CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Extraction of Biogenic Amines From Small Tissue Samples

Purpose

There is a growing need to assay small brain samples for their biogenic amine content. In fact, with existing microdissection techniques individual brain nuclei can be identified and isolated. Determinations of the biogenic amine content of these small samples require that the existing extraction (homogenization) methods be improved.

Reference

Determination of Monoamines in Brain Nuclei by HPLC with Electrochemical Detection: Young vs. Middle Aged Rats, K.J. Renner and V.N. Luine, Life Sci. 34(1984) 2193-2199.

Existing Methods

Brain samples are homogenized in 10 - 20 volumes (wt/vol) of ice-cold 0.1 N perchloric acid containing an internal standard (usually DHBA). The homogenate is centrifuged to remove the precipitated proteins and cell debris, and filtered through a 0.2 µm filter. The resulting supernatant is directly injected into an LCEC system.

Small brain samples handled in this fashion can be diluted to such an extent that biogenic amine determinations become difficult. Since enough $HClO_4$ has to be added to make sample handling convenient and quantitative, this results in sample dilution. Injecting larger sample volumes does not compensate for the dilution, as that will affect the subsequent chromatographic run.

Modified Method

The method described in the above reference maintains "the simplicity of sample preparation (existing methods) while allowing measurement of monoamines in as little as one 500 µm punch from a 300 µm thick brain section." Basically, in the modified method the brain punches are expelled into 60 µL of 0.1 M $HClO_4$ containing a known amount of DHBA. This suspension is frozen on dry ice, thawed (this fractures the cell membranes), and centrifuged at 15,000 g. The supernatant is removed and 40 µL are injected into the LCEC system.

The effectiveness of this technique was tested by homogenizing the freeze-thaw extracted pellet in $HClO_4$ (plus DHBA), centrifuging the homogenate, and assaying the supernatant. Only 5-HIAA, at 3% of the original sample levels, could be detected. This method results in higher sample amine concentrations and reduced amine degradation during sample preparation.

The primary disadvantage involves degradation of the indolamines during the time of sample preparation and analysis. The following modifications are proposed by the authors. Punched tissue is expelled into 60 µL of a sodium acetate solution (pH 5.0). After freeze-thaw and centrifugation, 2 µL of ascorbate oxidase (1 mg/10 ml of distilled water) is added to decrease the ascorbate contribution to the void volume response (see LCEC Capsule on this subject). The acetate buffer allows for the enzyme pretreatment of the sample, and the amine content is stable for up to 8 hours (5-HT and 5-HIAA degrade in $HClO_4$).

Related References

1. Simultaneous Multiple Electrode LCEC Assay for Catecholamines, Indoleamines and Metabolites in Brain Tissue, G.S. Mayer and R.E. Shoup, J. Chromatogr. 255(1983) 533-544.

2. Note that the separation presented in this report can be duplicated or even improved utilizing a BAS 400 or BAS 200 and a 3µm, C_{18}, 100 x 3.2 mm column.