IMPROVED HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF DILTIAZEM IN HUMAN PLASMA

Nisar-ur-Rahman, *Syed Saeed-ul-Hassan, *Uzma Niaz

Department of Pharmacy Islamia University Bahawalpur, *Faculty of Pharmacy University of the Punjab Lahore

ABSTRACT

A simple high-performance liquid chromatographic method was developed for determination of diltiazem in human plasma. Diltiazem and the internal standard, verapamil, were extracted from plasma samples using mixture of n-hexane and diethyl ether. The mobile phase was 0.1M ammonium dihydrogen phosphate - acetonitrile (62:38 v/v). Triethylamine (0.08% v/v in the mobile phase) was added before the pH was adjusted to 5.9 with 85% phosphoric acid. Analysis was run at a flow rate of 1.0 ml/min at a detection wavelength of 238 nm. The method was specific and sensitive with a detection limit of 2.5 ng/ml at a signal-to-noise ratio of 3:1. The limit of quantification was set at 5 ng/ml. The calibration curve was linear over a concentration range of 5-160 ng/ml. Mean recovery value of the extraction procedure was about 90%, while the within and between day coefficient of variation and percent error values of the assay method were less than 10%.

Keywords: Diltiazem, reversed phase HPLC assay

INTRODUCTION

Diltiazem is an orally and intravenously active therapeutic agent used in the treatment of chronic stable angina. Orally administered diltiazem is metabolized into two basic active metabolites N-demethyl diltiazem and deacetyl diltiazem. Their chemical structures are shown in fig. 1. The half-life of the metabolite N-demethyl diltiazem was found to be similar to that diltiazem whereas the half- life of deacetyl diltiazem was longer [1].

A review of the literature revealed that several high performance liquid chromatographic (HPLC) methods have been reported for the determination of diltiazem and its major metabolites in the plasma. Most of the methods involve liquid-liquid extractions with slight differences in the extraction solvent, mobile phase, the column and the run time for the analysis. Some authors employed reversed phase chromatography with either acid back-extraction [2,3] or solid-phase extraction procedure [4] and a normal-phase method [5]. A direct injection of plasma samples into the automatic chromatographic system for on-line clean up of plasma samples was also reported by Ascalone and Dalbo [6].

In this paper, we report a simple, sensitive and specific HPLC method for determination of diltiazem in human plasma using ultraviolet detection. The assay method was evaluated for accuracy, precision, recovery and linearity. The method employed a mobile phase of pH 5.9, permitting the use of a cheaper silica-based column. Also, a widely available compound, verapamil was used as the internal standard. The applicability of the method was demonstrated by applying it to analyze plasma samples obtained from a bioequivalence study.

Correspondence: Dr Nisar-ur-Rahman, Assistant Professor, Department of Pharmacy, Islamia University Bahawalpur.

MATERIALS AND METHOD

Materials

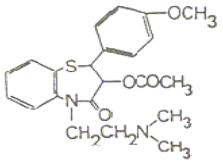
Diltiazem Hydrochloride (Sigma, USA), Verapamil Hydrochloride (USP, Reference standard), N-Monodemethyl diltiazem (Tanabe, Japan), Acetonitrile, HPLC grade (Malinckrodt, USA), Diethyl Ether, AR (BDH, England), N-Hexane, Methanol AR (Malinckrodt, USA), Ammonium Dihydrogen Ortho phosphate, AR (BDH, England), Triethylamine, AR (Fluka, Switzerland) and Phosphoric Acid, AR (BDH, England)

