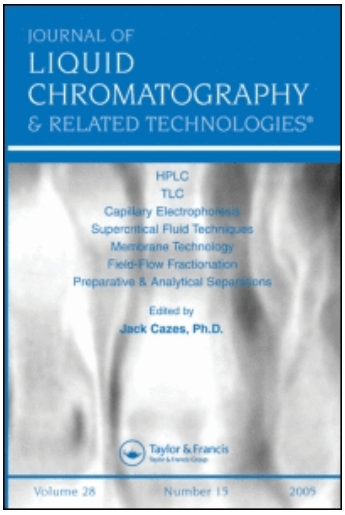
This article was downloaded by:
On: *25 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Rapid Isocratic HPLC Assay for Dipyridamole Using a Microbore Column Technique

A. R. Zoest[a]; J. E. Watson[a]; C. T. Hung[a]; S. Wanwimolruk[b]
[a] Zenith Technology Co. Ltd., Dunedin, New Zealand [b] School of Pharmacy University of Otago, Dunedin, New Zealand

To cite this Article Zoest, A. R. , Watson, J. E. , Hung, C. T. and Wanwimolruk, S.(1991) 'A Rapid Isocratic HPLC Assay for Dipyridamole Using a Microbore Column Technique', Journal of Liquid Chromatography & Related Technologies, 14: 10, 1967 — 1975
To link to this Article: DOI: 10.1080/01483919108049666
URL: http://dx.doi.org/10.1080/01483919108049666

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(10), 1967-1975 (1991)

A RAPID ISOCRATIC HPLC ASSAY FOR DIPYRIDAMOLE USING A MICROBORE COLUMN TECHNIQUE

A. R. ZOEST[1], S. WANWIMOLRUK[2]*,
J. E. WATSON[1], AND C. T. HUNG[1]
[1]*Zenith Technology Co. Ltd.*
[2]*School of Pharmacy*
University of Otago
Dunedin, New Zealand

Downloaded At: 10:04 25 January 2011

ABSTRACT

A simple, rapid and sensitive assay for the quantitation of dipyridamole in human plasma has been developed using a reversed-phase C18 microbore HPLC column. Dipyridamole and the internal standard (propranolol) were separated from the plasma by reversed-phase liquid chromatography following a direct plasma protein precipitation. For sample analysis 50 µl of the internal standard (propranolol 125 µg/ml) and 50 µl of perchloric acid (60% w/v) were added to the 500 µl of plasma. After brief vortex and centrifugation, the supernatant (100 µl) was injected onto the column. Chromatography was carried on a 5 µm ODS Hypersil C18 microbore column (2 mm I.D. x 10 cm) using an acetonitrile-water (48:52, v/v) mobile phase containing 20 mM Na_2HPO_4 and 50 mM sodium dodecyl sulfate adjusted to pH 2. The eluant was monitored at 305 nm. The run time for a plasma sample was less than 12 min. The intra- and inter-assay

*Address for correspondence: Dr. Sompon Wanwimolruk, School of Pharmacy, University of Otago, P.O. Box 913, Dunedin, New Zealand.

Copyright © 1991 by Marcel Dekker, Inc.

coefficients of variation were less than 9%. The lowest limit of detection for dipyridamole was 0.05 μg/ml (50 ng/ml). The specificity, sensitivity and reproducibility of the procedure are adequate and suitable for the clinical pharmacokinetic, bioavailability and therapeutic drug monitoring studies.

INTRODUCTION

Dipyridamole, a vasodilating and antiplatelet agent, has been widely used for the treatment of chronic angina pectoris and in conditions where modification of platelet function may be beneficial. Previous studies (1-3) have indicated that rational therapy with dipyridamole requires maintenance of plasma concentrations above a certain threshold level. For pharmacokinetic studies and therapeutic drug monitoring of dipyridamole, it is essential that a simple, rapid and specific method for the analysis of this drug be developed. Four high-performance liquid chromatographic (HPLC) assays have been reported for the determination of dipyridamole in plasma or blood (4-7). These former procedures were rather complicated and time-consuming as solvent extraction procedures were required before the chromatographic separation.

The present report describes a rapid, simple, sensitive and specific HPLC assay for the determination of dipyridamole in human plasma. This method involves a simple plasma protein precipitation procedure, requires no extraction steps and employs an internal standard.

Downloaded At: 10:04 25 January 2011

MATERIALS AND METHODS

Reagents and Chemicals

All chemicals were of analytical grade. Dipyridamole was kindly supplied by Douglas Pharmaceuticals Ltd, Auckland, New Zealand. The internal standard, propranolol hydrochloride was obtained from Pacific Pharmaceuticals Ltd (Auckland, New Zealand). HPLC-grade

acetonitrile and perchloric acid were purchased from BDH Ltd (Poole, UK). Sodium dodecyl sulfate was obtained from Sigma Chemicals Co (St Louis, MO, USA). Water was double glass distilled and MilliQ® filtered.

