RP-HPLC Method for Analysis of Diltiazem: Application to Drug Metal Interaction

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Summary: A simple and rapid high-performance liquid chromatographic (HPLC) method for the determination and quantification of diltiazem from bulk drug, pharmaceutical formulation and human serum has been developed. Shim-pack CLC-ODS (6.0 x 150 mm) column was used for separation at room temperature. Mobile phase consisted of acetonitrile/water (40:60 v/v), pH was adjusted to 2.8 with phosphoric acid and pumped at flow rate of 0.8 mL. min^{-1} with UV detection at 235nm. Method was linear in bulk drug and serum between the concentration range of 0.5-3.0 μg mL^{-1} and 1.0-5.0 μg mL^{-1}, respectively, with correlation coefficient of 0.9997. The limit of detection (LOD) and quantification (LOQ) for diltiazem in bulk drug and serum were 0.015 and 0.046, 0.02 and 0.07 μg mL^{-1}, respectively. Recovery values of diltiazem in bulk drug, dosage form and serum were 99.88 -101.15 %, 98.98-101.64 % and 98.99-102.58 %, respectively. The RSD was < 1 in all cases. Interference of other already administered common medicaments such as aspirin, caffeine, nicotine and other serum components were not found. This method was applied in drug metal interaction studies. Drug metal interaction studies were carried out at 37 and 60 °C to monitor the complexation of drug with metal ions. The study was helpful for the determination of drug in therapeutic concentrations inside human body as well its complexation with metal cations. The proposed method is rapid, accurate and selective, because of its sensitivity and reproducibility.

Introduction

Diltiazem hydrochloride (Fig. 1), (2*S-cis*)-3-acetoxy-2, 3-dihydro-5- [2-(dimethylamino) ethyl]-2-(*p*-methoxyphenyl)-1,5-benzonthiazepin-4-(5*H*)-one hydrochloride [1-3], belongs to the bezothiazepine class of compounds used in the treatment of angina pectoris, systemic hypertension, and supraventricular arrhythmias [1]. It is more effective against atrial than ventricular dysrhythmias, useful in treating reentrant supraventricular tachycardia and reducing ventricular rate in atrial flutter and fibrillation. It is also used to treat hypertension and angina [4, 5].

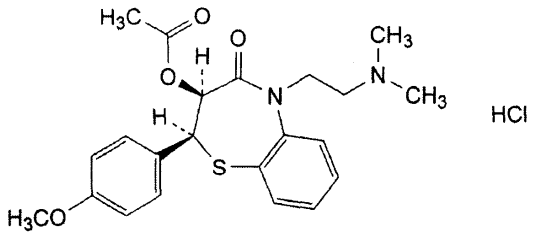

Fig. 1: Diltiazem hydrochloride.

A number of published articles described the determination of diltiazem, by RP-HPLC in serum [6-14] or tablets [15, 16] and by spectrophotometeric methods [17-21].

There are number of interactions reported with cephalosporins [22-25] and quinolinones with antacids containing *di-* and *tri-*valent metal cations [26]. The availability of these drugs can be affected by the concurrent ingestion of drugs containing multivalent cations. It is imperative to be aware of these interactions.

The present work describes a simple reverse phase HPLC method for the determination of diltiazem from bulk drug, dosage formulations, human serum and drug metal complexes. The method was found to be reproducible with good resolution between diltiazem and metformin (internal standard). The validity of method was determined over a wide range of period for any possible *intra-*day and *inter-*day variations. Moreover, the interaction of diltiazem with essential and trace

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elements which may either be present in low concentrations in human body or may be ingested as a result of multiple drug therapy have been carried out and the quantitation of diltiazem in these drug interactions has been monitored using the developed HPLC method.

