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High Performance Liquid Chromatographic Method for Determination of Dipyridamole in Human Plasma

DAVOOD BEIGI BANDARABADI, MORTEZA PIRALI HAMEDANI, MOHSEN AMINI, and ABBAS SHAFIEE.
Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, PO Box 14155/6451, Tehran, Iran

ABSTRACT

A simple, rapid and specific high-performance liquid chromatographic procedure is reported for quantitative determination of dipyridamole in human plasma. The assay uses a reversed-phase high-performance liquid chromatographic (HPLC) and UV detection at 280nm and has a limit of detection of approximately 5ng/mL. The mobile phase consists of MeOH-H_2O (60:40) adjusted to pH 3.3. Dipyridamole was extracted from plasma by back-extraction procedure, with propranolol as the internal standard. The reproducibility of the method is satisfactory.

Key words: HPLC, Dipyridamole, Human Plasma, UV Detection.

INTRODUCTION

Dipyridamole is widely used as; a coronary vasodilator in patients with high blood pressure, a prophylactic agent in patients with angina pectoris and an inhibitor of platelet aggregation in various thromboembolic conditions (1,2). However, the drug is known to have a delayed absorption pattern and a variation in bioavailability (3-5). Therefore, a simple and rapid method is necessary for the determination of dipyridamole in biological fluids and in various pharmaceutical formulations. Several methods have been reported for the analysis of dipyridamole in biological samples(6-9). Spectroflurometric procedures (6,7) have a drawback interference from plasma component and other fluorophores; furthermore, the required extraction procedures are tedious and complicated.

The chromatographic procedures based on fluorometric detection (8,9) suffer from the disadvantage of a limited linear dynamic range which complicates methods by requiring varying amounts of plasma. Furthermore, dipyridamole has endogenous fluorescence only at alkaline pH which can cause considerable difficulties with HPLC analysis due to instability of support at high pH (8). HPLC with UV detection has a wide linear dynamic range and dose not require alkaline pH. One HPLC method (9) with UV detection uses two different procedures for sample preparation i.e., ethanol protein precipitation for samples of expected concentrations higher than 0.1 µg/ml, and solvent extraction of a buffered sample with diethylether followed by partial evaporation and back-extraction into acid solutions of expected low concentrations. The use of two different procedures for sample preparation makes the method complcated, inconvenient and relatively time consuming and finally the method is externally calibrated

EXPERIMENTAL

Reagents:

Dipyridamole was supplied by Boehringer Inglheim (Ridgefield, CT, U.S.A) and the

Correspondence : M. Pirali Hamedani, Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, PO Box: 14155/6451, Iran

internal standard, propranolol was from Tolidarou (Tehran, Iran). Methanol, ethanol, diethylether, n-hexane and tris (hydroxymethyl)aminomethane (Tris buffer) were analytical grade from Merck (Darmstadt, Germany).

Apparatus:

The high-performance liquid chromatograph was Waters model 6000A solvent delivery system with a model U6K injector, a μBondapack phenyl column (30 × 0.39 cm I.D.; particle size 10μm), a 481 variable UV detector and a Millipore 746 integrator recorder.

HPLC Operating Condition:

The mobile phase consists of MeOH-H_2O (60:40) adjusted to pH 3.3. The mobile phase was degassed and filtered through a 0.45μm filter (Millipore, type HVLP). The flow rate was 1.5 ml/min. The UV detector was operated at 280nm.