Standard Solutions and Internal Standard

A stock solution containing 100 μg/ml of dipyridamole was prepared in methanol. The internal standard stock solution of propranolol hydrochloride (125 μg/ml) was prepared in 0.05 M HCl. Plasma standard solutions of dipyridamole for the calibration curves were prepared by appropriate dilution of the stock dipyridamole solutions with drug-free plasma so that concentrations of 0.05, 0.1, 0.2, 0.5, 1, 2 and 5 μg/ml were obtained. The plasma standards and the internal standard solution were freshly prepared each day of analysis.

Downloaded At: 10:04 25 January 2011

Analysis Procedure

A protein precipitation technique was used in the preparation of plasma standards and samples. To 0.5 ml of plasma sample or standard in a 1.5 ml plastic Eppendorf microcentrifuge tube, 50 μl of the internal standard solution (125 μg/ml propranolol HCl) and 50 μl of perchloric acid (60% w/v) were added. The contents were then vortexed for 10 seconds and centrifuged at 2000 g for 5 minutes. The clear supernatant was then transferred to a small plastic vial in the autoinjector and 100 μl of this solution was injected onto the HPLC column. The concentrations of dipyridamole in samples were determined from calibration plots of the chromatographic peak height ratios (dipyridamole/internal standard) versus dipyridamole concentration.

Chromatographic Conditions

The HPLC system consisted of an LKB 2150 pump (LKB, Stockholm, Sweden) connected to a Waters 712B (Milford, MA, USA) with samples stored at 4°C until injection. The detector used was a Linear UV is 200 spectrophotometer (Linear Instruments Corp., Nevada, USA) operated at 305 nm. The chromatographic response was recorded by a Shimadzu R3A integrator (Tokyo, Japan). A microbore HPLC column (2 mm I.D.

x 10 cm) packed with a reversed-phase C18 material, 5 μm ODS Hypersil (Shandon, London, UK) was used. Analysis of the samples of dipyridamole was performed using a mobile phase consisting of an acetonitrile-water mixture (48:52, v/v) containing 20 mM Na_2HPO_4 and 50 mM sodium dedecyl sulfate (SDS) adjusted to pH 2 with orthophosphoric acid. The flow rate of the mobile phase was 0.5 ml/min (back pressure approximately 100 bars). Chromatographic separations were performed at room temperature.

RESULTS AND DISCUSSION

Downloaded At: 10:04 25 January 2011

Dipyridamole is a base with pKa of 6.4 and is ionised in the acidic and neutral solvents commonly used in reversed-phase HPLC. In the present study, retention of dipyridamole was achieved by the addition of the anionic pairing ion sodium dodecyl sulfate (SDS), to the mobile phase at low pH. The variation of the capacity factor (k') of dipyridamole and the internal standard (propranolol) as a function of the mobile phase SDS concentration is illustrated in Figure 1. Both cationic solutes got through the predicted maxima. Such chromatographic behaviour can be explained by the ion-exchange desolvation mechanism (8). A mobile phase of an acetonitrile-water mixture (48:52, v/v) containing 20 mM Na_2HPO_4 and 50 mM SDS adjusted to pH 2 was chosen as it provides a good resolution between dipyridamole and the internal standard.

Figure 2 shows chromatograms of blank plasma, plasma spiked with 0.05 μg/ml (i.e., 50 ng/ml) of dipyridamole and a typical subject's plasma chromatogram 9 hours post dose. Under these chromatographic conditions, no endogenous sources of interference were observed and the resolution between dipyridamole and the internal standard was satisfactory. Blank plasma samples obtained from more than 25 volunteers were analysed and no plasma endogenous peaks co-eluting with dipyridamole and the internal standard were detected. The retention times for dipyridamole and the internal standard (propanolol) were 3.2 and 7.6 minutes, respectively.

