CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Eliminating Ascorbic and Uric Acid from Samples

Purpose

In many cases when analyzing a perchloric acid extract (homogenate) of brain tissue a large void volume response is encountered. The primary cause of this large response is ascorbic acid. This, of course, can interfere with the quantitation of norepinephrine (NE).

One can change the mobile phase composition to effect the separation of NE from the void volume response, but this will increase the time for the subsequent chromatographic run. Another approach is to remove the ascorbic acid prior to injection of the sample. This can be accomplished by the action of ascorbic acid oxidase (AAO, not to be confused with our acid washed alumina, also AAO). A study by L. McKay, C. Bradberry and A. Oke (J. Liq. Chromatogr. 311(1984) 167-169) utilized this approach. Samples of brain tissue are homogenized in pH 5.0 buffer containing DHBA. AAO was added and the homogenate centrifuged. The supernatant was then injected into the LCEC system. Perchloric acid precipitation of proteins following the ascorbic acid oxidase treatment may be of added benefit.

Ascorbic acid also can be removed with an ascorbate oxidase spatula (Boehringer Mannheim Biochemicals, Cat. No. #736 619). In this case the enzyme (AAO) is immobilized on a plastic spatula that is then stirred around in the sample solution to oxidize the ascorbate (to the electroinactive product dehydroascorbate).

Uric acid can also interfere with NE determinations in plasma and urine. In this case uricase can be added to the sample prior to LCEC analysis. Uric acid is oxidized to the non-electroactive product allantoin. This procedure can be found in the paper by G.C. Davis, P.T. Kissinger, and R.E. Shoup, Anal. Chem. 53(1981) 156-159.

Enzyme pretreatment of a sample is not a guaranteed solution to removal of a contaminant but it can be a viable approach for solving the type of problems outlined above.

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