Journal of Chromatography, 620 (1993) 278–280
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 7083

Short Communication

Simple direct injection high-performance liquid chromatographic method to determine quinidine in plasma

Jiu Li Huang and Denis J. Morgan*

Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville, Melbourne, Vic. 3052 (Australia)

(First received March 12th, 1993; revised manuscript received August 4th, 1993)

ABSTRACT

High-performance liquid chromatographic methods that use direct injection of plasma include column-switching procedures, modified mobile phases and small-pore modified stationary phases. By using a large-pore (300 Å) Selectosil C_{18} column, developed for the analysis of macromolecules, we have shown that quinidine in plasma and protein solutions can be assayed accurately and rapidly by directly injecting 2 μl plasma or protein solution onto the column. Column life is not reduced, and the limit of quantitation is 0.01 μM.

INTRODUCTION

To shorten and simplify sample handling, a number of different methods have been proposed for direct sample injection for the high-performance liquid chromatographic (HPLC) analysis of drugs in plasma and other biological fluids. These methods, which avoid column clogging by proteins and the consequent deterioration in chromatographic performance, have been summarized in a recent review [1]. They include the use of micellar mobile phases that solubilize the proteins following direct injection on standard columns [2,3]. Precolumn or two-column techniques have been used, which include a variety of column-switching methods that divert the proteins and other undesired compounds from the analytical column [4–7]. Other methods have used stationary phases designed to elute proteins in the void volume while retaining and separating small drug molecules. These include a C_{18} reversed-phase bonded to a small-pore silica support, in which denatured plasma proteins have been precipitated on the C_{18} phase [8]. There is the so-called internal-surface reversed-phase packing, where the outer surface of the particle is hydrophilic and non-adsorptive to proteins, whereas the inner surface is hydrophobic. The pore size is kept small so that large proteins are excluded and elute in the void volume [9]. Another packing material that excludes proteins is the shielded hydrophilic phase. This is a polymeric bonded phase containing hydrophobic regions enclosed by a hydrophilic network that small analytes can penetrate while the larger proteins are excluded [10].

* Corresponding author.

0378-4347/93/$06.00

Numerous HPLC methods have been reported for the assay of quinidine in plasma. These methods use either of the two conventional methods of sample preparation prior to chromatography. One method of sample preparation involves extraction of plasma with an organic solvent, followed either by back-extraction into an aqueous phase [11] or evaporation of the solvent and reconstitution of the residue in mobile phase [12–14]. The other method involves precipitation of plasma proteins with a precipitating agent, such as methanol, followed by centrifugation [15,16]. The latter "direct injection" method is superior to the former method in terms of speed and convenience.

This paper describes the use of a large-pore (300 Å) C_{18} column (Selectosil) for analysis of drugs in plasma and other protein solutions. This column packing was developed for the analysis of protein solutions, but we show, with the cardiac drug quinidine, that this column packing can also be used for the analysis of drugs by direct injection of plasma.

EXPERIMENTAL

Quinidine sulphate and dihydroquinidine hydrochloride were obtained from Sigma (St. Louis, MO, USA), 3-hydroxyquinidine was a gift from Dr. M. S. Ching (Department of Medicine, University of Melbourne, Victoria, Australia) and quinidine N-oxide was a gift from Professor G. R. Wilkinson (Department of Pharmacology, Vanderbilt University, TN, USA).

A standard C_{18} precolumn was used (ODS, 10 μm, 15 mm × 3.2 mm I.D.; New Guard, Applied Biosystems, Foster City, CA, USA). The analytical column was a large-pore (300 Å) C_{18} column (Selectosil ODS, 10 μm, 250 mm × 4.6 mm I.D.; Phenomenex, Torrance, CA, USA). Plasma or protein solution (2 μl) was injected directly via a loop injector (Model 7125, Rheodyne, Cotati, CA, USA). The mobile phase was water–acetonitrile–triethylamine–orthophosphoric acid (85.4:13:1:0.6, v/v/v/v) (pH 3.0), pumped at 1.0 ml/min by a Waters Model 510 pump (Waters, Milford, MA, USA). The detector was a fluorescence spectrophotometer (Model LS-3, Perkin Elmer, Norwalk, CT, USA) set at excitation 250 nm and emission 430 nm. Peak heights were recorded by a chart recorder (Omniscribe, Houston Instruments, Austin, TX, USA).

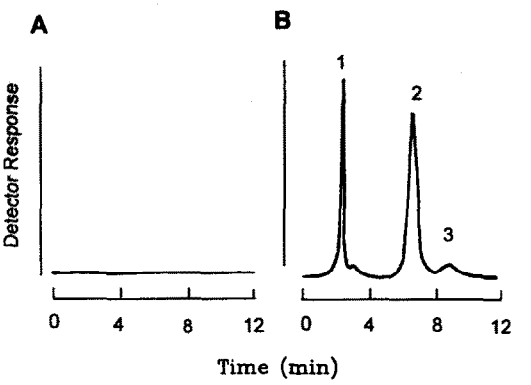

Fig. 1. Chromatograms obtained following direct injection of 2 μl of horse plasma on the Selectosil column: (A) blank; (B) 5 h following quinidine administration, showing 3-hydroxyquinidine (1), quinidine (2.8 μg/ml) (2), and dihydroquinidine (3).