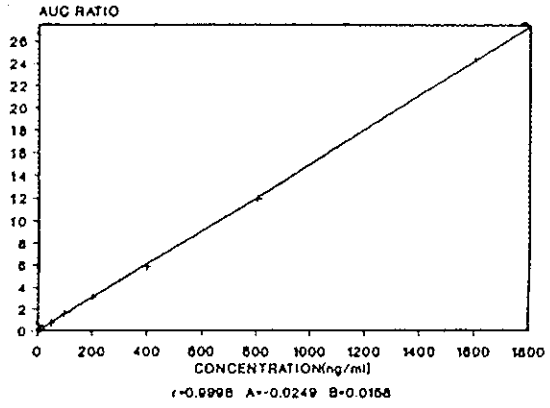

Fig 1. Calibration curve of dipyridamole with eight data points. A slope of 0.0158, an intercept of 0.0249 and a correlation coefficient of 0.9998 were calculated from the linear regression analysis

Standard Solutions:

A series of serial dilutions from the stock solution of dipyridamole (5mg/ml) was prepared in ethanol. A 50μl aliquot of each of these solutions was added to 5ml of plasma.

Sample Preparation:

To one ml of plasma was added 50μl of ethanol containing 500ng of propranolol. Twenty mg of sodium chloride was then added and the tube was shaked to insure saturation of the aqueous phase with the salt. One ml of 1M Tris buffer (pH=10) was added to the plasma solution and agitated to insure maximum dissolution of the sodium chloride followed by addition of 5 ml of diethyl ether. The mixture was shaken for 5min and centrifuged at 1500g for 10min. Using diethyl ether as the extraction solvent is technically advantageous: because of its lower specific gravity and freezing point than water and the ease of the separation from the aqueous phase by freezing.

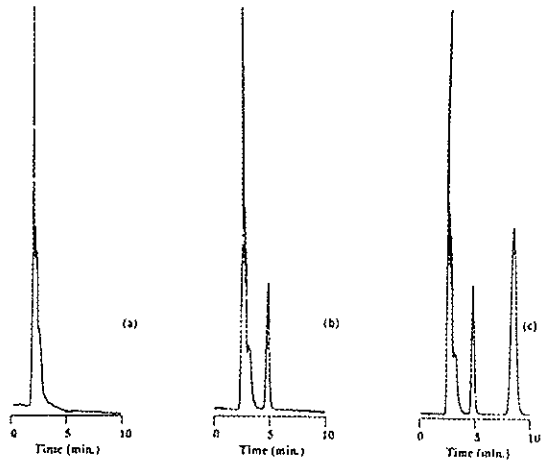

Fig 2. Chromatograms of (a) blank plasma, (b) plasma containing internal standard (4.78 min.) and (c) plasma with internal standard (9.25 min.) and dipyridamole.

The clear organic phase was transferred to a second tube containing 3ml of n-hexane. The dipyridamole and propranolol were then back-extracted into 50μl of 0.1N hydrochloric acid. The addition of n-hexan to the diethyl ether prior to back-extraction was necessary in order to have a high extraction efficiency into 50μl of 0.1N hydrochloric acid and was a technical improvement in that the

separation of the 50µl of aqueous from organic phase was free of emulsion. After shaking and centrifugation, the organic layer was removed by aspiration. An aliquot of the aqueous phase or the total (50µl) could be injected for analysis.

Calibration:

Eight different plasma samples containing 10,20,50,100,200,400,800 and 1600ng/ml of dipyridamole were prepared. Calibration curve was obtained by plotting concentration against peak area ratio (Fig. 1).

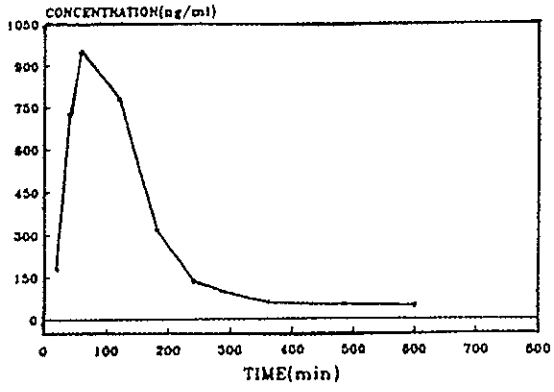

Fig 3. Mean plasma level of dipyridamole after a single oral dose of 75 mg in six individuals

RESULTS AND DISCUSSION

Under conditions described in the experimental section, propranolol and dipyridamole had retention times of 4.8 ± 0.2 and 9.2 ± 0.2 min, respectively (Fig. 2).. The use of an internal standard simplified the entire procedure by eliminating the need for precise transfer of solvent and precise injection volume. The reason for the use of propranolol was that the drug is readily available and its extraction characteristics are similar to dipyridamole (10). The reproducibility of the assay was verified by extracting six plasma samples with a concentration of 120ng/ml (Table 1).

