Waters
Lab Highlights

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ROUTINE DIAGNOSIS OF SOME DISEASES FACILITATED BY SIMPLIFIED ASSAY USING SEP-PAK® SILICA CARTRIDGES

A recent paper (1) reports that quantitative separations of bases from nucleosides were obtained with SEP-PAK^R Silica Cartridges from Waters. Other silica sources did not do as well. "Partial separations of bases from nucleosides were observed with four commercial preparations (Bio-Rad; Mallinckrodt; Clarkson; and Matheson Coleman and Bell), in that a variable amount of nucleosides eluted with the bases in the borate wash."

Because several genetic diseases are a result of aberrant nucleotide metabolism and are marked by altered concentrations of nucleotides, bases, or nucleosides in cells, serum, and urine, identification of the disease is dependent on quantitative measurements of contents of acid-soluble pools and/or accurate measurement of enzyme activity. Therefore, rapid and precise preparation of nucleotides, constituent bases and nucleosides from acid extracts or cells was developed. The procedure is described in a recent paper using SEP-PAK^R Silica Cartridges. This new method (5-10 minutes) will facilitate routine diagnosis of certain diseases by simplifying the assay and decreasing the assay time.

As an illustration of the usefulness of this procedure, nucleotides were purified from acid extracts of normal and transformed cell on the silica cartridges. The SEP-PAK^R cartridges (1-ml bed volume) were washed with 5 ml of water then with 5 ml of acetonitrile/water (90/10 by vol.). After applying 3-ml samples, adjusted to 900 ml/l acetonitrile content, the silica was washed with an additional 10 ml of the acetonitrile/water solvent. More than 95% of the amounts of bases and nucleosides present did not adsorb to silica under these conditions except for cytidine (92%). Charged nucleosides and nucleotides bind to the silica, but compounds without a charge at (neutral pH) are not retained. This procedure has been used to purify nucleotides from several normal and transformed cell lines. Also of note, is that the procedure using the SEP-PAK^R cartridges may replace the need to use the previously described, lengthy chromatographic techniques of ligand exchange, gel filtration, borate complexing and ion exclusion chromatography.

This report is also complementary to another description of the rapid group separation of nucleic acid components on Silica cartridges (2).

1. Lothrop, C. D., Jr. and Uziel, M., Clin. Chem. (1980) 26 (10), 1430-1434.
2. Uziel, M., Smith, L. H. and Taylor, S. H. Clin. Chem. (1975) 22, 1451-1455.

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