Journal of Chromatography, 574 (1992) 119–126
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6149

Direct analysis of whole blood by internal surface reversed-phase chromatography: an examination of the binding and metabolism of technetium dioxime complexes

Karen C. Rosenspire, Walter Hirth, Silvia Jurisson, David P. Nowotnik*, William C. Eckelman and Adrian D. Nunn

Bristol-Myers Squibb Pharmaceutical Research Institute, 1 Squibb Drive, New Brunswick, NJ 08903 (USA)

(First received May 29th, 1991; revised manuscript received September 6th, 1991)

ABSTRACT

We have developed a method using internal surface reversed-phase (ISRP) packing for rapid on-line separation of small hydrophobic compounds from cellular whole blood components. This is achieved by the use of 75-μm ISRP chromatographic material packed into a small high-performance liquid chromatographic (HPLC) column, in conjunction with column switching. We have applied this analytical method to study the *in vitro* metabolism of ^{99m}Tc-BATO (boronic acid adducts of technetium dioxime) cerebral and myocardial perfusion tracers in whole blood. The results from the ISRP procedure were compared with a conventional centrifugation method of analysis. This novel HPLC methods provides a rapid, convenient and reliable method for the analysis of radioactive and non-radioactive lipophilic components in whole blood.

INTRODUCTION

It is well established that the therapeutic effect of a drug is related to the concentration of circulating unbound drug and the kinetics of the bound–free equilibrium [1]. There are many techniques to establish the concentration of free and bound drug in blood: ultracentrifugation, ultrafiltration, equilibrium dialysis and solvent extraction [2–4]. The choice of method can have a profound effect on the numerical result [5]; the best technique for pharmacokinetic analysis would be one which least disrupts the equilibrium between bound and free drug.

High-performance liquid chromatography (HPLC) is frequently used in studies of metabolism of radioactive and non-radioactive compounds in blood and other biological samples [6]. However, because of the small particle size (< 10 μm) of the majority of HPLC column packing materials and the common use of organic solvents, analyses of drugs and metabolites in whole blood must use a pretreatment process to remove the protein and cellular material. These pretreatment steps may include centrifugation to remove cells and filtration or precipitation techniques to remove protein [1]. Pretreatment steps may be undesirable if they prevent rapid analysis in time-dependent studies and if they disturb delicate equilibria.

To avoid the alterations in cell and protein binding caused by sample preparation prior to HPLC analysis, alternative column packings have been developed to permit the application of whole blood samples [4,7]. For example, Tamai *et al.* [7] have developed a column with pores large enough to eliminate clogging. However, whole blood is hemolyzed on the column and the soluble components are selectively eluted. Such a procedure may cause unbound intracellular drug to be released with free extracellular drug during the procedure leading to a falsely high concentration of free drug.

Several HPLC packings have been developed

0378-4347/92/$05.00

for the analysis of plasma and serum samples including internal surface reversed-phase (ISRP) packings, dual-zone materials (DZMs) and shielded hydrophobic phases (SHPs) [8–11]. Normally, blood cells must be removed prior to analysis.

ISRP chromatography employs two retention mechanisms for the separation of analytes: size exclusion and hydrophobic interaction. Conventional ISRP packing is made of 5 μm GFF (glycine-phenylalanine-phenylalanine) glass beads. HPLC using columns packed with 5 μm ISRP packing have shown utility in the analysis of lipophilic drugs and their metabolites by direct injection of serum or plasma samples [9,10,12,13].

There are many applications in which direct injection of whole blood samples would be more suitable than the analysis of plasma or serum samples. However, 5-μm ISRP packing has interstices too small to allow the passage of blood cells. Larger particles would, of course, provide larger interstices, but interstices too large would decrease resolution. From data on the known size of circulating blood cells [14], we calculated that a packing with a particle size of 50–100 μm in diameter would provide interstices large enough to permit passage of cells in human or animal blood. Using a 75-μm GFF ISRP controlled-pore glass loaded into a small HPLC column, in conjunction with a switching valve and a second analytical HPLC column, we have developed a method for rapid on-line separation of small hydrophobic compounds from plasma proteins and cellular whole blood components.

