ORIGINAL ARTICLE

A VALIDATED METHOD FOR THE ANALYSIS OF DILTIAZEM IN RAW MATERIALS AND PHARMACEUTICAL FORMULATIONS BY RP-HPLC

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ABSTRACT

A simple, selective and rapid reversed phase high performance liquid chromatographic (HPLC) method for the analysis of diltiazem (DTZ) in bulk material and pharmaceutical formulations has been developed and validated. Sample was resolved on a Hypersil, ODS, C-18(150×4.6 mm, 5 micron) column. The mobile phase consisted of methanol-water (80:20 v/v, pH 3.1 adjusted with phosphoric acid) was delivered at a flow rate of 0.5 ml/min at ambient temperature and the retention time was about 2.6 minutes with symmetrical peaks. Studies were performed on an HPLC system equipped with a UV/ visible detector at 236nm. Flurbiprofen (FLR) was used as an internal standard. The developed method gave good resolution between diltiazem and internal standard. The method is specific to DTZ and able to resolve the drug peak from formulation excepients. The calibration curve was linear over the concentration range of 0.195-50 μg/ml (R^2 = 0.999). The proposed method is accurate (the accuracy results were 94.1-99.39 for diltiazem recoveries), precise (The intraday and interday precision CVs were 0.035-2.2 %) and linear within the desired range. The lower limits of detection for DTZ was found to be 0.0184μg/ml and the quantitation limit was about 0.056μg/ml and therefore could be employed as a more convenient and efficient option for the analysis of diltiazem and its related compounds in drug substance and formulations.

Keywords: Diltiazem; method validation; HPLC determination.

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INTRODUCTION

The calcium antagonist diltiazem has been marketed for the treatment of cardiovascular disorders for more than 20 years, and it remains one of the leading products in this therapeutic area (Leigh *et al*., 1991). Diltiazem is a benzothiazepine calcium-channel blocker with peripheral and coronary vasodilatator properties. It lowers the blood pressure and has some effect on cardiac conduction. It is administered orally in the treatment of angina pectoris and hypertension, and may be given intravenously in the treatment of arterial fibrillation or flutter and paroxysmal supra ventricular tachycardia (Martindale, 2005). Diltiazem has, like many other drug substances, a (β-dialkylamino)ethyl side-chain (Leigh *et al*., 1991). The drug substance (fig. 1) was originally developed as the hydrochloride, but the malate and maleate salts have also been described.

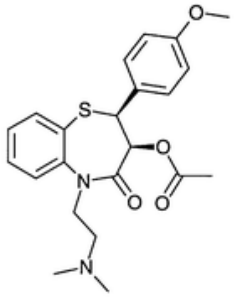

Fig. 1: Diltiazem

A number of methods have been used for analysis of DTZ in bulk drug, pharmaceutical dosage forms as well as in biological fluids such as extractive spectrophotometric method in pharmaceutical formations (Rahman and Hejaz-Azmi 2000, Kamath and Shivram1991; Shreedhar *et al*., 1995), HP-TLC (Agbaba *et al*., 1997) and GC (Sane *et al*., 1989). Several investigators have determined diltiazem by HPLC in pharmaceutical formulations (European Pharmacopoeia 2002**,** Lacroix *et al.,* 1989, Sidhu *et al*., 1987) in plasma and serum (Chaudhary *et al*., 1993; Hoglund and Nilsson 1987, Rustum 1989). All these methods are time consuming, expensive, complex in nature and damage the susceptibly of the column. In these methods mobile phases used are mostly expensive and hazardous for the column health and efficiency (theoretical plates). This article describes a simple, efficient and economical reverse phase HPLC method with UV detection for the determination of diltiazem from reference materials, bulk raw materials and dosage formulations using a C18 column. The method was found to be reproducible, with good resolution between diltiazem and flurbiprofen (internal standard). The developed method was validated for parameters such as linearity, recovery/accuracy, precision, specificity and selectivity, detection and quantification limits.

