Tissue Catecholamines

Simultaneous Assay for Norepinephrine, Epinephrine, and Dopamine

The most extensive application of liquid chromatography/electrochemistry (LCEC) to date has been the determination of tissue biogenic amines and metabolites. Numerous papers dealing with the analysis of brain parts, brain punchouts, whole brain, cerebrospinal fluid, dialysates and the circulatory system have been reported by the neuroscientific community.

Most methods rely on a simple alumina cleanup of the tissue homogenate supernatant. Usually ten to fifty milligrams of acid-washed aluminum oxide (AAO, Bioanalytical Systems) is added to each sample, the pH is rapidly raised to 8.5 with Tris buffer, and the samples are shaken for 10 minutes. The catecholamines are preferentially adsorbed onto the alumina at this pH. After shaking, the supernatant is aspirated to waste and washed twice with 3 mL of water. Each washing is also aspirated. The alumina is transferred to a Microfilter (Bioanalytical Systems) and centrifugally spun to dryness. Fifty to two hundred microliters of 0.1 M $HC1O_4$ are then added to the alumina, and the mixture is vortexed and then centrifugally filtered. This extract is injected. The assay is quick (40 min/10 samples) and requires no derivatives.

Typical output tracings using the cited procedure are shown in F1 and 2. Three milliliters of brain homogenate corresponding to 200 mg of tissue were assayed, and 50 µL of the final 200 µL extract was injected. The liquid chromatograph was fitted with a high resolution Biophase® ODS 5 µ column, permitting the separation of norepinephrine, epinephrine, dopac, dopamine, and internal standard in 13 minutes. A dual pen recorder permitted the recording of high as well as trace levels of catecholamines simultaneously. For example, both norepinephrine and dopamine were detected at a moderate sensitivity (20 nanoamperes full scale) while the detection of brain epinephrine, dopac, and the internal standard required a setting ten-fold more sensitive.

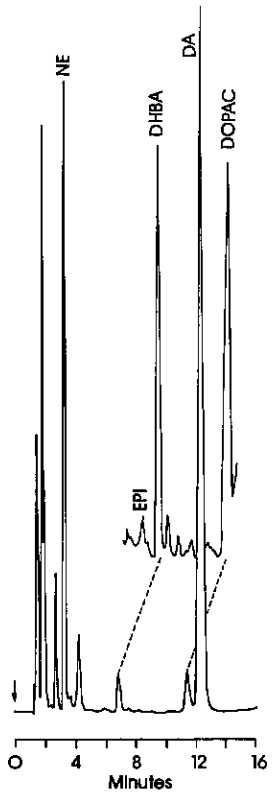

Figure 1. Dual pen recorder output of brain catecholamines. Concentrations: 157 ng/g norepinephrine, 2.4 ng/g epinephrine and 602 ng/g dopamine. Two hundred milligrams of tissue was assayed. Top tracing was run at 2 nA full scale; bottom, 20 nA full scale.

The catecholamines content of other types of tissue may be easily measured with the same methodology. F2A and 2B demonstrates dual-pen output for heart and lung, respectively. As expected, norepinephrine constituted the major catecholamine component in both types of tissue.

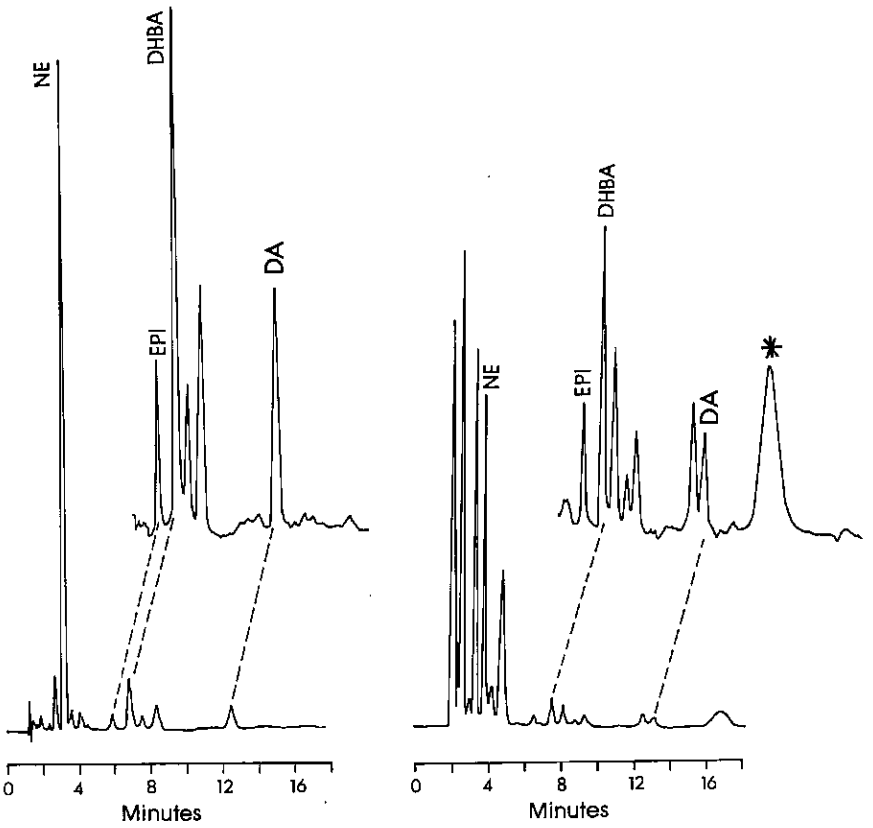

Figure 2. Chromatograms of (A) heart catecholamines (230 mg tissue assayed, 50 µL injected) and (B) lung catecholamines (650 mg, 35 µL injected). These samples contained: (A) 165 ng/g NE, 14 ng/g EPI, 20 ng/g DA, and (B) 22 ng/g NE, 6.9 ng/g EPI, 2.0 ng/g DA. The broad peak (*) was a late-eluting component from the heart extract.

Conditions

Instrument: LC-304T (Bioanalytical Systems Inc.) a BAS 200 or BAS 400

Detector: Thin-layer electrochemical detector, carbon paste or glassy carbon electrode

Stationary Phase: Biophase® ODS 5 µ, P/N 6017

Mobile Phase: 0.15 M monochloroacetate, pH 3.0, with 2.0 mM Na_2EDTA and 28 mg/L sodium octyl sulfate.

Temperature: 35°C, settable on LC-304T

Flowrate: 2.2 mL/min

Sensitivity: top trace, 2 nAmp full scale; bottom, 20 nAmp full scale

References

There are several other capsules related to this work. In addition, BAS maintains a file of nearly 2,000 neuroscience references. These are used to respond to "BAS Action Requests" which is the best approach to getting our assistance.