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Short communication

High-performance thin-layer chromatographic determination of diltiazem hydrochloride as bulk drug and in pharmaceutical preparations

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Abstract

A simple, rapid, selective and precise high-performance thin-layer chromatographic method for the analysis of diltiazem hydrochloride both as a bulk drug and in pharmaceuticals is reported. The mobile phase composition was ethyl acetate–methanol–strong ammonia solution (80:10:10, v/v). Densitometric analysis of diltiazem hydrochloride was carried out at 238 nm. The calibration curve of diltiazem hydrochloride in distilled water was linear in the range 40–400 ng. The mean value of correlation coefficient, slope and intercept were 0.997 ± 0.0008, 0.0617 ± 0.0012 and 7.16 ± 0.2562, respectively. The limits of detection and quantitation were 20 ng and 40 ng, respectively. The recovery of diltiazem hydrochloride was about 99.5%. The method was utilized to analyse diltiazem hydrochloride from conventional and sustained release tablets in the presence of commonly used excipients. © 1998 Elsevier Science B.V.

Keywords: Diltiazem hydrochloride

1. Introduction

Diltiazem hydrochloride (2*S*,3*S*)-3-acetyloxy-5-[2-(dimethyl-amino)ethyl]-2-(4-methoxy phenyl)-2,3-dihydro-1,5-benzothiazepin-4(5H)·1HCl (Fig. 1) is a calcium channel blocker. It is used in the management of classical and vasospastic angina pectoris and has also been used in the treatment of essential hypertension [1]. Spectrophotometry [2,3], gas chromatography [4], HPLC [5–7] and capillary zone electrophoresis [8] have been reported for its assay in pharmaceuticals. Spectrophotometric assays, although simple, are not stability indicating.

High-performance thin-layer chromatography (HPTLC) facilitates automated application and scanning in situ. Several samples can be run simultaneously using a small quantity of mobile phase, unlike HPLC and GLC. This lowers analysis time and cost per analysis. Substances are permanently stored on the plate. This makes it possible to repeat detection (scanning) of one or several fractions of the chromatogram with the same or different parameters.

This paper describes a simple, rapid, precise and specific HPTLC method for measurement of dil-

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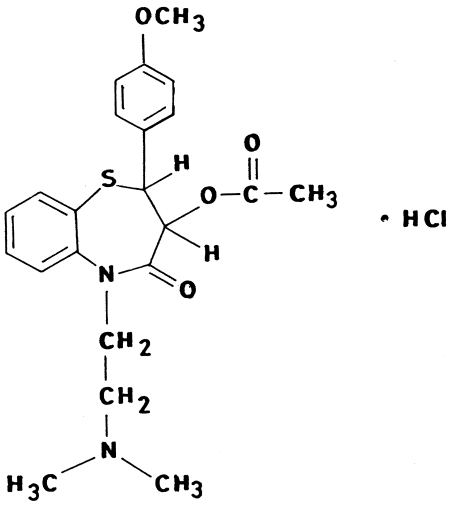

Fig. 1. Structure of diltiazem hydrochloride.

tiazem hydrochloride as bulk drug and from pharmaceutical dosage forms.

2. Experimental

2.1. Chemicals and reagents

Diltiazem hydrochloride U.S.P. grade (99.93% purity) was supplied by Cheminor (Hyderabad, India). Analytical grade solvents and reagents were purchased from Ranbaxy (New Delhi, India).

2.2. Preparation of standard solution

A stock solution of diltiazem hydrochloride (U.S.P grade) (1 mg/ml) was prepared in distilled water. Standard solution of 20 μg/ml was used.

2.3. Instrumentation

The samples were spotted on HPTLC aluminium plates (20×10 cm) precoated with silica gel 60 F_{254} (layer thickness 0.2 mm) (Merck) using Camag Linomat IV model. The plates were prewashed with methanol and air dried before use. The samples were streaked in the form of narrow bands of length 4 mm, 10 mm from the bottom edge, 5 mm from margin, 4 mm apart at a constant rate of 15 μl/s using a nitrogen aspirator. The migration distance was 7 cm with a migration time of 15 min. The separation was visualized with short wavelength (254 nm) ultraviolet lamp. Densitometric analysis of the separated components was carried out using Camag TLC scanner II in the absorbance mode at 238 nm. The slit dimensions were 6×0.3 mm and the sensitivity 230. Scanning speed was kept at 1 mm/s. Integration of chromatogram was performed using the Camag TLC scanner/integrator system.