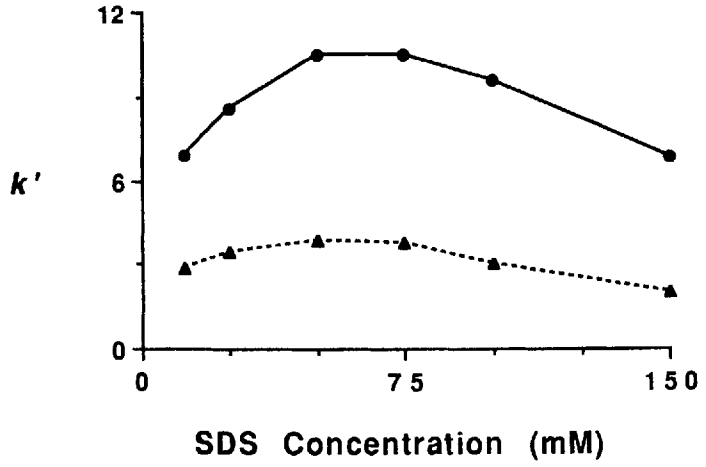


FIGURE 1

Variation in capacity factor (k') of dipyridamole (▲·····▲) and the internal standard propranolol (●——●) as a function of sodium dodecyl sulfate (SDS) concentration. The mobile phase was 48:52 (v/v) of acetonitrile-water containing 20 mM Na_2HPO_4; pH 2 and the flow rate was 0.5 ml/min.

Downloaded At: 10:04 25 January 2011

Absolute recovery of dipyridamole and the internal standard from plasma was assessed by comparing the peaks of the drugs in plasma samples with those obtained by direct injection of the pure drug standards. The mean recovery for dipyridamole (n=5) from plasma sample was 64.4 ± 1.6% (S.D.) at 0.5 μg/ml, 61.7 ± 1.8% at 1 μg/ml and 60.0 ± 0.7 at 2 μg/ml. The absolute recovery for the internal standard was 47 ± 2% (n=5).

The calibration curve for dipyridamole was linear over the concentration range of 0.05 to 5 μg/ml with the coefficient of determination (r^2) greater than 0.99. The typical linear relationship for the calibration curve can be expressed by the equation: y (peak height ratio) = 1.1052 x (concentration). The intercept (a) in all calibration curves were found to be statistically insignificant ($p > 0.05$) and were

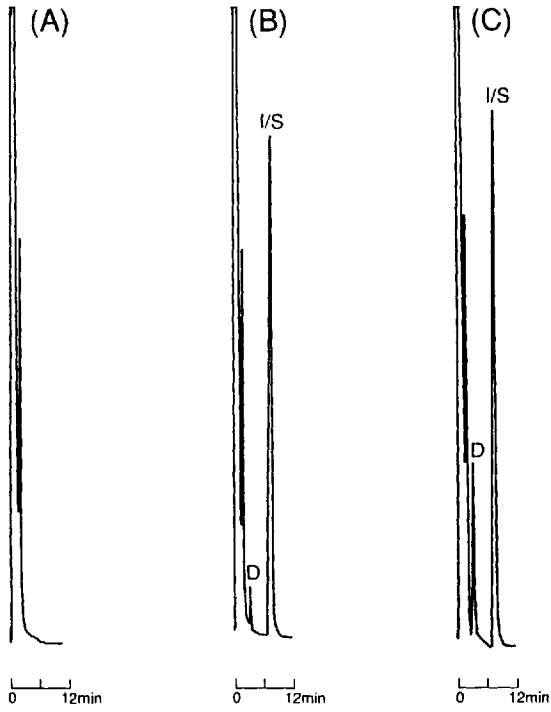


FIGURE 2

Typical chromatograms of human plasma : (A) blank plasma; (B) plasma spiked with 0.05 μg/ml dipyridamole and the internal standard; and (C) plasma with 0.21 μg/ml dipyridamole taken from a healthy subject 9 hours on day 6 after multiple doses of 150 mg dipyridamole slow-release (Persantin PL 150 mg capsule, Boehringer Ingelheim) twice a day for 5 days.
Peaks: D = Dipyridamole; I/S = Internal standard (propranolol)

thus not included for the calculations. The day-to-day coefficient of variation (C.V.) of the slope of the calibration curves of dipyridamole was 4.7% (n=5).

The reproducibility and precision of the assay together with the C.V. is shown in Table 1. At all concentrations studied the C.V. was less than 9%. The results indicate good precision of the assay. The

Downloaded At: 10:04 25 January 2011

TABLE 1

Within-day Reproducibility and Precision of the Assay for Dipyridamole in Plasma

Spiked Concentration (μg/ml)	n	Observed Concentration[1] (μg/ml)	C.V. (%)	Accuracy[2] (%)
0.05	5	0.045 ± 0.004	8.9	90.0
0.2	6	0.191 ± 0.008	4.2	95.5
1	6	1.012 ± 0.015	1.5	101.2
5	5	5.06 ± 0.19	3.8	101.2

1. Results given are mean ± S.D.
2. Accuracy (%) = $\frac{\text{Observed concentration}}{\text{Spiked concentration}} \times 100$

Downloaded At: 10:04 25 January 2011

measured value of five 0.05 μg/ml plasma dipyridamole standard gave values of 0.049, 0.042, 0.047, 0.048 and 0.040 μg/ml. The data gives a mean and S.D. of 0.045 ± 0.004 μg/ml. The C.V. of the assay at this concentration was 8.9% (with accuracy of 90%), which are lower than the generally accepted limit of 20% deviation from the mean for minimum quantifiable concentration (MQC). A typical chromatogram of 0.05 μg/ml plasma dipyridamole standard is shown in Figure 1(B). Thus, the MQC or the detection limit of sensitivity for this assay was assigned at 0.05 μg/ml (i.e., 50 ng/ml).

