CAPSULES
notes and applications from Bioanalytical Systems, Inc.
1990

Guaranteed Plasma Catecholamine Analysis

Purpose

Development of a mobile phase for catecholamines that excludes certain interfering compounds.

Ion pairing agents such as sodium octyl sulfate are commonly added to mobile phases to increase the retention of catecholamines on reverse-phase columns. However, under certain conditions, a number of compounds have been found to coelute with some of the catecholamines. Dihydrocaffeic acid, for example, may coelute with epinephrine or dopamine, especially in samples from coffee drinkers [1].

BAS has produced a proprietary mobile phase for the determination of catecholamines, which excludes uncharged and anionic interfering compounds. Dihydrocaffeic acid, caffeic acid, ascorbic acid and uric acid are all eluted before the first catecholamine of interest, epinephrine. The mobile phase is designed to be used exclusively with the Catecholamine Column, MF-6213CL. The column must, therefore, be used with only this mobile phase.

Existing Methods

Cation exchange chromatography, or reverse-phase chromatography with mobile phases containing ion pairing agents.

Conditions

System: BAS 200 Chromatographic Analyzer, refrigerated autosampler for automated operation.
Electrode: BAS Glassy Carbon
Potential: + 0.75 V vs Ag/AgCl
Column: Catecholamine Column, MF-6213CL
Mobile Phase: Proprietary mobile phase, CF-1100
Flow Rate: 1 mL/min.
Guaranteed Detection Limit: 10-15 pg for epinephrine, norepinephrine, DHBA and dopamine.
Linear Range: at least to 100 ng for epinephrine, norepinephrine, DHBA and dopamine.

Sample Preparation

Human plasma samples (fresh or thawed) were mixed with a solid proprietary Plasma Pretreatment Adsorbent (CF-1038) and clarified by centrifugation. The supernatants were subjected to an alumina extraction procedure (see application Capsule #227). Both human plasma samples and standards (catecholamines) were desorbed with Catecholamine Eluting Solution (CF-1039). Injection volume was 75 mL.

Notes

A calibration curve for epinephrine standards is presented in F1. Similar results were obtained for norepinephrine, DHBA and dopamine. Determination in biological materials normally requires calibration using standard addition to a pooled sample to account for recovery losses.

The standards eluted in the following order: epinephrine, norepinephrine, DHBA and dopamine (F2). Caffeic and dihydrocaffeic acid eluted much earlier than epinephrine, while uric and ascorbic acids eluted in the void volume.

The chromatogram of a sample obtained from a caffeine drinker (coffee, tea, or cola) is shown in F3.

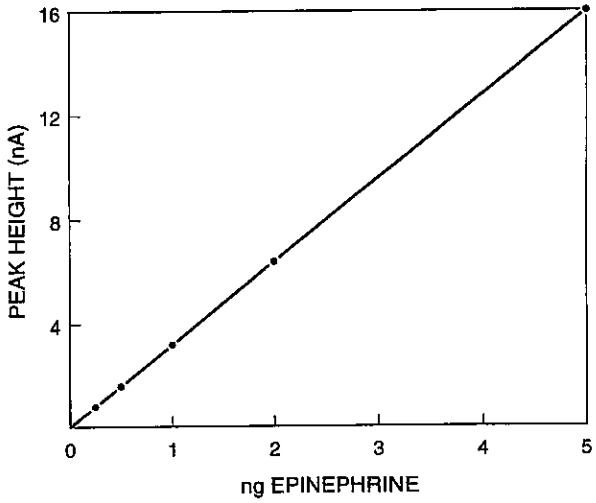

Figure 1. Calibration curve for injected epinephrine stand-

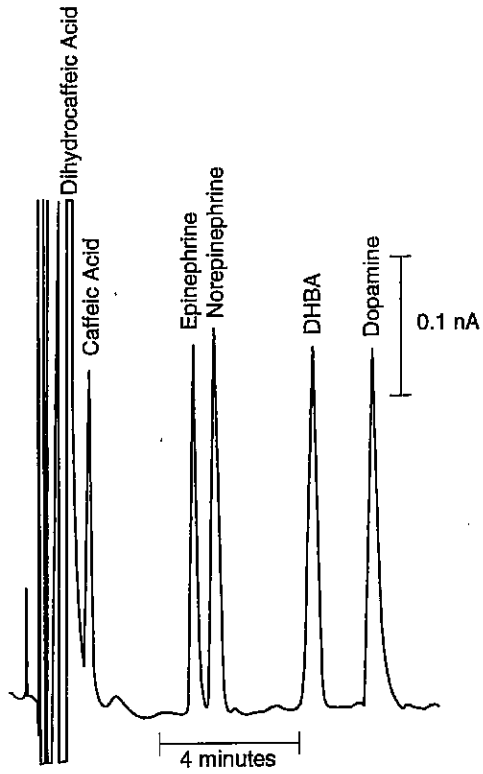

Figure 2. Chromatogram of injected standards (100 pg each catecholamine and DHBA, 50 pg caffeic acid and 25 pg dihydrocaffeic acid).

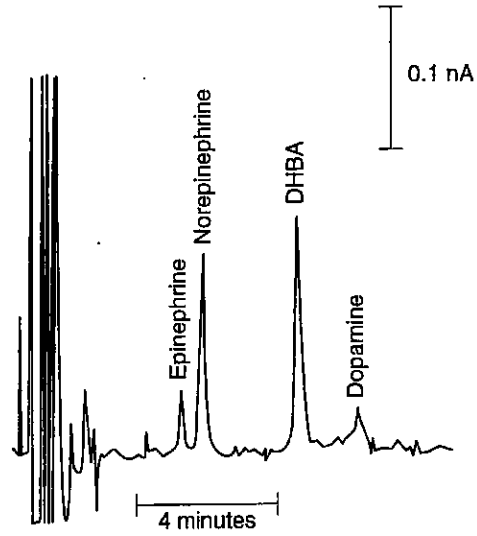

Figure 3. Sample from a human volunteer who had consumed a caffeine-containing drink.

Reference

1. *D.S. Goldstein and R. Stull, J. Chromatogr. 311 (1984) 148-153.*