2.4. Selection of mobile phase

Various solvent systems reported in the literature for TLC analysis of diltiazem hydrochloride were tried and an appropriate system consisting of ethyl acetate–methanol–strong ammonia solution (80:10:10, v/v) was selected.

2.5. Standard curve of diltiazem hydrochloride in distilled water

Appropriate quantities of standard solution (20 μg/ml) were spotted to obtain diltiazem hydrochloride in the concentration range of 40–400 ng (n=6).

2.6. Accuracy and precision of the assay

The accuracy of the assay was tested at 100 ng and 200 ng level of diltiazem hydrochloride. The concentration of diltiazem hydrochloride in the experimental samples (n=6) at each level was compared with the added concentration.

The intra-day precision was evaluated by analysing samples repeatedly at concentration of 100 ng and 200 ng of diltiazem hydrochloride (per spot) hourly for a period of 10 h (n=6 at each hour). The inter-day precision was similarly evaluated by repeated analysis of samples at concentrations of 100 ng and 200 ng of diltiazem hydrochloride (per spot) once a day for 10 days (n=6 each day).

2.7. Preparation of samples

The drug was extracted from conventional (C1; label claim-60 mg/tablet) and sustained release tablets (S1, S2; label claim-90 mg/tablet) as follows:

ten tablets were crushed to a fine powder and mixed well. Powder equivalent to 60 mg of drug for conventional tablets and 90 mg of drug for sustained release tablets was accurately weighed, dissolved in distilled water and shaken thoroughly. The contents were diluted to 50 ml with distilled water and then centrifuged for 10 min at 900 *g*. A 1-ml volume of supernatant was diluted to 100 ml with distilled water. A 10-μl volume of this solution was used for spotting and double spotting.

2.8. Recovery studies

The recovery studies were conducted by addition of 100 ng of standard diltiazem hydrochloride solution to various preanalysed tablet solution samples and the mixtures were reanalysed by the proposed method ($n=3$).

2.9. Degradation of diltiazem hydrochloride

Acid [7,9], light [7], temperature [7] and base [10] catalysed degradations of diltiazem hydrochloride have been reported. Diltiazem hydrochloride was degraded in solution by the following procedure: about 50 mg of diltiazem hydrochloride was accurately weighed and boiled vigorously with 15 ml hydrochloric acid buffer (pH 1.2) for 20 min. The contents were made up to 50 ml with water in a volumetric flask and stored at 60°C. A 2-ml volume of sample was withdrawn after 7 days and diluted to 50 ml with distilled water. A 20-μl volume of this solution was spotted.

3. Results and discussion

An R_f value of 0.54 was obtained using the solvent system ethyl acetate–methanol–strong ammonia solution (80:10:10, v/v). All standard curves ($n=6$) were linear over the range 40–400 ng. No significant difference was observed in the slopes of standard curves (ANOVA; $P>0.05$). The standard curve is depicted in Fig. 2. The mean values (±S.D.) of correlation coefficient, slope and intercept were 0.997±0.0008, 0.0617±0.0012 and 7.16±0.2562, respectively. The limit of detection for diltiazem hydrochloride was 20 ng. The limit of quantitation

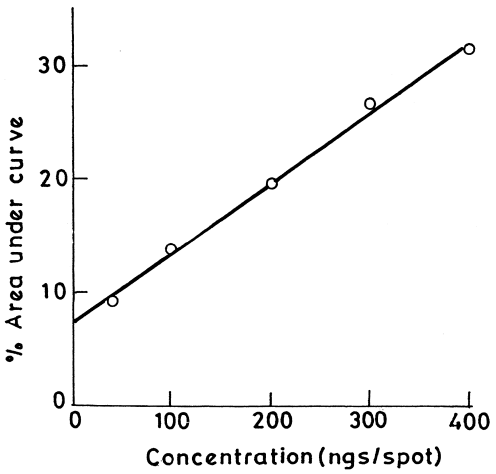

Fig. 2. Standard curve of diltiazem hydrochloride.

was 40 ng with coefficient of variation 1.05% ($n=6$). A typical chromatogram at the limit of quantitation is depicted in Fig. 3a.