Instrumentation

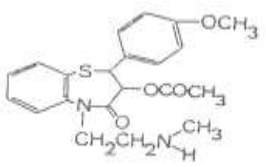
The HPLC system comprised a Jasco PU-980 Intelligent HPLC pump, a Gilson 119 UV/VIS detector (Gilson Medical Electronics, Villiers-le-Bel, France) connected to a Hitachi D-2500 integrator (Hitachi, Tokyo, Japan) and a Rheodyne 7125 sample injector fitted with a 50 µl sample loop. The detector was operated using a sensitivity range of 0.005 AUFS and wavelength of 237 nm. A LiChrospher 100 RP-18e reversed phase column (5µm, 250-x 4.6 mm ID) (Merck, Germany) fitted with a refillable guard column (Upchurch Scientific, Oak Harbour, WA, USA) and packed with Perisorb RP-18; 30-40 µm pellicular stationary phase (Upchurch Scientific, Oak Harbour, WA, and USA) was used for the separation. The mobile phase comprised 0.1M ammonium dihydrogen phosphate and acetonitrile (62:38 v/v). Triethylamine (0.08% v/v in the mobile phase) was added before the pH was adjusted to 5.9 with 85% phosphoric acid. Analysis was run at a flow rate of 1.0 ml/min and the detection wavelength was 238nm with a sensitivity range of 0.005 aufs.

Standard Solutions

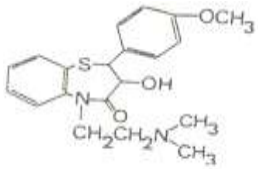
Stock solutions of diltiazem were prepared by dissolving 100 mg of diltiazem with 100 ml methanol. The standard curve was prepared by spiking drug free plasma with a known weight of diltiazem at



Diltiazem HCI



N-Demethyl Diltiazem



Deacetyl Diltiazem

Fig.1: Chemical structure of diltiazem HCl and its major metabolites

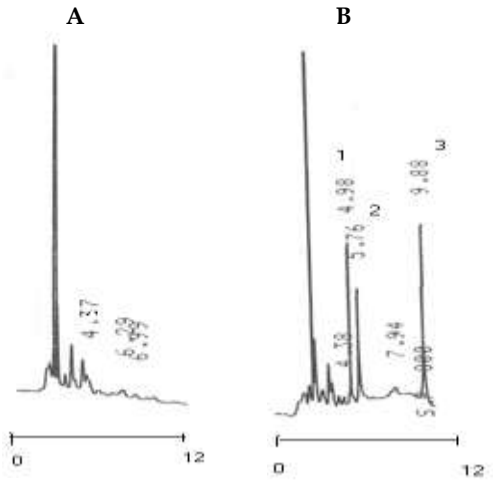


Fig.2: Chromatograms of (A) blank plasma and (B) plasma sample obtained from a volunteer at 4 hours after oral administration of 90 mg controlled release diltiazem showing peaks for N-demethyl diltiazem (1), diltiazem (2) and the internal standard, verapamil (3).

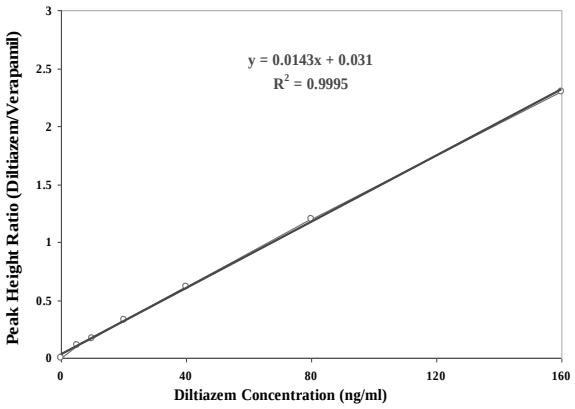


Fig. 3: Mean standard curve of diltiazem HCI

concentration levels of 5, 10, 20, 40, 80 and 160 ng/ml. The standard plasma samples were stored at –200C in glass bottles.