Plasma samples stored at -70°C for up to 3 months showed no signs of decomposition and practically the same concentration values were obtained (n=5). This suggests that dipyridamole is stable under these storage conditions for at least 3 months.

In addition, the sensitivity of this assay can be increased for the studies that are required to measure concentrations lower than 0.05 μg/ml. This could be done by using a fluorescence detector at an excitation wavelength of 293 nm and emission wavelength of 468 nm.

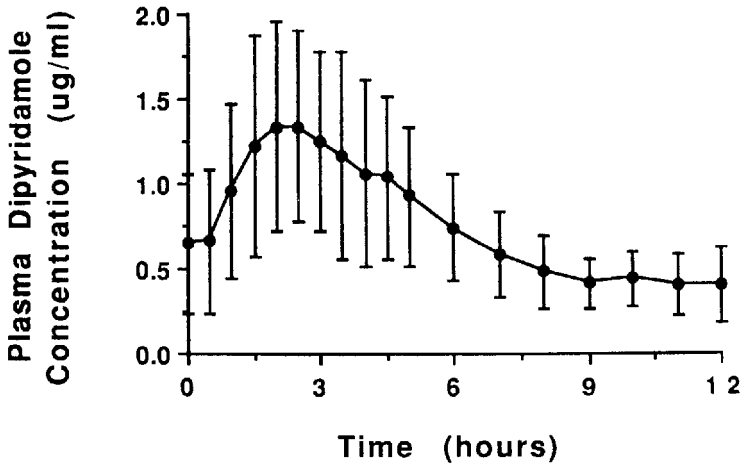


FIGURE 3

Mean plasma concentrations of dipyridamole versus time profile in 14 healthy subjects on day 6 after an oral administration of 150 mg dipyridamole slow-release capsule (Persantin PL). The subjects received multiple doses of dipyridamole 150 mg twice a day for 5 days. The vertical bars represent the standard deviation of the mean plasma concentrations.

Downloaded At: 10:04 25 January 2011

We found that there was no endogenous sources of interference from plasma and the detection limit of the assay can be assigned at 1 ng/ml.

The present HPLC method has been applied to determine the plasma dipyridamole concentrations in a bioavailability study. More than 600 plasma samples from healthy subjects participating in the bioavailability study were analysed by this procedure. The mean (± S.D.) plasma dipyridamole concentration-time profile observed in 14 subjects on day 6 after multiple doses of 150 mg dipyridamole twice a day for 5 days is shown in Figure 3. The mean peak plasma concentration was 1.55 ± 0.62 μg/ml. The mean area under the plasma concentration-time curve (AUC) was 9.01 μg.h/ml.

In summary, the present HPLC method is sufficiently simple, sensitive and rapid to permit the analysis of plasma sample of 100

samples per day as the sample preparation is simple, required no extraction and the run time per sample is less than 12 min. The assay was proved to be suitable for the routine analysis in clinical and pharmacokinetic studies.

REFERENCES

1. Tyce, G.M., Fuster, V. and Owen, C.A. Res. Commun. Chem. Pathol. Pharmacol. **16**, 495, 1979.

2. Mahoney, C., Wolfram, K.M. and Bjornsson, T.D. Clin. Res. **28**, 240A, 1980.

3. Rajah, S.M., Crow, K.M., Penney, A.F., Ahmad, R. and Watson, D.A. Brit. J. Clin. Pharmacol. 4, 129, 1977.

4. Pedersen, A. J. Chromatogr. **162**, 98, 1979.

5. Schmid, J., Beschke, K., Roth, W., Bozler, G. and Koss, F.W. J. Chromatogr. **163**, 239, 1979.

6. Wolfram, K.M. and Bjornsson, T.D. J. Chromatogr. **183**, 57, 1980.

7. Rosenfeld J., Devereaux D., Buchanan, M.R. and Turpie, A.G.G. J. Chromatogr. **231**, 216, 1982.

8. Hung, C.T. and Taylor R.B. J. Chromatogr. **209**, 175, 1981.

Downloaded At: 10:04 25 January 2011