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ORIGINAL RESEARCH PAPER

High-performance thin-layer chromatography–spectrodensitometric determination of diltiazem hydrochloride and its commonly occurring degradation impurity

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Abstract
The present work encompassed the assay quantification of diltiazem hydrochloride and its degradation impurity F by high-performance thin-layer chromatography (HPTLC). HPTLC was performed with chloroform–methanol–formic acid (7.5:1.5:0.2, *V/V*) as the mobile phase and aluminum-backed thin layer chromatography (TLC) plates precoated with a 200 µm layer of silica gel 60 F_{254} as the stationary phase. The densitometric chromatograms were performed at 254 nm, and the method was validated as recommended in the International Council for Harmonisation (ICH) guidelines. Statistical data from the analysis showed that the method is precise and highly sensitive. The correlation coefficient values of diltiazem hydrochloride and its impurity F were 0.9998 and 0.9975, respectively. The limit of detection was 76.81 ng/zone for diltiazem hydrochloride and 29.21 ng/zone for impurity F, while the limit of quantification was 232.77 ng/zone for diltiazem hydrochloride and 87.05 ng/zone for impurity F. The preciseness of the method was demonstrated and calculated as the % relative standard deviation for diltiazem hydrochloride and its impurity F. The mean recovery of the accuracy for diltiazem hydrochloride was between 98.23% and 100.29%, whereas for impurity F it was 100.02–100.29%. The simplicity of the method proves its applicability for the quantification of impurity F during the synthesis of diltiazem hydrochloride.

Keywords High-performance thin-layer chromatography · HPTLC · Method development · Validation · Diltiazem hydrochloride · Impurity F

1 Introduction

Diltiazem hydrochloride is chemically (2S,3S)-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl-acetate hydrochloride (Fig. 1A). The molecular formula of diltiazem hydrochloride is $C_{22}H_{27}ClN_2O_4S$, which corresponds to the relative molecular mass 450.97. It appears as a white to off-white powder which is soluble in water, methanol, ethanol, dimethyl sulfoxide, chloroform, and formic acid [1–4].

Diltiazem hydrochloride is one of the members of the non-dihydropyridine calcium channel blockers (CCB), which employs a variety of slow-release delivery systems to increase its duration of action and thereby decrease the dosing frequency [5]. Diltiazem hydrochloride decreases the myocardial contractility and peripheral vascular resistance of smooth muscle [6, 7].

There may be many known and unknown organic impurities associated in the synthesis of the said drug molecule. The current version of the US Pharmacopeia (USP) and the European Pharmacopoeia (EP) both summarize the identification and allowable limits for identified (impurity A–F) and non-identified organic impurities.

With regards to USP, the details and passing criteria of impurity A–F are well described, and clearly specify the discrete liquid chromatography test and allowable limits of impurity F, that is desacetyl diltiazem [7]. Similarly, EP indicates

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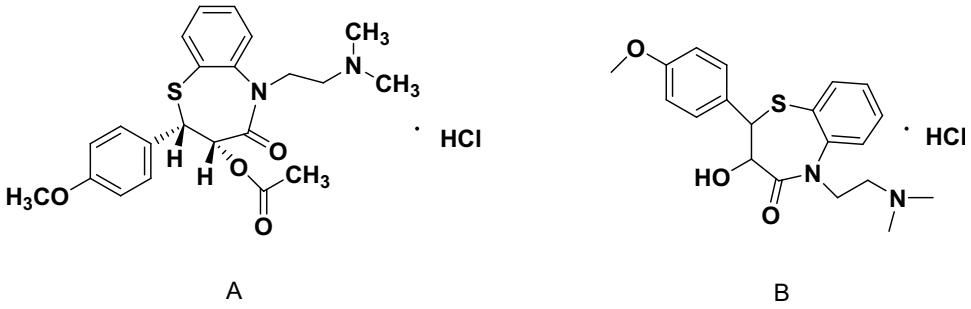

Fig. 1 **A** The chemical structure of diltiazem hydrochloride; **B** impurity F of desacetyl diltiazem hydrochloride

the impurity F as specified impurity where the allowable limits of the said impurity are three times higher than other identified and non-identified impurities occurring during the synthesis [8].