Extraction Procedure

A I ml aliquot of the plasma was accurately measured into a glass tube with a Teflon lined screw cap, followed by addition of 50 µl of internal standard (4 µg/ml of verapamil HCl in aqueous solution and 4.0-ml mixture of diethyl ether and n-hexane (1:1). The mixture was vortexed for 1 min using a vortex mixer and then centrifuged (Labofuge 200, Heraeus Sepatech, Germany) at 3500 rpm for 10 minutes. The upper organic layer was transferred into a reactivial (Pierce Reacti-vial, USA) and then evaporated to dryness at 40°C under a gentle stream of nitrogen gas. The residue was reconstituted with 75 µl of mobile phase and from this 50 µl injected onto the column.

Assay Validation

Samples were quantified using height ratio of diltiazem over the internal standard. Extraction recovery, within-day and between-day precision and accuracy studies (n=6) of the method were carried out using these plasma standards. The recovery of the extraction procedure for diltiazem and internal standard were calculated by comparing the peak height obtained after extraction with that of aqueous solution of corresponding concentrations without extraction. The accuracy was expressed as percentage error, obtained by calculating the percentage of difference between the measured and the spiked concentration over that of the spiked value, whereas the precision was denoted using the coefficient of variation (CV).

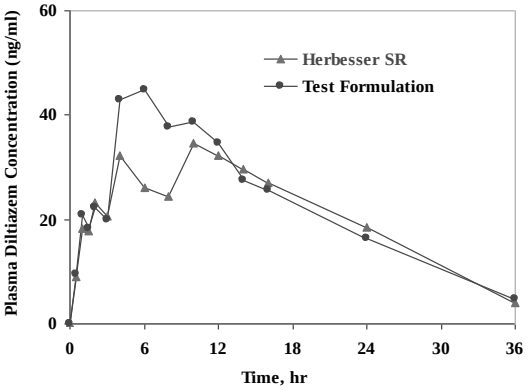


Fig. 4: Mean plasma diltiazem concentration versus time profiles of herbesser SR and test

Table 1: Extraction recovery of diltiazem within-day and between-day accuracy and precision (n=6)

Concentration (ng/ml)	Recovery (%)	CV (%)	Within day Accuracy (%)	Within day Precision CV (%)	Between Day Accuracy (%)	Between Day Precision CV(%)
5	93.6	9.4	92.8	9.1	96.7	8.8
10	95.0	9.9	100.1	8.6	101.5	6.9
20	97.5	6.3	91.5	8.8	103.1	6.5
40	91.4	6.5	93.4	7.3	104.8	8.3
80	99.2	3.1	92.4	6.7	104.1	6.1
160	100.2	3.8	90.5	2.8	102.3	8.5

CV = Coefficient of Variation

RESULTS AND DISCUSSION

Diltiazem and its metabolites are weakly basic amines and the amino groups are known to interact strongly with the stationary support, causing peak tailing and broadening [7, 8]. Some short-chain tertiary amine modifiers such as triethylamine (TEA) are very effective in improving peak symmetry and reducing retention time of solutes with amino functional groups [9]. Therefore, TEA in 0.08% v/v was incorporated into the mobile phase to improve the resolution as well as peak symmetry of diltiazem and its metabolite. By increasing the ratio of the mobile phase different effects upon separation of diltiazem and its metabolite were observed such as by increasing the ratio of water in the mobile phase, the retention time of the components was increased. By increasing the ratio of acetonitrile the retention time was decreased and peaks were sharp instead of broadening of peaks.

Chromatograms obtained with blank plasma and plasma sample of a healthy volunteer after 4 hours dosing with 90 mg diltiazem have been shown in fig. 2 A and B. The retention times of diltiazem HCl and internal standard (verapamil HCl) were 5.78 and 9.88 minutes respectively. The blank sample was clean and no interfering peak was observed at the retention times of diltiazem and verapamil HCl. The peak at 4.98 minute was found to be N-monodemethyl diltiazem, a major metabolite of diltiazem HCl and did not interfere with the drug or internal standard and hence this was verified by injecting a standard solution of the metabolite on to the column.