Results and Discussion

In the recent past, use of HPLC methods for the quantitation of drugs has received considerable attention. In the present study, the best separation of diltiazem and internal standard was achieved using a Shim-pack CLC-ODS (6.0 X 150 mm) column. Best results were obtained for the determination of diltiazem and its separation from metformin using mobile phase acetonitrile/water (40:60 v/v). Long analysis duration results when lower percentage of acetonitrile in mobile phase used while higher percentage of acetonitrile in mobile phase results in very little analysis duration. When pH of mobile phase was adjusted to 2.8 with 85 % phosphoric acid, optimal retention times (metformin - 2.68 minutes and diltiazem - 4.26 minutes) were achieved (Fig. 2). Higher pH of the mobile phase also results in peak tailing and at a lower pH retention time of metformin and diltiazem was delayed. The method has been successfully used for the determination of diltiazem in concentrations, as low as 0.5 μg mL^{-1}, with retention time of only 4.47 minutes and internal standard was separated in serum earlier at 2.73 minutes (Fig. 3). The retention time of other common interfering components of serum is 4.02 minutes for caffeine and 6.30 minutes for aspirin (Fig. 4). However, nicotine was not detected in the chromatographic conditions used in this method.

Linearity and Sensitivity

Linearity was demonstrated at six concentrations over the range of 0.5-3.0 μg mL^{-1} for four consecutive days and gave correlation coefficient (r^2) of 0.9997 and in serum over the concentration range of 1.0-5.0 μg mL^{-1} the correlation coefficient (r^2) was 0.9997. The mean linear regression equations of standards curves are: $y = 23838x - 549.73$ for diltiazem in bulk form and $y = 25671x - 1068.8$ in serum. The limit of detection (LOD) and quantification (LOQ) for diltiazem in bulk form and serum were 0.015 and 0.046, 0.02 and 0.07 μg mL^{-1}, respectively.

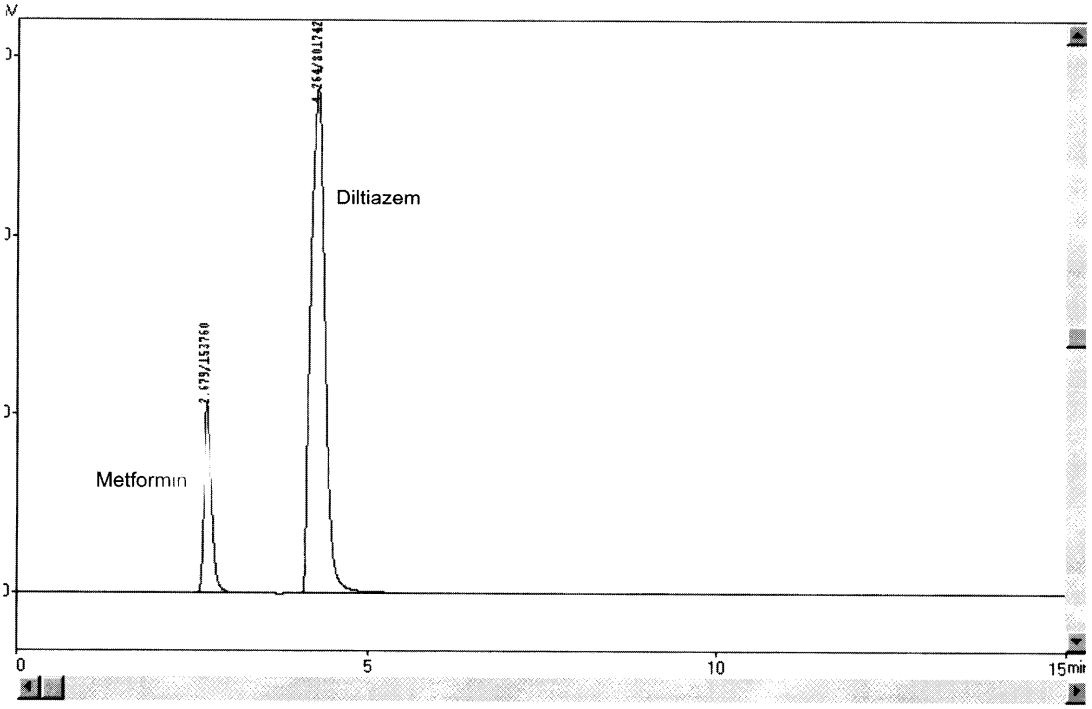

Fig. 2: A typical chromatogram showing metformin and diltiazem peaks.