The hydrophobic compounds studied are ^{99m}Tc-BATO (boronic acid adducts of technetium dioxime) complexes. These γ-emitting radiolabeled complexes have shown utility as myocardial and cerebral perfusion imaging agents and one, CardioTec (^{99m}TcCl(CDO)$_3$BMe), is approved by the FDA for routine clinical use in the USA [15–18]. While ^{99m}Tc-BATOs are stable in kit form, axial ligand exchange (with exchange of the native chloro form to hydroxy) has been shown to occur in aqueous solution *in vivo* and *in vitro* and the chloro and hydroxy forms have slightly different biodistributions [19]. We have developed an ISRP-HPLC method to characterize the metabolism and binding characteristics of ^{99m}Tc-BATOs in whole blood.

EXPERIMENTAL

Materials

All solvents were of HPLC grade. Sodium citrate (trisodium salt), ammonium acetate, sodium hypochlorite and 5-sulfosalicyclic acid were purchased from Aldrich (Milwaukee, WI, USA). ^{99m}TcCl(DMG)$_3$B2MP, ^{99m}TcCl(CDO)$_3$BMe, ^{99m}TcOH(CDO)$_3$BMe, ^{99m}TcCl(DMG)$_3$BMe (CDO = cyclohexanedione dioxime, DMG = dimethylglyoxime, BMe = methylboronic acid and B2MP = 2-methylpropylboronic acid) were prepared as previously reported from lyophilized kits containing dioxime, boronic acid, stannous chloride and ^{99m}Tc-pertechnetate (Squibb Diagnostics, Princeton, NJ, USA). ^{99m}Tc-Human serum albumin (HSA) and ^{99m}Tc-Choletec (Squibb Diagnostics) were prepared according to the package insert. ^{99m}Tc-Labeled rat red blood cells (RBCs) were prepared by gently mixing washed RBCs with stannous pyrophosphate (Phosphotec, Squibb Diagnostics) followed by sodium hypochlorite for 5 min at room temperature. ^{99m}Tc-Labelled pertechnetate was then added to the RBCs and gently mixed for another 5 min [20].

Blood preparation

Heparinized whole blood drawn from the abdominal aorta of pentobarbital-anesthetized male Sprague–Dawley rats was incubated at 37°C with ^{99m}TcCl(DMG)$_3$B2MP, ^{99m}TcCl(CDO)$_3$BMe or ^{99m}TcCl(DMG)$_3$BMe (1 mCi/ml of blood). At designated intervals (1, 5, 15, 30 min, 1, 2, 3, 4 h) aliquots of whole blood were analyzed by HPLC (see below) to separate the ^{99m}Tc-labeled components into bound (protein plus RBCs) and free fractions. An aliquot of whole blood was also removed and separated into RBC and plasma fractions by centrifugation (see below). The plasma was then separated into protein-precipitable and -soluble fractions. In addition, as a measure of the fragility of the RBCs to the HPLC conditions, RBCs were collected after passage through the HPLC system and centrifuged (see below); the resulting supernatant was visually checked for hemoglobin as an indication of cell lysis.

Chromatography system

The HPLC apparatus used was a Perkin-Elmer 410 pump (Perkin-Elmer, Norwalk, CT, USA) with a flow-through NaI radiation detector (Tennelec, Oak Ridge, TN, USA). An ISRP column (75 μm, 50 mm × 4.6 mm I.D.; Regis, Morton Grove, IL, USA) and a Nucleosil C_8 (5 μm, 250 mm × 4.6 mm I.D., Macherey-Nagel, Cambridge, UK) with a Brownlee C_8 (Applied Biosystems, Foster City, CA, USA) or Nucleosil C_8 (Macherey-Nagel) guard cartridge were connected via a Rheodyne column switching valve (Rheodyne, Cotati, CA, USA) (Fig. 1). Whole blood samples and standards (20 μl) were injected into a biocompatible Rheodyne injector with a 100-μl injection loop prefilled with 0.1 *M* sodium citrate (pH 7.0). Both columns were equilibrated with 0.1 *M* sodium citrate. For the first 3 min, the ISRP column was eluted with 0.1 *M* sodium citrate at 1 ml/min (Fig. 1A). At 3 min the flow was directed from the ISRP column to the C_8 column and the mobile phase was changed using a step gradient to acetonitrile–0.1 *M* sodium citrate (72:28) at 1 ml/min for 17 min (Fig. 1B). For the determination of total drug bound and free (without resolving the free metabolites), no second column is required. In this case only a 50-mm ISRP column was used (0.1 *M* sodium citrate at 2 ml/min for 1 min, followed by a step gradient to acetonitrile–0.1 *M* sodium citrate (95:5) at 2 ml/min for 4 min, then returning to initial conditions of 0.1 *M* sodium citrate at 5 ml/min).