EXPERIMENTAL

Instrumentation

A Shimadzu HPLC system equipped with LC-10 AT VP pump and SPD-10 A VP UV–VIS detector was utilized. Chromatographic system was integrated via Shimadzu model CBM-102 Communication Bus Module to P-IV computer loaded with Shimadzu CLASS-VP software (Version 5.03) for data acquisition and mathematical calculations. Rheodyne manual injector fitted with a 20 µl loop, a Hypersil, ODS, C18 (150×4.6 mm, 5 micron) column and DGU-14 AM on-line degasser. In addition, Mattler Toledo electronic balance, microliter syringe and micropore filtration assembly were used in this study.

Materials and reagents

Diltiazem was a kind gift sample from Hilton Pharma (Private) Limited, and flurbiprofen from Pharm. Evo Pvt. Ltd. Four different formulations of DTZ namely Dilzem (Parke Davis Pvt. Ltd.), Tiazem (Hilton Pharma Pvt. Ltd), Etizem (Getz Pharma Pvt., Ltd.) and Herbesser (Tanab Seiyaku Co., Ltd) were obtained from retail pharmacies. Each product was labeled to contain 30 mg of DTZ and had an expiry of not less than 365 days at the time of study. All reagents used were of HPLC grade. Methanol and phosphoric acid 85% (Merck, Germany) and HPLC grade water were used to prepare the mobile phase. Stock solutions of diltiazem were prepared in mobile phase and solution of flurbiprofen was prepared in methanol. Fresh working solutions were prepared daily. All solutions were filtered (0.45µm) and degassed by sonicator.

Preparation of solutions

A stock solution of 100 µg/ml of DTZ and flurbiprofen were prepared in mobile phase and serially diluted to required concentrations. Twenty tablets of Tiazem weighed, finely powdered and an amount equivalent to 5 mg was mixed with 50 ml mobile phase to give a concentration of 100 µg/ml. This mixture was allowed to stand for 1 hour with intermittent sonication to ensure complete solubility of the drug. This stock solution was filtered and clear filtrate was diluted to desired concentrations.

Chromatographic conditions

The mobile phase was methanol/water (80:20 v/v). The pH of this mobile phase was adjusted to 3.1 with phosphoric acid (85%). Prior to delivering into the system it was filtered through 0.45 µm filter and degassed using a sonicator. The samples were introduced by injector with a 20-µliter sample loop. The analysis was carried out under isocratic conditions using a flow rate 0.5 ml/min at ambient temperature. Chromatograms were recorded at 236 nm using a detector SPD-10AV VP Shimadzu UV visible.

Validation procedures

The method was validated for the parameters like system suitability, specificity, range and linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, ruggedness and robustness.

The system suitability was assessed by five replicate analyses of the drug at a concentration of 50 µg/ml. System suitability of the method was evaluated by analyzing the repeatability, peaks symmetry (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 chromatographic method was determined to ensure separation of internal standard and diltiazem. Specificity was also determined in the presence of excepients used in formulation, DTZ, was spiked (at a concentration of 12.5µg/ml) in drug product and chromatogram was observed and compared with that of raw material. The purity of the peak was checked using a UV-Visible detector. To evaluate the linearity, the LOD and LOQ of the method in reference drug, serial dilutions were made from the standard stock solution in the working range of 0.195-50 µg/ml and volume was made up with diluent which was a mixture of 80:20 MeOH:H_2O and resolved on a C18 column with UV detection at 236nm. To determine accuracy of the method and recovery of diltiazem in dosage formulation, working standard of DTZ was prepared in the range of 0.195-50µg/ml. Samples for recovery studies were also prepared by spiking known amount of drug in the placebo at three concentration levels (80, 100 and 120%) and analyzed. The precision of the method was investigated with respect to repeatability. To determine intermediate precision, standard solutions of DTZ at nine concentration levels were analyzed three times within the same day (intra-day variation) and on three different days (inter-day variation). Generally acceptable repeatability of the results within one day and day-to-day was observed. Robustness studies were performed on method precision sample concentration 25µg/ml by making slight variations in flow rate, amount of methanol, and pH changes one at a time.