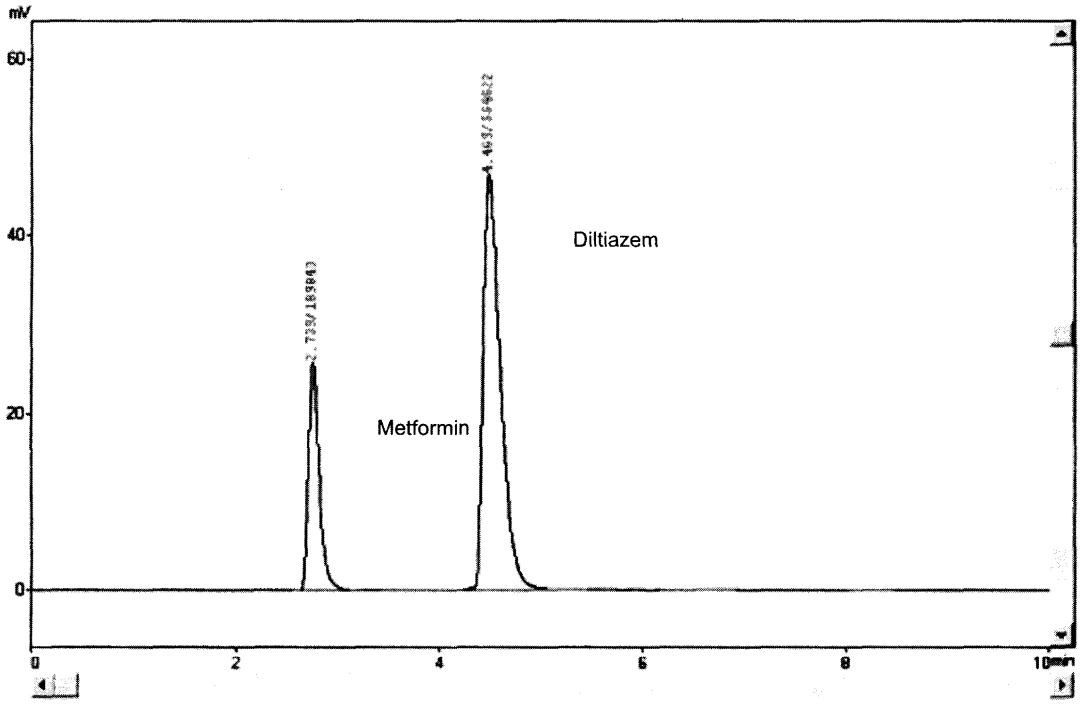

Fig. 3: A typical chromatogram showing diltiazem and metformin peaks in serum.

