and DOPA, and of dopamine, after derivatization with o-phthalaldehyde-N-acetyl-L-cysteine (OPTA-AcCys). The difficult-to-separate enantiomers of important neurotransmitters were reacted with the chiral derivatization reagent OPTA-AcCys (F1), allowing rapid and efficient separation.

### Reference

Separation of Norepinephrine Enantiomers, DOPA Enantiomers and Dopamine Derivatized with o-Phthalaldehyde-N-acetyl-L-cysteine by High-Performance Liquid Chromatography, N. Nimura, K. Iwaki and T. Kinoshita, J. Chromatogr., 402(1987) 387-391.

### Conditions

Detector: Fluorescence

Wavelengths: Excitation: 340 nm (slitwidth 20 nm)

Emission: 450 nm (slitwidth 10 nm)

Column: Develosil ODS-5 (100 x 6.0 mm)

Mobile Phase: Phosphate buffer (pH 6.5): Methanol, stepwise gradient from 35-50-60-35% methanol over 15 min. Flow rate was 1 mL/min.

Detection Limit: 65-100 pg (S/N = 2)

Linear Range: 0.2 - 10.0 ng

### Sample Preparation

Commercial standards were incubated for 5 min with OPTA-Ac-Cys in borate buffer (pH 9.5), then injected in 5  $\mu$ L aliquots.

### Notes

Each enantiomeric pair was readily separated (F2).

Dopamine is non-chimeral, but its derivative could be separated from the four other species present.

The separation of catecholamine enantiomers presented in this report can be duplicated with the BAS 200 Problem Solver. Catecholamines and their

isoindole derivatives are highly electroactive and can be readily detected with BAS amperometric detectors.

The information in this publication is supplied as a service to our customers. Performance of the methodology has not necessarily been verified by BAS technical staff.

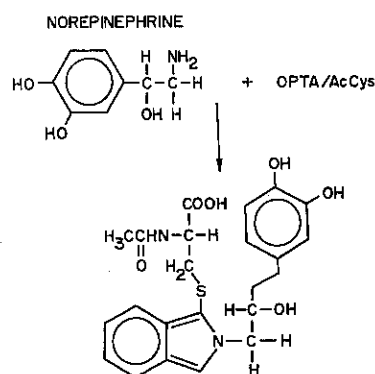


Figure 1. Derivatization of Norepinephrine.

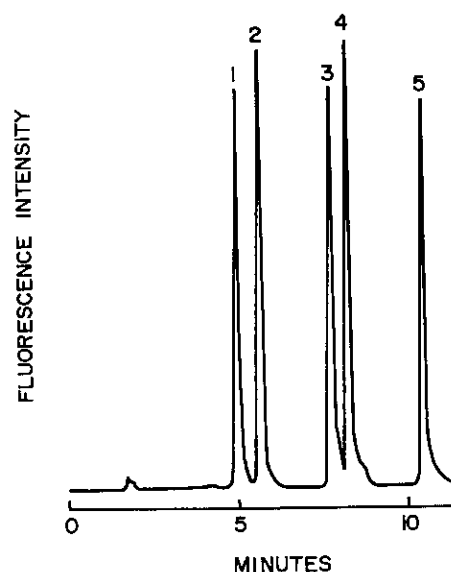


Figure 2. Separation of catecholamine enantiomers. 1 - L-DOPA, 2 = D-DOPA, 3 = L-norepinephrine, 4 = D-norepinephrine, 5 = dopamine.

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