Assay in formulations

In case of marketed formulations, twenty accurately weighed tablets of each brand were crushed to a fine powder and an amount equivalent to 10 mg of DTZ was added into different 100 ml volumetric flasks and volume was made up with MeOH:H_2O mixture to give a concentration of approximately 100 µg/ml. This mixture was allowed to stand for 1 hour with intermittent sonication to ensure complete solubility of the drug (stock solution). The samples were filtered through a 0.45-µm-membrane filter and serial dilutions (0.195, 0.315, 0.78, 1.56, 6.25, 25, 50µg/ml) were made in 25ml volumetric flask (containing 50 µg/ml flurbiprofen as internal standard) and were injected for HPLC analysis.

RESULTS AND DISCUSSION

The use of HPLC methods for the quantitation of drug has become a routine consideration in the quality control of drugs and drug products. The goal of this study was to develop a rapid, easy accurate, precise reliable and least time consuming HPLC method for the analysis of diltiazem from raw materials, bulk drug samples, tablets or other formulations using the most commonly employed C-18 column with UV detector.

Development and optimization of isocratic HPLC conditions

Initial method development was conducted on a Hypersil, ODS, C-18 (150×4.6 mm, 5 micron) column for

Table 1: System suitability parameters

Parameters	Minimum	Maximum	Average	%RSD
Retention time (min)	2.45	2.60	2.57	2.40
Peak area of DTZ	435719	446160	442021	0.87
Capacity factor (*K*')	0.00	0.00	0.00	0.00
Tailing factor (*T*)	1.37	1.39	1.373	0.59
Theretical plates (*N*)	1688	1918	1863	4.65
Resolution (R)	4.65	4.76	4.71	1.23

Table 2: Regression statistics of the proposed method

Concentration	Goodness-of-fit	Slope	Intercept	Standard error of estimate	P value
range(µg/ml)	(*R*2)				
0.195-50	0.999549	16759.035	16630.46	0.5571	0.000

separation at ambient temperature. This column provides efficient and reproducible separation of the components while minimizing solvent usage. Consequently, it was selected for the method development. Among the suitable isocratic conditions to resolve diltiazem on C_{18} column, the mobile phase methanol-water (80:20 v/v) adjusted pH with phosphoric acid to 3.1 was found to provide a reproducible, baseline and resolved peak. The lower percentage of methanol in mobile phase results in peak tailing of both components. Optimal retention times (diltiazem =2.6 minutes and flurbiprofen = 3.99 minutes) were achieved when the pH of mobile phase was adjusted to 3.1 with 85% phosphoric acid.

The chromatographic conditions were optimized with respect to specificity, resolution and time of analysis. The specificity of the method was established through the study of resolution factor of diltiazem peak from the internal standard peak. Peaks were identified using retention times compared with those of standards. The method has been successful in determining diltiazem in concentration, as low as 0.195 μg, with retention time of only 2.6 minutes. The internal standard, flurbiprofen was separated later at 3.99 minutes.

Method validation

The newly developed method has been validated and holds well for the determination of drug in raw materials and other dosage formulations.. For validation of analytical methods, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use [ICH 1996] and [USP 2004] have recommended the accomplishment of accuracy test, precision, specificity, linearity, ruggedness and robustness of the method.

System suitability testing

Typical system suitability results are summarized in table 1. All the values for the system suitability parameters were within limits **(**Swartz and Krull, 1998). System suitability test are an integral part of chromatographic methods and are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed.

Range and linearity

The range of an analytical method is the interval between the upper and lower analytical concentration of a sample where the method has shown to demonstrate acceptable accuracy, precision, and linearity **(**Krull and Swartz, 1997). The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range (United States Pharmacopeia 2000, USP 2004) The linearity of the method was observed in the expected concentration range demonstrating its suitability for analysis.The calibration curve was constructed with nine concentrations including the LOQ ranging from 0.195 to 50 μg/ml (fig. 3). The regression statistics are shown in table 2. The goodness-of-fit(R^2) was found to be > 0.999 and value of intercept was less than 2% of the response of 100% concentration in all the cases indicating functional linear relationship between the concentration of analyte and area under the peak.