The lipophilicity of the complexes was determined by HPLC using a PRP-1 column (10 μm; 150 mm × 4.1 mm I.D.; Hamilton, Reno, NV, USA) and eluted with 0.1 *M* ammonium acetate (pH 4.6)–acetonitrile (35:65) at 2 ml/min [21]. Sodium nitrate was used to determine the void volume and detected at 210 nm.

Radiochemical purity of $^{99m}TcCl(DMG)_3$-B2MP, $^{99m}TcCl(CDO)_3BMe$, $^{99m}TcOH(CDO)_3BMe$ and $^{99m}TcCl(DMG)_3BMe$ was determined by reversed-phase HPLC using a Nucleosil C_8 column (without the ISRP column) (5 μm; 250 mm × 4.6 mm I.D.), a mobile phase of 0.1 *M* sodium citrate–acetonitrile (70:30) at 1 ml/min and flow through radiation detector.

Centrifugation method

Heparinized whole blood (1 ml containing 1 mCi of one of the ^{99m}Tc-labeled agents) was centrifuged (Beckman 11 microfuge, Beckman, Palo Alto, CA, USA) at room temperature (280 *g*; 5 min) to separate RBCs from plasma. The RBCs were washed three times in cold normal saline to remove trapped plasma. The plasma and saline washings were added to 100 mg of 5-sulfosalicylic acid to precipitate proteins and inhibit further protein binding. This procedure required 20–25 min. The RBCs and plasma fractions were analyzed for radioactivity. The deproteinized plasma was kept on ice until counted and centrifuged (10 000 *g*; 5 min), with the resulting plasma protein precipitate and supernatant analyzed for radioactivity.

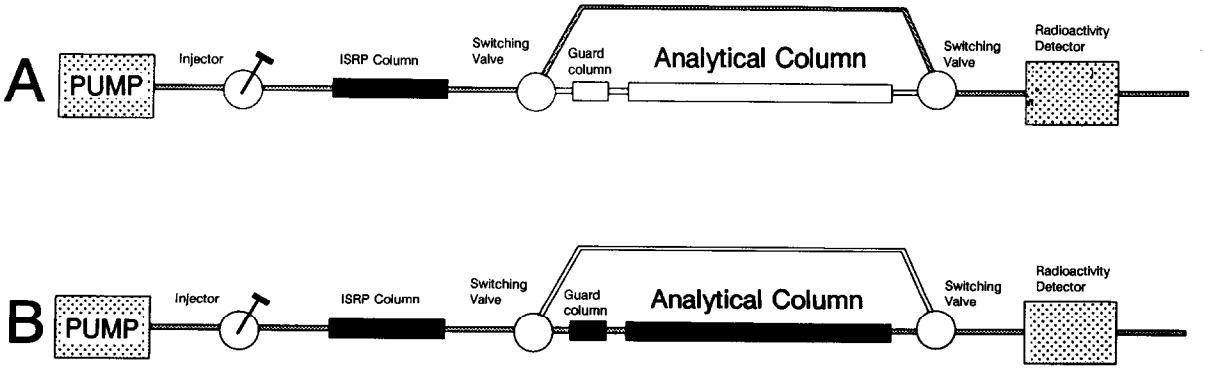

Fig. 1. (A) Initially, eluent flow proceeds directly from the ISRP column to the radioactivity detector. Free analyte is retained by the column, while analyte bound to blood cells and plasma proteins is eluted. (B) After all blood cells and proteins have been eluted, flow is redirected through the analytical column, increasing the organic solvent content to remove lipophilic components from the ISRP column, with subsequent separation of these components by the analytical column.

Statistical analysis

Statistical significance was determined using the Student's *t*-test.

RESULTS

Sample size and chromatographic conditions

Only small aliquots (20 μl) of whole blood were applied to the 75-μm ISRP column. As has been reported for the 5-μm ISRP column, back-pressure is related to sample size, due to frit performance [22]. It was observed in some of our studies that using 100-μl blood samples resulted in very high back-pressure on the ISRP column after only seven or eight injections. The pressure would return to normal after the inlet frit had been changed. The use of 20 μl extended the number of injections before back-pressure problems were observed to 50–75.

