CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Apparent Loss of Internal Standard (DHBA) in Dog Plasma

Purpose

To account for the apparent disappearance of internal standard, dihydroxybenzylamine (DHBA), from stored dog plasma.

The use of LCEC for the determination of plasma catecholamines is now routine in a great many labs. As is common practice in an analytical procedure involving sample storage and clean up, an internal standard is added to account for any handling loss.

Dihydroxybenzylamine has been commonly used for this purpose in plasma catecholamine determination. DHBA elutes in a clean region of the catecholamine chromatogram, between EPI and DA. Other internal standards generally elute later and can reduce sample through-put. In isolated cases, it has been noted that the efficiency of extraction of DHBA is low or variable. Dog plasma is one instance where DHBA appears to "disappear" from stored plasma, independent of the storage temperature. This phenomenon does not occur in human plasma.

Reference

Electrochemical Detection for Plasma Catecholamines: Apparent Loss of Dihydroxybenzylamine in Dog Plasma, N.W. Robie and K.S. DuSapin, J. Chromatogr. 270(1983) 366-370.

Conditions

Detector: BAS LC-4B/17
Electrode: glassy carbon
Potential: +0.65 V vs. Ag/AgCl
Column: 5 µm C_{18}, reverse phase
Mobile Phase: 0.15 M monochloroacetic acid (pH 3.0), 2 mM EDTA, 75 mg/L SOS. Flow rate was 2.2 mL/min.

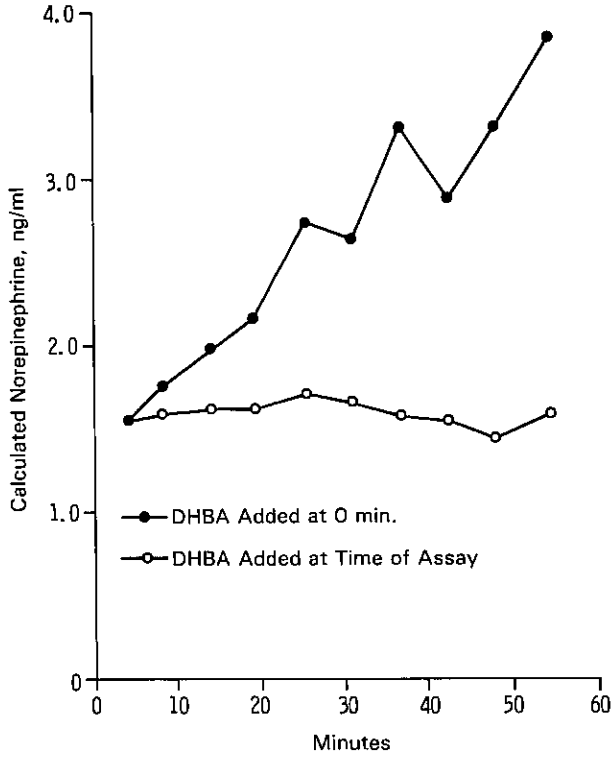

Figure 1. Calculated values for NE when DHBA was added either immediately before the Tris buffer (0), or at zero time (•). Reproduced with permission of authors (see Reference).

Sample Preparation

Plasma extraction was as described in Capsule XXX. A 50 µL volume of the final acidic catecholamine preparation was injected into the LCEC System.

Experimental

An initial experiment eliminated the possibility that DHBA is adsorbed to the polyethylene tubes used during the alumina extraction of the catecholamines.

A second study examined the stability of DHBA at -30°C and -80°C for up to 12 days of storage. Stabilized plasma was spiked with NE and DHBA and base levels determined before storage was initiated. The recovery of DHBA was erratic and decreased at both storage temperatures. On the other hand, the recovery of NE remained constant over the 12 day storage period.

A third study examined the short term storage loss of DHBA. Replicate plasma samples were spiked with NE and DHBA at zero time and analyzed at 4-6 min. intervals. The timed interval is that period from the addition of DHBA to the time Tris buffer is added to the sample. There was a progressive loss of extractable DHBA with time. This resulted in an erroneous increase in the calculated NE value (based on the extracted internal standard, DHBA). Replicate samples that had DHBA added immediately before addition of the Tris buffer yielded consistent calculated values for NE (Fig. 1).

The mechanism for the observed loss was not examined, but may be due to irreversible binding of DHBA to plasma protein or a specific enzymatic breakdown of DHBA. A standard solution of DHBA in phosphate buffer did not change. The authors concluded with the suggestion that, when catecholamines are measured in dog plasma DHBA should be added immediately before the addition of Tris buffer in the assay procedure.

When human plasma was subjected to a similar series of experiments, no apparent loss of DHBA was noted. This also applies to human plasma stored at -30°C for 3 days.

Comments

Although DHBA is the internal standard that is most widely used for plasma catecholamine determinations, N-methyldopamine and isoproterenol have also been utilized. Both have a capacity factor greater than dopamine.

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