

NOTE NO. 42 - August 30 1993 Page 1 of 2

**AN ISRP-REVEALED TIME-DEPENDENCE OF DRUG BINDING.
INJECTION OF UNFILTERED WHOLE BLOOD.**

Analytes: Technetium dioxime complexes

Sample Matrix: Whole blood, heparinized but unfiltered

Sample Size: 20 µl whole blood, preinjected into 100 µl sample loop prefilled with A

Detection: 254 nm

First Column: 75 micron GFF ISRP, 5 cm x 4.6 mm I.D.

Second Column: Guarded 5 micron C8, 5 cm x 4.6 mm I.D.

Regis Product Number: 785008

Mobile Phase: Before starting, equilibrate both columns with 0.1 M sodium citrate (A). Thereafter, in use:

With ISRP only:

First minute: A, 2 mL/min;

Next 4 min: CH₃CN/A 95/5, 2 mL/min;

Thereafter: A, 5 mL/min.

With both columns:

First 3 mins: ISRP--->detector: A, 1 mL/min

Next 17 mins: ISRP--->C8--->detector: CH₃CN/A 72/28, 1 mL/min.

Discussion: This paper is unusual in calling for the direct injection of unfiltered whole blood into a special 75-micron ISRP GFF column (custom-developed by Regis, given to the researchers). The column was packed with glass beads of controlled porosity and 75-micron diameter. Such a particle diameter should and did allow the passage of blood cells through the column, thus avoiding on-column hemolysis.

The distribution of the total drug between bound and free was determined by use of the ISRP column alone (Figure 1A) (1). The binding and metabolism of the technetium dioxime complexes were determined by use of the upstream ISRP-downstream C8, coupled-column array (Figure 1B) (1).

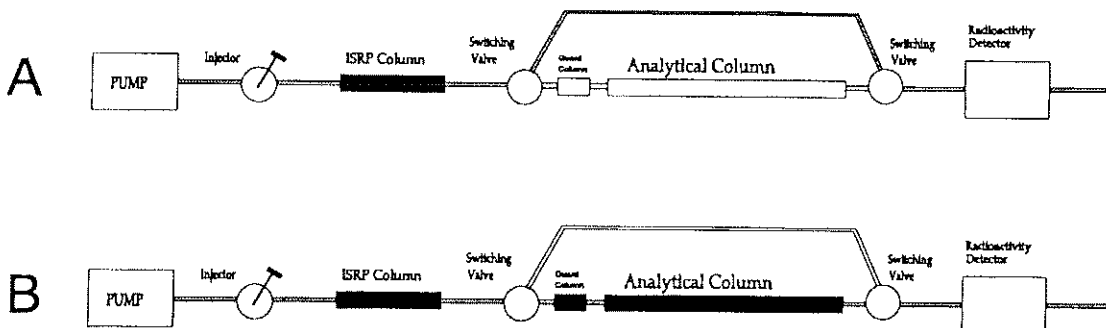


Figure 1A and 1B: In mode A, the effluent from the ISRP column is sent directly to the detector. In mode B, the ISRP effluent is sent into the C8 column. (Reproduced with permission from reference 1. Copyright Elsevier Publishing Company.)

For a given drug, the pharmacokinetic organ extraction and blood clearance of the drug may be crucial (2). With the technique described in this paper, differing degrees of equilibrium time-dependence could be shown for the different metabolites, and more realistic values for the shorter binding times (see Figure 2). Such results could not be obtained by centrifugation, which lasts 25 minutes and thus inherently conceals binding variations within that time.

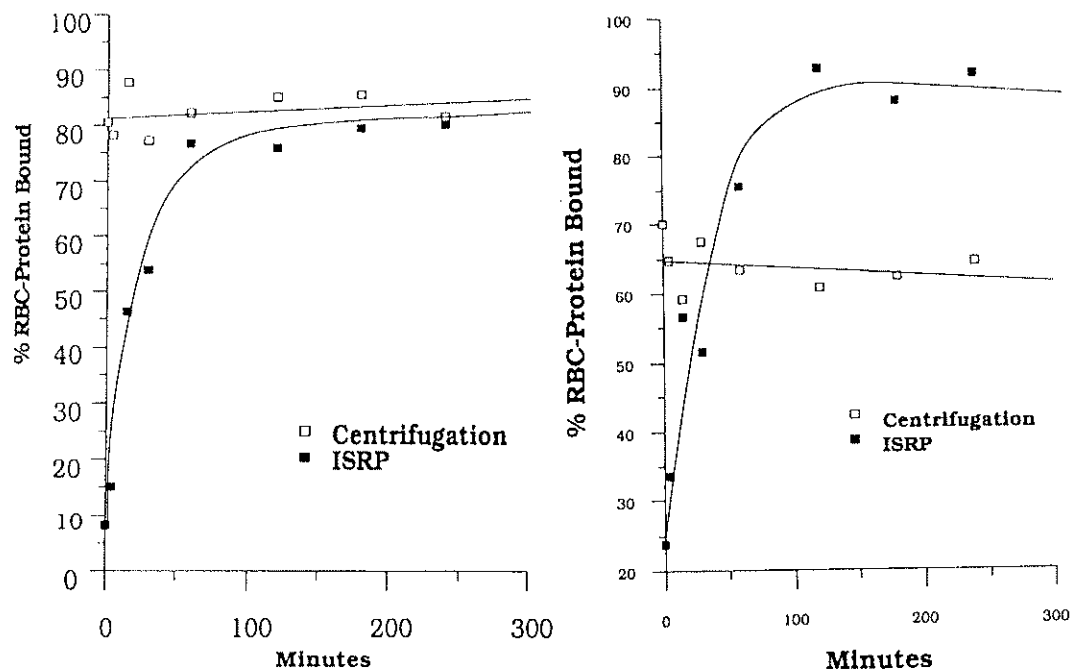


Figure 2: Drug-protein binding that increases early and sharply can be revealed by ISRP chromatography but not by centrifugation, which requires 25 minutes of processing. The two graphs show binding time dependences for two technetium dioxime complexes. The authors suggest that centrifugation disturbs the binding more than the ISRP method and therefore, for the complex the time behavior of which is shown at the the right, a lower equilibrium binding value. (Reproduced with permission from reference 1. Copyright Elsevier Publishing Company.)

References: (1) Rosenspire, K. C.; Hirth, W.; Jurisson, S.; Nowotnik, D. P.; Echelman, W. C.; Nunn, A. D. J. *Chromatogr.* **1992**, *574*, 119-126.

(2) Narra, R. K.; Nunn, A. D.; Kuczynski, B. L.; Di Rocco, R. J.; Feld, T.; Silva, D. A.; Eckelmann, W. C. *J. Nucl. Med.* **1990**, *31*, 1370.