The extraction recovery of diltiazem was determined by comparing the peak height obtained by direct injection of standard aqueous solutions to those obtained after the plasma extraction procedure. A mixture of diethyl ether and n-hexane (1:1 v/v) gave better recoveries for diltiazem and internal standard as well as provided cleaner chromatograms compared to using n-hexane, diethyl ether, methyl tertiary butyl ether, ethyl acetate or chloroform alone as the extracting solvent. The recovery of diltiazem as well as the internal standard was more than 90%.

The accuracy and precision of the method assessed by analysis of plasma samples within-day and between-day at various concentration levels, together with the recovery values of the extraction procedure are given in table 1. The values for the CV were all less than 10% at the concentration range determined. Similarly, the accuracy was within 90-105% at these concentration values. It is interesting to note that, the between-day CV values appeared to be relatively smaller than those of the within day, suggesting that the precision was not compromised during between-day analysis. The limit of detection was approximately 2.5 ng/ml at a signal-to-noise ratio of 3:1. However, the limit of quantification was set at 5 ng/ml being the lowest concentration used in the construction of the standards curve. The mean standard curve (n=6) is shown in fig. 3. A linear correlation was found between the peak height ratio of diltiazem and the internal standard Vs diltiazem concentration in the plasma in the range of 5-160 ng/ml with a coefficient of correlation (r) 0.9995.

The method was applied to analyze plasma samples obtained from a bioequivalence study of a test preparation of diltiazem versus the innovator preparation Herbesser SR 90 mg. fig. 4 shows the plasma profiles of both preparations that diltiazem could still be detected after 36 h after dosing.

CONCLUSION

The present HPLC method is sensitive, simple, specific and appropriate to be used for determination of plasma diltiazem in pharmacokinetic/bioavailability studies. The pH of the mobile phase used was well within the practical working pH range of common reversed phase C18 columns, which is typically between pH 3-7, thus avoiding the use of more expensive pH sensitive columns. Moreover, the assay method was well evaluated to other reported methods.

REFERENCES

1. Hoglund P. and Nilsson L G. Phamacokinetcs of diltiazem and its metabolite after single and multiple dosing in healthy volunteers. **Ther Drug Monit 1989; 11: 558-566.**

2. Abernethy D R. Schwartz J B. Todd E L. Diltiazem and desacetyldiltiazem analysis in human plasma using high-performance liquid chromatography: improved sensitivity without derivatization. **J Chromatogr 1985; 342: 216-220.**

3. Montamat S C. Abernethy D R. Mitchell J R. High-performance liquid chromatographic determination of diltiazem and its major metabolites, N-monodemethyldiltiazem and desacetyldiltiazem, in plasma. **J Chromatogr 1987; 415: 203-207.**

4. Boulieu R. Bonnefous J L. Ferry S. Solid-phase extraction of diltiazem and its metabolites from plasma prior to high-performance liquid chromatography. **J Chromatogr 1990; 528: 542-546.**

5. Kinney C D and Kelly J G. Estimation of concentrations of diltiazem in plasma using normal-phase column liquid chromatography with ultraviolet detection. **J Chromatogr 1986; 382: 377-381.**

6. Ascalone and Dalbo L. Automated high-performance liquid chromatography and column-switching technique for on line clean up and analysis of diltiazem in human plasma. **J Chromatogr 1987; 423: 239-249.**

7. Bij K E. Horvath Cs. Melander W R. Nahum A. Surface silanols in silca-bonded hydrocarbonaceous stationary phases. II. Irregular retention behaviour and effect of silanol masking. **J Chromatogr 1981; 203: 65-85.**

8. Nahum A. and Horvath, Cs. Surface silanols in silica-bonded hydrocarbonaceous stationary phases. I. Dual retention mechanism in reversed-phase chromatography. **J Chromatogr 1981; 203: 53-63.**

9. Kiel J S. Morgan S L. Abramson R K. Effects of amine modifiers on retention and peak shape in reversed phase high performance liquid chromatography **J Chromatogr 1985; 320: 313-323.**