It is important when using the ISRP column with column switching to completely remove protein and RBCs from the ISRP before adding organic modifier and switching to the analytical column. While the labeled RBCs and protein were apparently removed from the ISRP by 1 min, it was observed that adding acetonitrile to the mobile phase at less than 2 min caused a steady increase in back-pressure with successive injections. Delaying the addition of organics to the

TABLE I

CAPACITY FACTORS AND LOG *P* FOR ^{99m}Tc-LABELED AGENTS

Agent	Retention time[a] (min)	Capacity factor (k')[a]	Log P[b]
^{99m}Tc-RBC	0.7	–	N.D.[c]
^{99m}Tc-HSA	0.7	–	N.D.
^{99m}Tc-pertechnetate	1.0	0.4	−1.8
^{99m}Tc-Choletec	14.3	19.4	N.D.
^{99m}TcCl(CDO)$_3$BMe	16.6	22.7	4.6
^{99m}TcOH(CDO)$_3$BMe	16.9	23.1	4.0
^{99m}TcCl(DMG)$_3$B2MP	17.3	23.7	3.5
^{99m}TcCl(DMG)$_3$BMe	19.1	26.3	2.3

[a] ISRP–Nucleosil C_8 columns with Brownlee guard cartridge as described in the text.

[b] PRP-1 column as described in the text.

[c] Not determined.

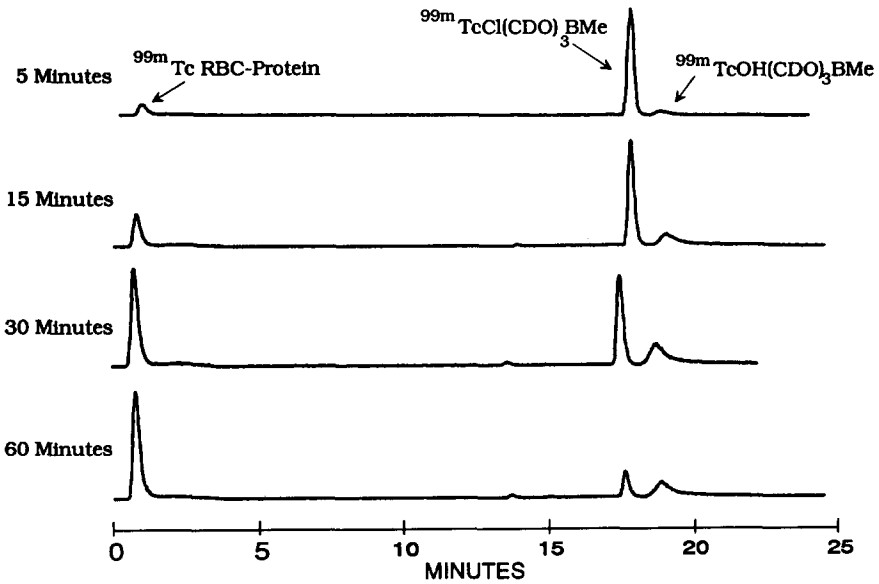

Fig. 2. Representative chromatograms showing the *in vitro* metabolism of ^{99m}TcCl(CDO)$_3$BMe incubated in rat blood at 37°C. These chromatograms show an increase in percentage RBC–protein binding with time. The remaining free activity shows metabolism of ^{99m}TcCl(CDO)$_3$BMe to the hydroxy form (^{99m}TcOH(CDO)$_3$BMe). Chromatographic conditions: both the ISRP and Nucleosil columns with a Brownlee guard cartridge are equilibrated with 0.1 *M* sodium citrate. For the first 3 min, the ISRP column was eluted with 0.1 *M* sodium citrate at 1 ml/min. At 3 min the flow was directed from the ISRP column to the Nucleosil C_8 column and the mobile phase was changed to acetonitrile–0.1 *M* sodium citrate (72:28) at 1 ml/min for 17 min.

mobile phase until after 3 min ensured complete removal of all protein and cellular material from the ISRP and avoided their precipitation in the system. Column problems were minimized by following these simple precautions.