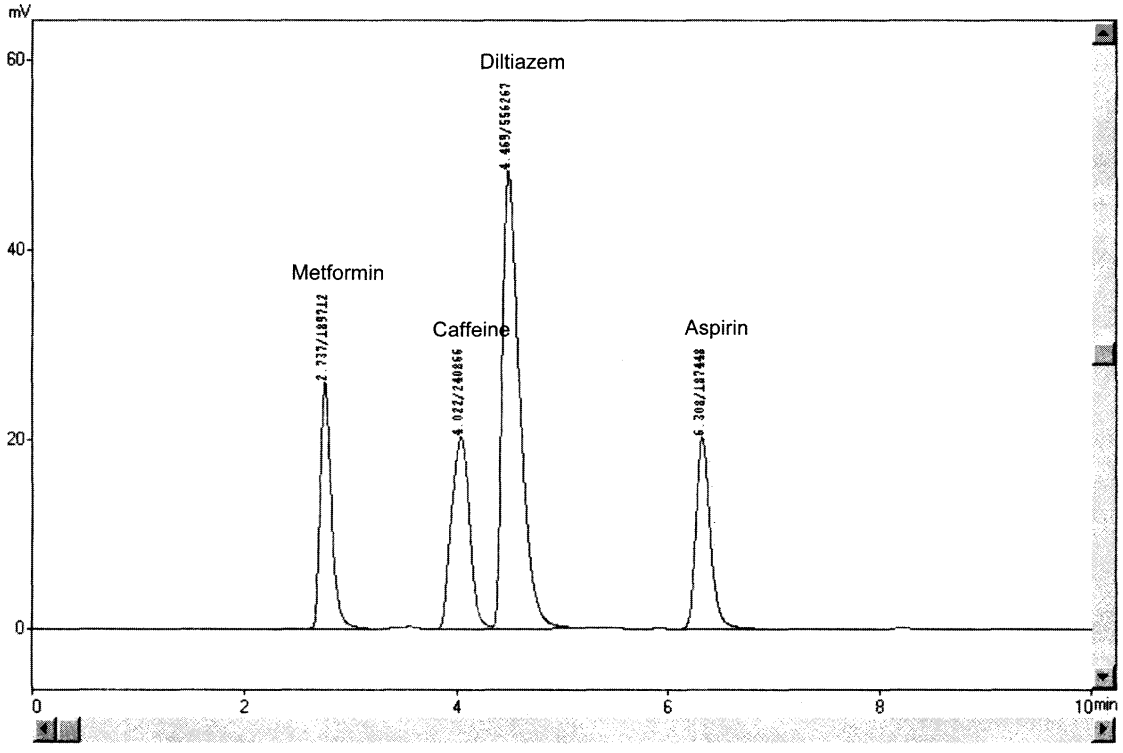

Fig. 4: A typical chromatogram showing diltiazem, metformin, caffeine and aspirin peaks in serum.

Precision

The precision of the assay was determined by (repeatability) *intra*-day and *inter*-day (intermediate precision) analysis. Precision was expressed as relative standard deviation (RSD). The results are shown in Table-1.

Table-1: Interday Precision of diltiazem by proposed method.

Con. Injected ($\mu g\ mL^{-1}$)	Day 1 RSD	Day 2 RSD	Day 3 RSD	Day 4 RSD
0.5	0.0087	0.0076	0.0043	0.0026
1.0	0.0076	0.0125	0.0008	0.0011
1.5	0.0046	0.0065	0.0074	0.0013
2.0	0.0028	0.0076	0.0014	0.0014
2.5	0.0032	0.0051	0.0037	0.0045
3.0	0.0017	0.0044	0.0014	0.0031

Accuracy (Recovery)

Accuracy was determined by analyzing independently prepared solutions of diltiazem in triplicates at different concentration levels covering the entire linearity range. Accuracy was expressed as percent (%) recoveries. In serum the relative recoveries of diltiazem were calculated by comparing the concentrations obtained from drug supplemented plasma to the actual added concentrations. The results are shown in Tables-2 and 3.

Table-2: Precision and recovery of diltiazem in raw material and dosage form.

	Raw Material		Dosage Form	
Conc. Injected ($\mu g\ mL^{-1}$)	Conc. Found ($\mu g\ mL^{-1}$)	Recovery (%)	Conc. Found ($\mu g\ mL^{-1}$)	Recovery (%)
0.5	0.499	99.88	0.508	101.64
1	1.007	100.75	0.999	99.92
1.5	1.508	100.50	1.490	99.33
2	2.023	101.15	1.980	98.98
2.5	2.515	100.61	2.521	100.84
3	3.024	100.82	2.990	99.67

Table 3: Precision and recovery of diltiazem in serum.

