Waters
Lab Highlights
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A RAPID SCREENING PROCEDURE USING RADIAL-PAK™ RESOLVE C_{18} FOR COMMON ANTICONVULSANTS IN SERUM

A method for the fast analysis of three common anticonvulsants in serum has been developed using a Radial-PAK™ RESOLVE™ C_{18} column (5 mm I.D. X 100 mm) and C_{18} Guard-PAK™ cartridge. The procedure, developed by Udo Rupprecht of Millipore, Australia, has the advantage of rapid sample preparation along with a short (under 6 minutes) analysis time. More than ten laboratories in Australia use this as a screening procedure for anticonvulsants.

The procedure monitors amounts of phenobarbital, phenytoin and carbamazepine along with an internal standard, 5-(4 methyl phenyl)-5-phenyl hydantoin (5-MPPH). The 5-MPPH is dissolved in acetonitrile at a concentration of 0.05 mg/ml. Sample preparation requires taking 200 µl of the internal standard (5-MPPH) and adding 200 µl of serum. The sample is then vortexed for 10 seconds, centrifuged for 10 minutes and 20 µl of the supernantant is injected. A typical chromatogram obtained from this procedure is shown in Figure 1.

The mobile phase consists of 300 ml of methanol, 180 ml of acetonitrile, and 0.88 g of KH_2PO_4 in 650 ml of water (pH = 6.0 with H_3PO_4 or KOH) at a flow rate of 3.0 ml/min. Detection of the peaks is monitored at 214 nm, 0.1 AUFS.

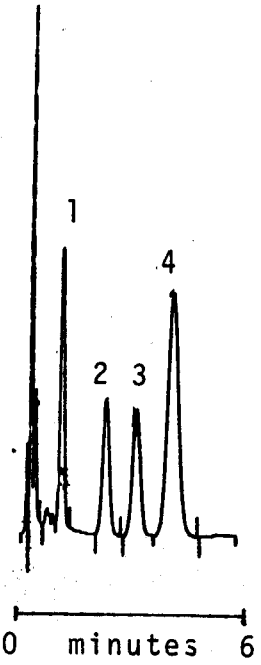

FIGURE 1: Chromatogram of three anticonvulsants in serum plus internal standard. Sample concentrations are (1) phenobarbital 100 µmoles/L, (2) phenytoin 65 µmoles/L, (3) carbamazepine 40 µmoles/L, (4) 5-MPPH, internal standard. Instrument: Model 320 QA-1™ Analyzer. Other chromatographic conditions are in the text.

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