Column retention and lipophilicity

The retention times, capacity factors and log *P* values for all standards are shown in Table I. The radiochemical purity of ^{99m}TcCl(DMG)$_3$B2MP, ^{99m}TcCl(CDO)$_3$BMe, ^{99m}TcOH(CDO)$_3$BMe, ^{99m}TcCl(DMG)$_3$BMe was greater than 82, 92, 90 and 90%, respectively. The capacity factor (k' was calculated for each standard assuming ^{99m}Tc-RBCs and ^{99m}Tc-HSA were unretained. Labeled RBCs proteins and hydrophilic compounds such as ^{99m}Tc-pertechnetate are not well retained by the ISRP column, whereas lipophilic compounds are retained by the ISRP column and can subsequently be removed by adding acetonitrile to the eluent. They are resolved by the reversed-phase column. The lipophilic compounds are not necessarily eluted from the ISRP–Nucleosil system in order of lipophilicity, whereas using the Nucleosil C_8 column (alone or with a Nucleosil guard column) the elution order of the ^{99m}Tc-BATOs paralleled lipophilicity, as measured by a PRP-1 HPLC system [23]. This suggests that the ISRP retains compounds by additional mechanisms, not just hydrophobic interaction. For example, it has been demonstrated that GFF ISRP columns can be used in a weak cation-exchange mode, presumed to be due to the terminal carboxylate groups on the phenylalanine packing [24]. It is unlikely that the neutral BATO complexes could utilize this mode of retention; instead they might be involved in some hydrogen-bonding interactions with the peptide interior of the ISRP packing. The Brownlee C_8 guard column with the Nucleosil C_8 analytical column had the same effect on elution order as the ISRP–Nucleosil combination. In the case of the Brownlee precolumn, it is presumed that uncapped silanol influences the order of retention.

Typical radiochromatograms of ^{99m}TcCl(CDO)$_3$BMe incubated at 37°C *in vitro* in rat blood at 5, 15, 30 and 60 min are shown in Fig. 2. As the incubation time is increased, the percentage radioactivity present as labeled RBCs and protein increases while the percentage radioactivity as ^{99m}TcCl(CDO)$_3$BMe decreases. The metabolite, ^{99m}TcOH(CDO)$_3$BMe, formed by axial ligand exchange [21] increases only slightly with time. Similar chromatograms, showing increasing RBC and protein binding with time, were also obtained using ^{99m}TcCl(DMG)$_3$B2MP and ^{99m}TcCl(DMG)$_3$BMe (data not shown). The retention times of these peaks were the same as that of fully characterized standards [21], thus confirming the assignment of the chloro and hydroxy peaks in the chromatogram.

Recovery of red blood cells

HPLC eluent containing protein and RBCs, collected for the first 2 min after the injection of whole blood onto the ISRP column, was centrifuged to determine whether cell lysis was occurring on the column. The resulting supernatant showed that little if any hemolysis was occurring and that the cells were eluting intact.

Comparison of HPLC and centrifugation methods

Because the RBCs and protein coelute, the radioactivity associated with this peak represents the sum of RBC- and protein-bound drug. Thus for comparison with the HPLC method, radioactivity associated with the RBC- and protein-precipitable fractions after centrifugation was summed. A comparison of the percentage RBC–protein-associated radioactivity *versus* time for the two methods is shown in Figs. 3–5 for ^{99m}TcCl(CDO)$_3$BMe, ^{99m}TcCl(DMG)$_3$B2MP and ^{99m}TcCl(DMG)$_3$BMe, respectively.

As can be seen in Figs. 3–5, the percentage RBC–protein-bound radioactivity increases with time over the first 30–60 min for the HPLC method and then plateaus to an equilibrium value. However, for the centrifugation method, the percentage bound remains almost constant over the entire time of the study. For both ^{99m}TcCl(CDO)$_3$BMe and ^{99m}TcCl(DMG)$_3$BMe the binding values at equilibrium for the two methods are not significantly different. The binding values are ~80% and ~68% for ^{99m}TcCl(CDO)$_3$BMe and ^{99m}TcCl(DMG)$_3$BMe, respectively. However, for ^{99m}TcCl(DMG)$_3$B2MP the equilibrium binding value for the two methods are significantly different ($p < 0.01$), with the

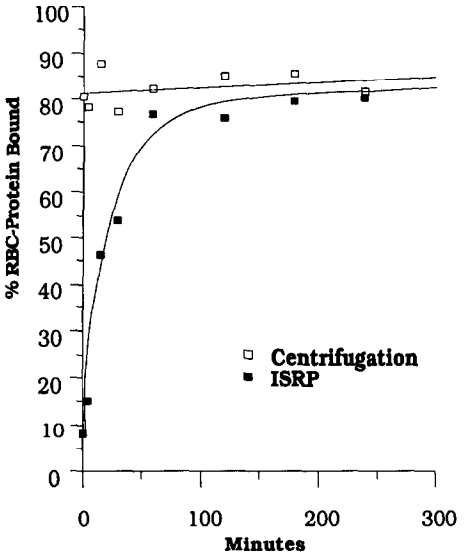

Fig. 3. Comparison of the ISRP method (□) with centrifugation (■) for ^{99m}TcCl(CDO)$_3$BMe (n = 2). The values for equilibrium binding from the ISRP and centrifugation methods are in good agreement. At early time points, the ISRP method is able to demonstrate the rise in percentage binding with time. The centrifugation method cannot provide these data at early times due to the long processing time associated with this method.