Accuracy

Accuracy of an analytical method is the closeness in agreement between the accepted true value or a reference value and the actual result obtained. Accuracy studies are usually evaluated by determining the recovery of a spiked sample of the analyte into the matrix of the sample to be analyzed **(**Krull and Swartz 1997). The results of accuracy studies are shown in table 3. Recoveries of diltiazem were 94.1-99.39% and coefficients of variation ranged from 0.034% to 3.09% which is evident that the method is accurate within the desired range.

Precision

Precision is a measure of the ability of the method to generate reproducible results. The precision of a method is evaluated using three separate determinations for repeatability, intermediate precision, and reproducibility (Krull and Swartz 1997). Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment and is expressed as the % RSD. The results of intra-and inter-day variations are shown in table 4. The accuracy of the method ranged from 0.035% to 2.26%. The results obtained from intermediate precision (inter-day) also indicated a good method precision. All the data were within the acceptance criteria.

Detection and quantitation limit

The detection limit or LOD is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated. LOD was expressed as a concentration that gives a signal to noise ratio of 2:1 or 3:1 (United States Pharmacopeia, 2000; Aboudan *et al*., 2001). Quantitation limit or LOQ, on the other hand is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. LOQ is measured in terms of signal to noise ratio of 10:1 (United States Pharmacopeia 2000, Aboudan *et al.,* 2001). LOD and LOQ were calculated by the equation given in ICH guidelines (ICH guideline Q2B, 2003). This may be expressed as LOD = 3.3 σ /S and LOQ = 10 σ / S, where σ is the standard deviation of the response, S is the slope of the calibration curve which may be estimated from the calibration curve of the analyte. The limit of detection and limit of quantification of the proposed method at 236 nm were found to be 0.0184 μg/ml and 0.056 μg/ml respectively.

Specificity

Specificity is the ability of a method to discriminate between the analyte of interest and other components that are present in the sample (USP 2004, Krull and Swartz 1997). The specificity of the method was evaluated to ensure separation of diltiazem and was demonstrated by assaying samples of diltiazem and flurbiprofen. The method demonstrated good resolution between the peaks and was found to be free of interference. For demonstrating the specificity of the method for drug formulation, the drug was spiked, wherein the excepients used in different formulation products did not interfere with the drug peak and thus the method was specific for DTZ.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of the test results obtained by the same samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different lots of reagents and different days (Plackett and Burman 1943-1946, ASTM E 1169-89). The assay result indicated that the method was capable with high precision (table 4). Results of % RSD (0.084-1.912) of three different days prove the ruggedness of developed method.

Robustness

Robustness is a measure of the performance of a method when small, deliberate changes are made to the conditions of method (Plackett and Burman 1943-1946, ASTM E

Table 3: Accuracy / Recovery data for diltiazem

Parameters	Concentration (μg/ml)	% RSD	% Recovery	Recovered concentration
Assay	0.195	1.14	95.37	0.19
	0.39	1.63	95.20	0.37
	0.78	1.84	94.54	0.74
	1.56	3.09	99.39	1.55
	6.25	2.71	97.83	6.11
	25	0.70	98.67	24.67
	50	1.74	98.39	49.19
Spiking method (level)				
80%	10	0.098	95.5	9.5
		0.162	97.7	9.7
		0.133	98.0	9.8
100%	12.5	0.18	97.6	12.2
		0.21	95.8	11.97
		0.29	97.0	12.12
120%	15	0.072	96.4	14.46
		0.034	94.1	14.11
		0.199	99.0	14.85

Table 4: Intra-and inter-day, variation /intermediate precision of the method

Concentration	Day 1	Day 2	Day 3
(μg/ml)	(%RSD)	(%RSD)	(% RSD)
0.195	0.679	0.347	0.315
0.39	1.384	1.384	0.722
0.78	1.797	1.797	2.266
1.56	0.135	0.135	0.154
3.12	0.487	0.035	0.035
6.25	0.117	0.117	0.088
12.5	0.099	0.123	0.669
25	0.383	0.383	0.153
50	0.309	0.309	0.123

1169-89). The results of the robustness study are summarized in table 5.