Conc. Injected ($\mu g\ mL^{-1}$)	Conc. Found ($\mu g\ mL^{-1}$)	RSD	Recovery (%)
1.0	0.990	0.00828	98.99
2.0	2.046	0.00577	102.28
3.0	2.997	0.00500	99.91
4.0	4.103	0.00950	102.58
5.0	5.123	0.00188	102.45

System Suitability and Specificity

System suitability of the method was evaluated by analyzing the symmetry of the peaks (symmetry factor), theoretical plates of the column, resolution between the peaks of diltiazem and internal standard, mass distribution ratio (capacity factor) and relative retention. The specificity of the method was evaluated to ensure separation of diltiazem and was demonstrated by assaying a sample of diltiazem HCl and metformin HCl. Solutions of diltiazem containing aspirin, nicotine and caffeine were prepared and then injected to check for interference from these commonly used drugs. The method demonstrated good resolution and was found to be free of interferences.

Ruggedness

Ruggedness of this method was evaluated in two different labs with two different instruments. Lab 1 was in the Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy University of Karachi, while Lab 2 was in the Department of Chemistry, Faculty of Science, University of Karachi. The method did not show any notable deviations in results from acceptable limits.

Assay of Diltiazem in Different Brands

Three different brands of diltiazem tablets were assayed, the results were 100.38 %, 99.66 % and 104.46 % for Dilzem tablets (Parke Davis), Etizem tablets (Highnoon) and Herbesser Tablets (ICI) respectively, while % error were 0.38, 0.33 and 4.40, respectively as shown in Table 4.

Table-4: Assay of diltiazem in dosage forms (tablets of different brands) by the proposed method.

Dosage Formulation Label claim 30mg	Amount Recovered	% Assay	% Error
Dilzem Tablets (Parke Davis)	30.11	100.38	0.38
Etizem Tablets (Highnoon)	29.90	99.66	0.33
Herbesser Tablets (ICI)	31.33	104.46	4.40

Metal Solutions Preparation for Drug-Metal Interaction Studies

Diltiazem has one tertiary amine group, carbonyl group and acetoxy group, which may act as reacting site. The probability of acetoxy group reacting with any other molecule is much more as compared to that of tertiary amine. No apparent change was found in the chromatograms of interaction studies with respect to standard

Table-5: Quantitation of Diltiazem metal complexes at 37 & 60 °C by proposed method.

Drug/metal	% availabilities	
	at 37°C	at 60°C
Dil blank	100.00	100.00
Dil + Mg(II)	97.50	102.78
Dil + Ca(II)	97.86	104.63
Dil + Cr(III)	97.47	110.54
Dil + Mn(II)	95.46	104.84
Dil +Fe(II)	99.72	112.80
Dil +Fe(III)	98.81	106.95
Dil +Co(II)	100.03	109.41
Dil +Ni (II)	101.19	108.26
Dil +Cu(II)	99.93	110.35
Dil +Zn(II)	99.88	105.39
Dil +Cd(II)	99.44	108.64

Dil = Diltiazem

chromatograms which showed that diltiazen did not interact with any metal as the availability of diltiazem is almost 100 % in presence of metals at both temperatures *i.e.* 37 °C and 60 °C.