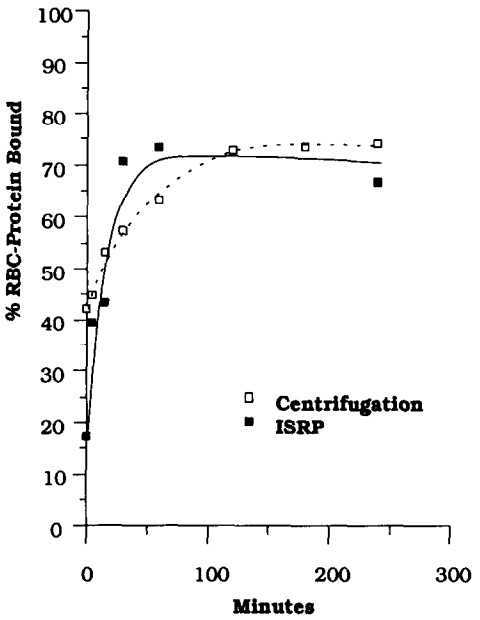

Fig. 4. Comparison of the ISRP method (□) with centrifugation (■) for ^{99m}TcCl(DMG)$_3$BMe (n = 2). These results are similar to those for ^{99m}TcCl(CDO)$_3$BMe with good agreement in the equilibrium binding.

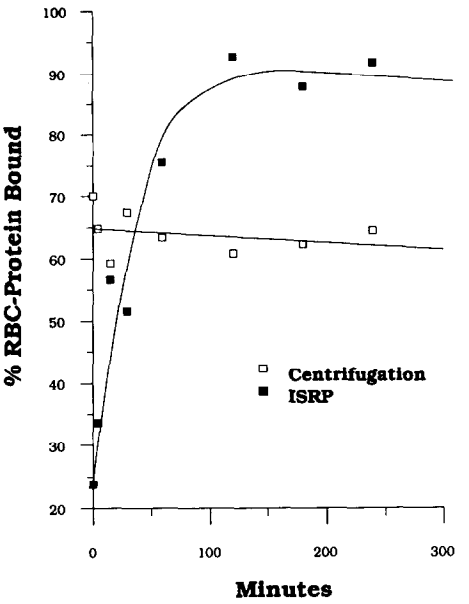

Fig. 5. Comparison of the ISRP (□) and centrifugation (■) methods for ^{99m}TcCl(DMG)$_3$B2MP (n = 1). The equilibrium binding value by centrifugation is lower (*ca.* 60%) than the value obtained from the HPLC method (*ca.* 90%). This may indicate that the centrifugation method disturbs the RBC–protein binding more than the HPLC method for this agent.

centrifugation method providing a lower value (62%) than the HPLC method (90%).

DISCUSSION

The 75-μm ISRP column used in conjunction with a reversed-phase column was found to separate bound from free drug under the conditions used. The method is simple and easy to use as no sample processing is required. The LRBCs remained intact on passage through the ISRP column in isotonic buffer. Thus, the method is rapid with minimal disruption of the equilibrium binding to the RBCs.

Typical chromatograms showing good separation of the bound (RBCs and protein) and free drug (^{99m}TcCl(CDO)$_3$BMe and ^{99m}TcOH (CDO)$_3$BMe) are seen in Fig. 2. These separations were obtained using the ISRP column with the Nucleosil C_8 column. Under these conditions, the lipophilic compounds elute from the ISRP around 4 min. Hydrophilic compounds such as ^{99m}Tc-pertechnetate coelute with the

bound peak. Increasing the column length might increase the resolution between the bound and hydrophilic agents, however, it has been shown that increasing the ISRP column length may remove weakly bound ^{99m}Tc-labeled species from serum proteins [19,25,26]. Thus a short column is required to minimize perturbations of the bound–free equilibrium. When analyzing other drugs, alterations of the second column and eluent may be used to modify the separation of the free components. However, because the ISRP column must use an aqueous mobile phase with organic modifier, the secondary column should be a reversed-phase column or other support, such as ion exchange, which is compatible with an aqueous mobile phase.