Table 5: Robustness data for diltiazem

Parameter	% RSD	% Recovery
Flow rate 0.49ml/min	0.61	91.32
Methanol:water (82:18 v/v)	0.19	90.26
pH 3.0	0.39	90.67

Applicability of method for DTZ analysis

This developed HPLC method was sensitive and specific for the quantitative determination of diltiazem from dosage formulations. The method was applied to the determination of DTZ content in marketed formulations. The assay results are shown in table 6 demonstrating the suitability of method.

CONCLUSION

A rapid, precise, accurate, low cost and least time consuming RP-HPLC method for the qualitative and quantitative analysis, determination and quantification of diltiazem in raw materials as well as dosages formulations

Table 6: Assay of DTZ in marketed formulations

S. No.	Product	DTZ label claim (mg per tablet)	% RSD	% Recovery	Found (mg)
1	Dilzem	30	0.770	91.54	27.65
2	Tiazem	30	0.016	94.56	27.76
3	Etizem	30	0.067	93.61	28.08
4	Herbesser	30	0.057	92.80	27.97

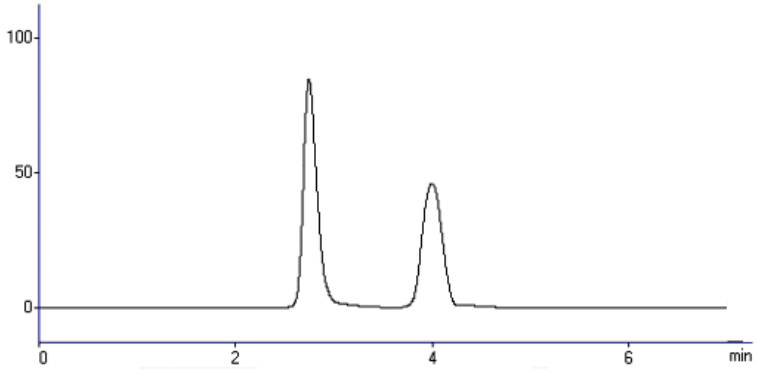

Fig. 2: Chromatogram showing resolution of DTZ from its internal standard. Retention time of DTZ –2.6 & flurbiprofen 3.99 minutes.

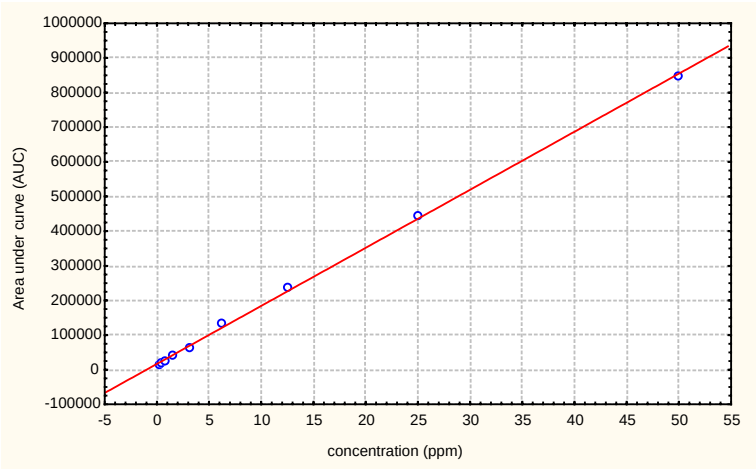

Fig. 3: Calibration curve showing linearity over the concentration range of 0.195-50 µg/ml.

has been successfully developed. The proposed RP-HPLC method enables the determination of diltiazem because of good separation and resolution of chromatographic peaks from internal standard. The accuracy and precision of the method were confirmed by the statistical parameters. Reliability, rapidness, simplicity, sensitivity, economical nature and good recovery of this HPLC method gives it advantage over to the other reported HPLC methods for determination of diltiazem.

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