RESULTS AND DISCUSSION

Typical chromatograms from the analysis of horse plasma subjected to this procedure are shown in Fig. 1. The chromatogram of blank plasma shows no interference because of the selectivity of the fluorescence detector (Fig. 1A). However, use of a UV detector (250 nm) showed that, under these experimental conditions, the proteins eluted in the void volume. On this system, the quinidine metabolites 3-hydroxyquinidine and quinidine N-oxide elute well before quinidine, and dihydroquinidine, an impurity of quinidine, elutes well after quinidine. Peaks for 3-hydroxyquinidine and dihydroquinidine are evident in Fig. 1B, which is a chromatogram of plasma from a horse following administration of quinidine.

Calibration curves for plasma and albumin solutions were constructed over the range 0.2–17 μ*M*. A typical calibration curve was $y = 0.102 + 31.2x$ ($r^2 = 0.999$). In a solution of bovine serum albumin (60 g/l) the intra-assay coefficient of variation ($n = 6$) was 2.0%, 0.7% and 1.3% at

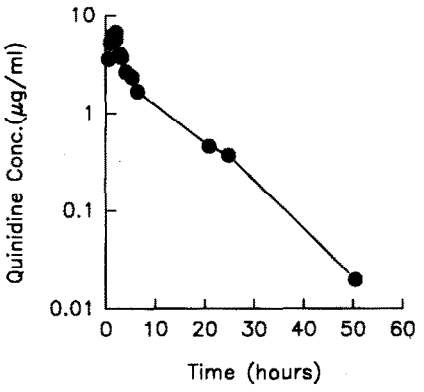

Fig. 2. Plasma quinidine concentrations following i.v. injection of 300 mg in a horse.

quinidine concentrations of 0.2, 1 and 100 μM, respectively. The minimum quantifiable concentration was 0.01 μM (3 ng/ml) (signal-to-noise ratio greater than 4:1).

We used this method to rapidly assay quinidine in the perfusate output of an isolated perfused rat heart preparation, in which the perfusate contained quinidine in bovine serum albumin (1–60 g/l) or α_1-acidglycoprotein (0.1–1.5 g/l). The method is so rapid and simple that the perfusate quinidine concentration can be available within a few minutes of collection. This enables the perfusate quinidine concentration profile to be monitored during the experiment.

We also used this method to assay quinidine in horse plasma following intravenous quinidine injection, and an example is shown in Fig. 2. So far, we have assayed more than 1000 plasma and protein samples with no evidence of column deterioration, in contrast to our experience with a conventional C_{18} column. We used a conventional precolumn with the Selectosil analytical column, and this precolumn has lasted for at least 1000 samples. We have also used a precolumn packed with the Selectosil C_{18} 10 μm, 100 Å pore size packing. As this performed no better than the conventional precolumn and was much more expensive, we preferred to use the conventional precolumn.

In conclusion, the Selectosil column presents a convenient and readily available alternative direct injection method for the analysis of drugs, such as quinidine, in plasma and protein solutions.

ACKNOWLEDGEMENTS

We thank Ms Cao Ling Xu for technical assistance. This work was supported by Pharmasearch Ltd, and Jiu Li Huang was supported by a University of Melbourne Postgraduate Scholarship and an Australian Government Overseas Postgraduate Research Scholarship.

REFERENCES

1 R. D. McDowall, *J. Chromatogr.*, 492 (1989) 3.
2 F. D. DeLuccia, M. Arunyanart and L. J. Cline Love, *Anal. Chem.*, 57 (1985) 1564.
3 Y.-N. Kim and P. R. Brown, *J. Chromatogr.*, 384 (1987) 209.
4 W. Roth, K. Beschke, R. Jauch, A. Zimmer and F. W. Koss, *J. Chromatogr.*, 222 (1981) 475.
5 W. Voelter, Th. Kronbach, K. Zech and R. Huber, *J. Chromatogr.*, 239 (1982) 475.
6 R. Huber, K. Zech, M. Worg, Th. Kronbach and W. Voelter, *Chromatographia*, 16 (1982) 233.
7 A. Nazareth, L. Jaramillo, B. L. Karger, R. W. Giese and L. R. Snyder, *J. Chromatogr.*, 309 (1984) 357.
8 H. Yoshida, I. Morita, T. Masujima and H. Imai, *Chem. Pharm. Bull.*, 30 (1982) 2287.
9 I. H. Hagestam and T. C. Pinkerton, *Anal. Chem.*, 57 (1985) 1757.
10 D. J. Gisch, B. T. Hunter and B. Feibush, *J. Chromatogr.*, 433 (1988) 264.
11 G. R. Gotelli and J. H. Wall, in P. M. Kabra and L. J. Marton (Editors), *Clinical Liquid Chromatography*, CRC Press, Boca Raton, FL, Vol. I, 1984, p. 57.
12 T. W. Guentert, in P. M. Kabra and L. J. Marton (Editors), CRC Press, Boca Raton, FL, Vol. I, 1984, p. 63.
13 G. W. Mihaly, K. M. Hyman, R. A. Smallwood and K. J. Hardy, *J. Chromatogr.*, 415 (1987) 177.
14 J. J. MacKichan and B. J. Shields, *Ther. Drug Monitor.*, 9 (1987) 104.
15 T. W. Guentert and S. Øie, *J. Pharmacol. Exp. Ther.*, 215 (1980) 165.
16 R. Leroyer, C. Jarreau and M. Pays, *J. Chromatogr.*, 228 (1982) 366.