In pharmacokinetic studies, knowledge of the changes in blood concentration of drug and metabolites are essential. Analyses ignoring the bound fraction can, at best, be misleading, and could give rise to large errors. As an example in this study, analyses of the relative amounts of free chloro BATO and hydroxy BATO [^{99m}TcX(CDO)$_3$BMe] on incubation in whole blood suggest that the ^{99m}TcCl(CDO)$_3$BMe concentration is decreasing with time and the ^{99m}TcOH(CDO)$_3$BMe concentration is increasing over time. By also taking into account the percentage bound using the ISRP–Nucleosil method, it can be seen in Fig. 2 that the concentration of free ^{99m}TcOH(CDO)$_3$BMe changes very little with time, and loss of free ^{99m}TcCl(CDO)$_3$BMe results from increased cell–protein binding. Thus, the more complete analysis afforded by the ISRP method allows for a more accurate interpretation of data. The nature of the species bound to RBCs and protein cannot be determined by this method, but from a related study [27] it can be assumed that the change in percentage binding with time is associated in part with the metabolism of the complexes.

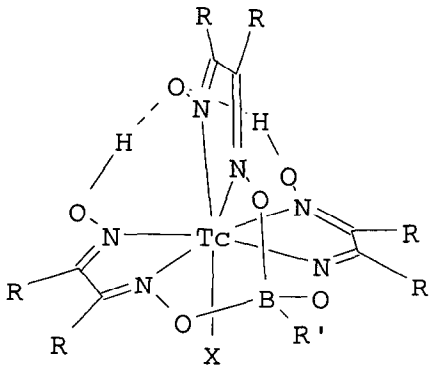

Fig. 6. General structure for ^{99m}Tc-BATOs. R = CH_3 for DMG; R = $(CH_2)_2$ for CDO; R′ = CH_3 for BMe; R′ = $CH_2CH(CH_3)_2$ for B2MP. X = Cl or OH.

The RBC–protein equilibrium binding values compared favorably between the ISRP and centrifugation methods for ^{99m}TcCl(CDO)$_3$BMe and ^{99m}TcCl(DMG)$_3$BMe. However, the equilibrium binding values for ^{99m}TcCl(DMG)$_3$B2MP between the two methods were significantly different ($p < 0.01$) (Fig. 5). While it is understandable that each agent has a different equilibrium binding value, it was not anticipated that the binding value would be so different between methods as was observed for ^{99m}TcCl(DMG)$_3$B2MP. This finding could not be related to lipophilicity since the log P for ^{99m}TcCl(DMG)$_3$B2MP is intermediate to that of ^{99m}TcCl(CDO)$_3$BMe and ^{99m}TcCl(DMG)$_3$BMe. The difference in binding values for ^{99m}TcCl(DMG)$_3$B2MP may be related to differences in structure. Fig. 6 shows the structures of the ^{99m}Tc-BATOs. The ^{99m}Tc-BATOs studied have two structural differences in either the oxime or boronic acid groups. The oxime group is either cyclohexanedione dioxime (CDO) or dimethylglyoxime (DMG) and the boronic acid has either a methyl (BMe) or 2-methylpropyl (B2MP) substituent. This branched-chain boronic acid has a greater potential to interact with the hydrophobic regions of proteins than does one with a methyl group. Such weak binding may be more sensitive to alteration during the centrifugation process. It has been reported that dilution of plasma or blood samples has been shown to disrupt the equilibrium between bound and free for some drugs [1], such as may occur during the washing of the RBCs labeled from the ^{99m}Tc-BATOs.

For all three agents the centrifugation method provides an essentially constant equilibrium binding value at all times, whereas the ISRP method shows a time dependence of drug binding to RBCs and protein. The equilibrium binding value is actually a condition-dependent equilibrium binding value, as noted for ^{99m}TcCl(DMG)$_3$B2MP. The centrifugation method re-

quires ~25 min during which time additional RBC–protein binding may take place. The ISRP method which rapidly separates bound and free drug components prevents additional binding and thus gives a more realistic value especially at early times. Accurate data on binding is important in pharmacokinetic studies. Correction of the arterial input function for pharmacokinetic analysis is important especially for drugs which have a high first-pass organ extraction and rapid blood clearance, such as $^{99m}TcCl(CDO)_3BMe$ and $^{99m}TcCl(DMG)_3B2MP$ [16].

This novel HPLC method provides a rapid, convenient and reliable method for the analysis of radioactive and non-radioactive lipophilic compounds added to whole blood without the need for prior separation methods. Our studies indicate that equilibria between bound and free forms are not greatly disturbed by the ISRP method compared to a centrifugation method. This simple and rapid HPLC analysis method allows time-dependent studies without alteration of delicate equilibria. Variation in the secondary analytical column, solvents and detection system should allow this system to be used in many drug metabolism studies.

