

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

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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-H₂O (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). Spectrofluorometric 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 does 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 complicated, inconvenient and relatively time consuming and finally the method is externally calibrated

EXPERIMENTAL

Reagents:

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

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 μ Bondapak phenyl column (30×0.39 cm I.D.; particle size $10\mu\text{m}$), a 481 variable UV detector and a Millipore 746 integrator recorder.

HPLC Operating Condition:

The mobile phase consists of MeOH-H₂O (60:40) adjusted to pH 3.3. The mobile phase was degassed and filtered through a $0.45\mu\text{m}$ filter (Millipore, type HVLP). The flow rate was 1.5 ml/min. The UV detector was operated at 280nm.

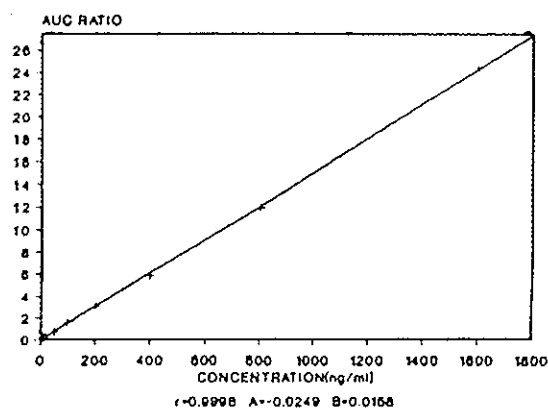


Fig 1. Calibration curve of dipyrindamole with eight data points. A slope of 0.0158, an intercept of 0.0249 and a correlation coefficient of 0.9998 were calculated from the linear regression analysis

Standard Solutions:

A series of serial dilutions from the stock solution of dipyrindamole (5mg/ml) was prepared in ethanol. A $50\mu\text{l}$ aliquot of each of these solutions was added to 5ml of plasma.

Sample Preparation:

To one ml of plasma was added $50\mu\text{l}$ of ethanol containing 500ng of propranolol. Twenty mg of sodium chloride was then added and the tube was shaken 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.

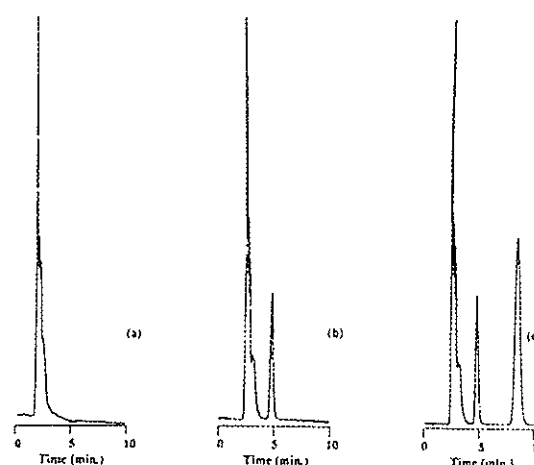


Fig 2. Chromatograms of (a) blank plasma, (b) plasma containing internal standard (4.78 min.) and (c) plasma with internal standard (9.25 min.) and dipyrindamole.

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

separation of the 50 μ l 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 (50 μ l) 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).

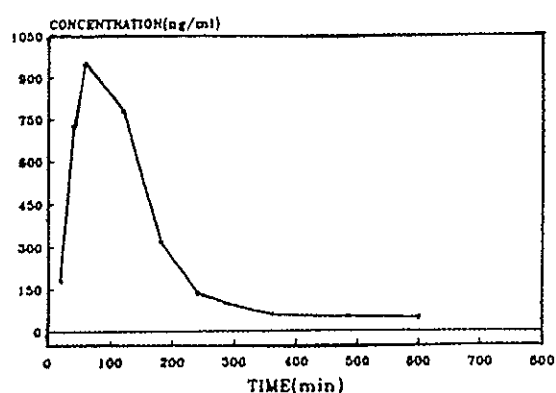


Fig 3. Mean plasma level of dipyridamole after a single oral dose of 75 mg in six individuals

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 $87 \pm 5\%$.

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

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