The results in Table 1 revealed excellent accuracy and high precision of the assay method. The low coefficient of variation was indicative of acceptable intra-day and inter-day precision of the assay. The recovery of diltiazem hydrochloride was greater than 99.5% and is reported in Table 2. There was no

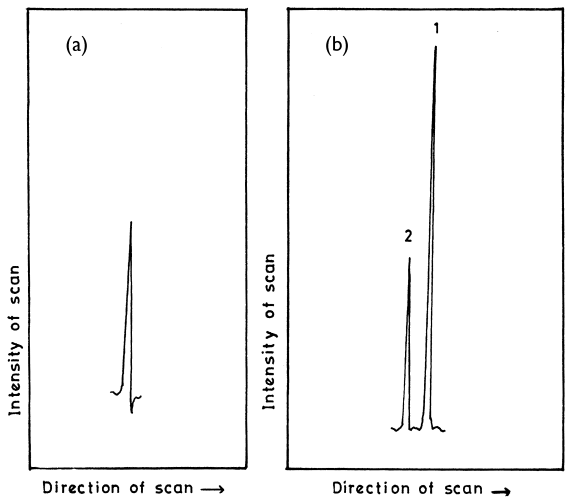

Fig. 3. Typical chromatogram of (a) diltiazem hydrochloride at limit of quantitation–40 ng, (b) diltiazem hydrochloride (1) and its degradation product (2).

Table 1
Accuracy and precision of the assay

Added concentration (ng/spot)	Concentration found (mean±S.D.) (ng/spot)	Coefficient of variation (%)
Accuracy[a]		
100	98±3	3.06
200	197±2	1.02
Intra-day precision[b]		
100	97±2	2.06
200	198±3	1.51
Inter-day precision[c]		
100	98±4	4.08
200	196±5	2.55

[a] $n=6$.
[b] $n=6$ at each hour.
[c] $n=6$ each day.

interference from the common excipients present in conventional tablets or sustained release tablets.

A stability indicating ion-pair reversed-phase high-performance chromatography assay for diltiazem hydrochloride has been reported by Abdel-Hamid et al. [7] wherein desacetyldiltiazem, being more polar, eluted faster than diltiazem hydrochloride. Analogously our HPTLC method revealed an additional spot with an R_f value of 0.1, which was well separated from the spot of diltiazem hydrochloride ($R_f=0.54$). Based on the report of Suleiman et al. [9] this additional spot was assumed to be desacetyldiltiazem obtained by hydrolysis of the more labile acetyl group of diltiazem hydrochloride. A typical chromatogram of diltiazem hydrochloride and its hydrolysed degradation product is shown in Fig. 3b. The developed analytical method could therefore be considered a stability indicating method for analysis of diltiazem hydrochloride. A single spot at an R_f value of 0.54 was observed in the drug samples extracted from the conventional and sustained release tablets. It could therefore be suggested that no degradation of diltiazem hydrochloride occurred in the conventional or in the sustained release tablets.

Table 2
Recovery studies

Sample	Amount found (mg/tablet)	Recovery[a] (%) (mean±S.D.)
C1	59.90	99.62±0.57
S1	89.85	99.50±0.60
S2	89.67	99.54±0.40

[a] $n=3$.
C1=60 mg conventional tablet; S1,S2=90 mg sustained release tablet.

4. Conclusion

The proposed method is simple, rapid, sensitive and precise. It could be used as a stability indicating assay technique for analysis of diltiazem hydrochloride as the bulk drug and from pharmaceutical dosage forms. It could also be extended to study the degradation kinetics of diltiazem hydrochloride and for its estimation in plasma and other biological fluids.

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