ACKNOWLEDGEMENTS

We gratefully acknowledge the technical assistance of Lillian Belnavis and Christine Hood and the help in data analysis by Bruce Kuczynski and Al Bauer. In addition, we thank the Regis Chemical Co. (Morton, IL, USA) for their generosity in supplying the 75-μm ISRP columns.

REFERENCES

1 G. W. Peng and W. L. Chiou, *J. Chromatogr.*, 531 (1990) 73.
2 R. D. McDowall, E. Doyle, G. S. Murkitt and V. S. Picot, *J. Pharm. Biomed. Anal.*, 7 (1989) 1087.
3 B. Sebille, R. Zini, C. V. Madjar, N. Thuaud and J. P. Tillement, *J. Chromatogr.*, 531 (1990) 51.
4 M. J. Koenigbauer, *J. Chromatogr.*, 531 (1990) 79.
5 O. Jeghers, A. Piepsz and H. R. Ham, *Eur. J. Nucl. Med.*, 17 (1990) 101.
6 S. H. Y. Wong, *J. Pharm. Biomed. Anal.*, 7 (1989) 1011.
7 G. Tamai, H. Yoshida and H. Imai, *J. Chromatogr.*, 423 (1987) 147.
8 D. J. Gisch, B. T. Hunter and B. Feibush, *J. Chromatogr.*, 433 (1988) 264.
9 H. Hagestam and T. C. Pinkerton, *Anal. Chem.*, 57 (1985) 1757.
10 Z. K. Shihabi, *J. Liq. Chromatogr.*, 11 (1988) 1579.
11 D. E. Williams and P. M. Kabra, *Anal. Chem.*, 62 (1990) 807.
12 T. C. Pinkerton, *J. Chromatogr.*, 544 (1991) 13.
13 J. Haginaka, *Trends Anal. Chem.*, 10 (1991) 17.
14 A. B. McNaught and R. Callander, *Illustrated Physiology*, Churchill Livingstone, Edinburgh, 1975.
15 R. K. Narra, A. D. Nunn, B. L. Kuczynski, T. Feld, P. Wedeking and W. C. Eckelman, *J. Nucl. Med.*, 30 (1989) 1830.
16 R. K. Narra, A. D. Nunn, B. L. Kuczynski, R. J. Di Rocco, T. Feld, D. A. Silva and W. C. Eckelman, *J. Nucl. Med.*, 31 (1990) 1370.
17 D. W. Seldin, L. L. Johnson, D. K. Blood, M. J. Muschel, K. F. Smith, R. M. Wall and P. J. Cannon, *J. Nucl. Med.*, 30 (1989) 312.
18 J. S. Zielonka, P. Cannon, L. Johnson, D. Seldin, R. L. Bellinger, E. Chua, R. E. Coleman, R. C. Reba, A. Wasserman, W. Drane, C. Williams, M. L. Goris, J. A. Leppo, R. Aktar, V. S. Bamrah and A. J. Krubsack, *Nuklearmedizin*, Suppl. 26 (1990) 211.
19 D. P. Nowotnik, W. Hirth, S. Jurisson, K. E. Linder, R. K. Narra, A. D. Nunn and W. C. Eckelman, in M. Nicolini, G. Bandoli and U. Mazzi (Editors) *Technetium and Rhenium in Chemistry and Nuclear Medicine 3*, Cortina International, Verona, 1990, p. 393.
20 S. C. Srivastava and R. F. Straub, in D. J. Maddalena, G. M. Snowdon and G. R. Boniface (Editors), *Advances in Radiopharmacology*, International Association of Radiopharmacology, Wallongong, 1990, Ch. 1, p. 4.
21 S. Jurisson, W. Hirth, K. Linder, R. J. Di Rocco, R. K. Narra, D. P. Nowotnik and A. D. Nunn, *Nucl. Med. Biol.*, 18 (1991) 735.
22 *5 μm ISRP Column Package Insert*, Regis, Morton Grove, IL.
23 T. Feld and A. D. Nunn, *J. Labelled Compd. Radiopharm.*, 26 (1989) 274.
24 T. C. Pinkerton and K. A. Koeplinger, *J. Chromatogr.*, 458 (1988) 129.
25 L. L. Weyman and E. Deutsch, *Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Atlanta, GA, March 6–10, 1989.*
26 T. C. Pinkerton, T. D. Miller and L. J. Janis, *Anal. Chem.*, 61 (1989) 1171.
27 W. L. Rumsey, K. C. Rosenspire and A. D. Nunn, *J. Nucl. Med.*, (1992) in press.