Experimental

Materials and Reagents

Samples of diltiazem and metformin were a kind gift from Parke Davis and Aventis Pharma, respectively, which were used without further purification. Different brands of diltiazem tablet i.e. Dilzem tablets 30mg (Parke Davis), Etizem tablets 30 mg (ICI Pakistan) and Herbesser tablets 30 mg (Highnoon Pharma) have been purchased from the market. The essential and trace elements used were in the form of their hydrated salts as magnesium chloride hexahydrate ($MgCl_2.6H_2O$), calcium chloride dihydrate ($CaCl_2.2H_2O$), chromium chloride hexahydrate ($CrCl_3.6H_2O$), manganese chloride tetrahydrate ($MnCl_2.4H_2O$), Ferrous sulphate heptahydrate ($FeSO_4.7H_2O$), ferric chloride hexahydrate ($FeCl_3.6H_2O$), cobalt chloride hexahydrate ($CoCl_2.6H_2O$), nickel chloride hexahydrate ($NiCl_2.6H_2O$), copper chloride dihydrate ($CuCl_2.2H_2O$), zinc chloride ($ZnCl_2$) and cadmium chloride monohydrate ($CdCl_2.H_2O$). HPLC grade acetonitrile and phosphoric acid of analytical grade were used. HPLC grade water was used to prepare the mobile phase.

Apparatus

The liquid chromatographic system consisted of Shimadzu model LC-10 AT VP gradient pump, a Shimadzu model SPD-10AV, VP variable wavelength UV-Visible detector (Shimadzu Corporation, Kyoto, Japan). Chromatographic system was integrated via Shimadzu model CBM-102 Communication Bus Module. Analysis was conducted on a Shim-pack CLC-ODS (6.0 X 150 mm) analytical reverse phased column.

Chromatographic Conditions

The mobile phase consisted of acetonitrile-water (40:60) whose pH was adjusted to 2.8 with phosphoric acid (85 %). Prior to delivering into the system it was filtered through 0.45 μm filter and degassed using an ultrasonic bath. The analysis was carried out under isocratic conditions using a flow rate of 0.8 mL min^{-1} at room temperature. The samples were introduced by injector with a 20-µL sample loop. Chromatograms were recorded at 235 nm using a detector SPD-10AV VP Shimadzu UV visible.

Analytical Procedure

Preparation of Standard Solutions

Stock solution of diltiazem or the internal standard was prepared by accurately weighing 10 mg of the compound and dissolving in 10ml distilled water and diluting to volume in a 100 ml volumetric flask to give a concentration of 100 µg mL^{-1} diltiazem or internal standard. The diltiazem standards were sequentially diluted with distilled water to yield 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0-µg mL^{-1} working standard solutions for preparation of calibration curves. The internal standard stock solution was diluted with water to yield 10 µg mL^{-1} internal standard working solution.

Serum Drug Analysis

The recoveries of diltiazem from pooled human serum were determined by the stated chromatographic conditions. Multiple blood samples (10 mL) of ten healthy non-smoker volunteers (age ranging from 22-25 years) not involved in any strenuous activity and not taking any other medicaments were collected in evacuated glass tubes. The blood was then centrifuged at 3000 rpm for 10 minutes and the plasma separated and deprotinated by acetonitrile. The supernatant obtained was filtered through a 0.45 µ filter. Serum thus obtained was mixed in ratio of 1:1 with drug solutions; these were stored at –20 °C pending drugs analysis.

Drug-Metal Interaction Studies

Drug and metal standard solutions of 100 μg mL^{-1} were mixed in ratio of 3:1 (v/v). Reaction mixture was allowed to react at 37 °C and 60 °C on a water bath for 30 minutes. Resulting solution mixture was filtered through 0.45 μ membrane filter and then introduced in HPLC system by means of Rheodyne injection system with 20 μL loop using the above described chromatographic system.

Conclusion

An accurate, precise and least time consuming RP-HPLC method for the analysis of diltiazem in bulk drug, pharmaceutical formulations, human serum has been successfully developed. The proposed RP-HPLC method also enables for the determination of diltiazem in its metal interaction studies. The study was beneficial to determine the drug in therapeutic concentrations inside human body as well its complexation with metal cations. Results are accurate and precise and are confirmed by the statistical parameters. Rapidness, simplicity, sensitivity, economical nature, good recovery and precision of this HPLC method give it advantage over to the other reported HPLC methods for determination of diltiazem.

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