The coefficient of variation of the peak area ratio of these samples was 2.4%. The percent recovery of dipyridamole from these standard plasma samples was 87 ± 5%.

The assay method was tested by monitoring the plasma concentration-time profile for dipyridamole in six healthy volunteers following oral administration of 75 mg dipyridamole as a film coated tablet made by Tolidarou (Tehran, Iran) (Fig. 3).

CONCLUSION

This paper describes a rapid, simple and specific HPLC method for the analysis of dipyridamole which is based on reversed-phase chromatography and UV detection. The method is internally calibrated and uses uniform volumes of plasma, so it is suitable for routine analysis of bioavailability and clinical pharmacokinetic studies.

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Table 1. Accuracy and precision of the method for the determination of dipyridamole in plasma samples.

Concentration (ng/ml)	Intraday (n=5) Mean ± S.D. (ng/ml)	C.V. (%)	Interday (n=5) Mean ± S.D. (ng/ml)	C.V. (%)
50	51.3 ± 1.88	3.67	48.7 ± 1.86	3.86
120	120.7 ± 2.89	2.4	122.0 ± 2.94	2.41
300	302.9 ± 7.02	2.26	297.4 ± 6.72	2.32

REFERENCES

1-Morris, G.K., Mitchell, J.R.A. (1977), Preventing venous thromboembolism in elderly patients with hip fractures; studies of low-dose heparin, dipyridamole, aspirin and flurbiprofen, Br. Med. J., 1: 535-541.

2- Wu, K. K., Hoak, J. C. (1976), Spontaneous platelet aggregation in arterial insufficiency, Thromb. Haemostas. 35: 702-705.

3- Markieudricz, A., Semenowicz, K., Szczyrba, E., and Kocek, B.(1978), Comparison of bio-availability of two dipyridamole formulations, Pol. J. Pharmacol. Pharm. 30: 603-611.

4- Williams, C., Mayer, P.R., Gonzalez, M.A., Erb, R.J., and Kildsig, D.O. (1982), Comparative bioavilability of dipyridamole from two tablet formulation, Curr.Thrap. Res., 32: 236-241.

5- Mahony, C., Wolfram, K.M., Cocchetto, D.M., and Bjornsson, T.D. (1982), Dipyridamole kinetics, Clin Pharmacol. Ther. 31: 330-338.

6- Zak, S.B., Tallan, H.H., Quine, G.P., Fratta, I. and Greengard, P.G. (1963), Determination and physiological distribution of dipyridamole and its glucoronides in biological material, J. Pharmacol. Exp. Ther. 141: 392-397.

7- Mellinger, T.J., and Bonorfoush, J.C. (1966), Blood levels of dipyridamole in humans, Arch. Int. Pharmacol., 163: 471-476.

8- Schmid, J., Beschke, K., Roth, W., Bozler, G., and Wilhem, F. (1979), Rapid, sensitive determination of dipyridamole in human plasma by HPLC, J. Chromatogr., 163, 239-243.

9- Pedersen, A.K. (1979), Specific determination of dipyridamole in serum by HPLC, J. Chromatogr., 162: 98-103.

10- Rosenfeld, J., Devereaux, D., and Buchanan, M.R. (1982), High-performance liquid chromatographic determination of dipyridamole, J. Chromatogr., 231: 216-221.