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DIRECT CHROMATOGRAPHIC ANALYSIS OF METABOLITES OF LIPOPHILIC TRACERS IN WHOLE BLOOD BY GFF-ISRП CHROMATOGRAPHY

Analytes: Boronic acid adducts of Technetium diOxime

Sample Matrix: Heparinized whole blood

Column: 75 micron GFF-ISRП glass beads, 5 cm x 4.6 mm ID

Discussion: HPLC is frequently used in studies of metabolism of radioactive (and non-radioactive) compounds in whole blood and other biological samples. Normally, a pretreatment step (which involves either separation of cellular material from plasma, or solvent extraction of analytes, or both) is required because of the small particle size (<10 micron) of the majority of HPLC column packing materials. These pretreatment steps are undesirable. They restrict rapid analysis in time dependent studies. They may alter delicate equilibria. Results may be perturbed due to different solvent extraction efficiencies of the hydrophobic drugs and their metabolites.

Previously, HPLC using columns packed with 5 micron GFF-ISRП silica have shown utility in the analysis of lipophilic drugs and their metabolites by direct injection of serum or plasma samples. Now, we report a novel HPLC method based on GFF-ISRП material for the rapid on-line separation of lipophilic drugs and their metabolites from whole human blood. This is achieved through the use of 75 micron GFF-ISRП glass bead material packed into a small HPLC column, and used in conjunction with a HPLC system containing a switching valve and a second analytical column (Figure 1). When heparinized whole blood is applied to the GFF-ISRП column, and the column eluted with an isotonic eluent, lipophilic compounds free in plasma are retained by the column. Plasma proteins, blood cells, and compounds bound to these blood components are not retained and pass through the ISRП column, and are directed to a radioactivity detector (Fig. 1a). Following the elution of these components, the solvent flow is redirected to pass from the ISRП column to an analytical column, and then to the radioactivity detector (Fig. 1b). The lipophilic compounds retained on the ISRП column are eluted by increasing the percentage of organic solvent in the eluent, and are separated by the analytical column.

Figure 1. HPLC system for Analysis of Compounds in Whole Blood

a. Initially, eluent flow proceeds b. After all blood cells and proteins have directly from the ISRP column to been eluted, flow is redirected through the radioactivity detector. the analytical column, increasing organic content of the eluent to remove lipophilic compounds from the ISRP column.

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Discussion (cont.): We have applied this analytical method to the study of metabolism the ^{99m}Tc -BATO (Boronic Technetium diOxime) cerebral perfusion tracers, previously described by Treher et al (1). The ^{99m}Tc -BATO complex was mixed with heparinized whole blood. At set periods, a 20-40 ul sample of the whole blood was injected onto the 5 cm x 4.6 mm GFF-ISRP glass bead column, which was eluted with 0.1M ammonium citrate (pH 5.0) at a flow rate of 1.0 ml/min. The column eluant was directed to the radiometric detector to measure the amount of radioactivity which is associated with the whole blood components. After 1.1 minutes, eluate from the column was redirected through a C8 analytical column. After 2.0 min. the system flow rate was increased to 1.5 ml/min and a solvent gradient begun to a final solvent composition of 72:28 acetonitrile:0.1M ammonium citrate.

The following Results were obtained following incubation of $^{99m}\text{TcCl}(\text{DMG})_3\text{2MP}$ in whole human blood at 37 deg. C, (DMG=dimethylglyoxime; 2MP=2-methylpropylpylboronic acid) and analysis by the ISRP/C8 HPLC method described above. In whole human blood, the $t_{1/2}$ for loss of $^{99m}\text{TcCl}(\text{DMG})_3\text{2MP}$ is 10.4 min., with the generation of at least two metabolites (unidentified) in addition to the formation of $^{99m}\text{TcOH}(\text{DMG})_3\text{2MP}$ (by exchange of the axial chloro ligand by hydroxyl(2) in vivo) and protein bound ^{99m}Tc .

Time * (minutes)	% bound Rt = 0.6	% Cl ** Rt = 11.5	Metabolites		
			Rt = 7.6	Rt = 8.9	Rt = 10.5
1	2.5	93.7	0.4	1.1	1.5
5	22.4	53.6	4.8	11.3	5.6

Conclusion: This novel HPLC method provides a rapid, convenient, and reliable method for the analysis of radioactive and non radioactive lipophilic components in whole blood. Variation in the secondary analytical column, solvents, and detection system should allow this system to be used in many drug metabolism studies.

References: (1) E.H. Treher, L.C. Francesconi, J.Z. Gougoutas, M.F. Malley, A.D. Nunn, *Inorg. Chem.*, 28, 3411-3416 (1989).
(2) W. Hirth, S. Jurisson, K. Linder, T. Feld, A.D. Nunn, *J. Nucl. Med.*, 29, 800, (1988).

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