Diltiazem hydrochloride impurity F (Fig. 1B) is chemically known as (2S,3S)-5-[2-(dimethylamino)ethyl]-3-hydroxy-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepin-4(5H)-one) and commonly known as desacetyl diltiazem. The relative molecular mass and molecular formula of impurity F are 408.94 and $C_{20}H_{25}ClN_2O_2S$, respectively. In the aqueous medium, diltiazem hydrochloride easily turns into desacetyl diltiazem owing to hydrolysis under moderate to slightly high temperatures. It may also lead to the easy formation of degradation product in various pharmaceutical dosage forms [9]. Thus, controlling and monitoring impurity F in bulk and the pharmaceutical dosage form are of utmost importance.

A detailed survey of literature reveals that many analytical methods have been reported for the qualitative and quantitative determination of diltiazem hydrochloride in bulk and marketed formulation using ultraviolet−visible (UV−Vis) spectroscopy, high-performance liquid chromatography and high-performance thin-layer chromatography (HPTLC) [10–15]. A few validated methods are also reported for the quantification of diltiazem hydrochloride in the presence of its known impurities. In this work, we developed and validated a method for the simultaneous determination of diltiazem hydrochloride and its potent impurity F using HPTLC. The work was conducted in accordance with the International Council for Harmonisation (ICH) guidelines [17]. Thin layer chromatographic (TLC) technique plays an essential role during initial drug development to identify the purity of the drug and presence of entities other than the drug. Moreover, HPTLC meets all the quality requirements of current analytical laboratories [16].

2 Experimental

2.1 Instruments

CAMAG HPTLC system connected with Linomat 5 sample applicator (CAMAG, Muttenz, Switzerland), twin-trough developing chamber (10 × 20 cm), dual lamp containing UV chamber (CAMAG), winCATS software (Version 1.4.6, CAMAG), Hamilton syringe (100 µL capacity; Bonaduz, Switzerland), aluminum-backed TLC plates (5 × 10 cm) precoated with a 200 µm layer of silica gel 60 F_{254} (E. Merk, Darmstsdt, Germany) were used for chromatographic method development. After development of the HPTLC plate, it was further scanned using CAMAG TLC Scanner 3.

2.2 Chemicals and reagents

The samples and working standards of purified diltiazem hydrochloride (99.95%) and impurity F were procured from Anlon Healthcare Pvt. Ltd. (Rajkot, India). All the spectroscopic-grade solvents and analytical-grade reagents were purchased from Loba Chemicals Pvt. Ltd. (Mumbai, India). High-purity deionized water was obtained from Merck Milli-Q Direct-Q 3 water purification system (Merck Millipore, Burlington, MA, USA).

2.3 Chromatographic development

The HPTLC plates were prewashed with methanol and dried for 5 min in an oven at 110 ℃ prior to sample application. Linomat 5 semi-automatic autosampler was used as a sample applicator on the HPTLC plate, 15 mm from the bottom edge with 8 mm band size. After sample application, the development chamber was saturated with mobile phase at ambient temperature for 20 min. The HPTLC plate was monitored until 70 mm development distance. After complete development of the HPTLC plate, it was dried and scanned by TLC Scanner 3 with 6 × 0.2 mm slit dimension at 254 nm. The scanning speed of the instrument was 10 mm/s. The developed chromatograms were operated by winCATS software (version 1.4.6).

2.4 Preparation of standard solution of diltiazem hydrochloride

The standard solution (1000 µg/mL) of the powdered diltiazem hydrochloride was prepared by dissolving 10 mg of working standard in 10 mL methanol. An aliquot of

about 5% of standard impurity F was added to the powdered diltiazem hydrochloride for identification and method validation.

2.5 TLC analysis of impurity F

The procured sample of diltiazem hydrochloride (5 mg) was dissolved in 1 mL methanol to make a sample solution. Similarly, the standard solution of impurity F (5 mg) was prepared by dissolving it in 1 mL methanol. A precoated silica gel G TLC plate (Merck) of dimensions 2.5 × 5 cm was taken. The sample and standard solutions were simultaneously applied to the TLC plate and developed using the solvent system ethyl acetate−*n*-hexane (1:1, *V/V*). The resultant TLC was visualized under UV light (254 nm) and R_F was calculated and found to be the same.

2.6 Method validation

The linearity of the method was demonstrated by measuring the correlation coefficient of five different concentrations ranging from 1800 to 4200 ng/zone for diltiazem hydrochloride and 90 to 210 ng/zone for impurity F. All the samples were applied to HPTLC plates twice to cover the concentration range and establish linearity. The correlation coefficient value was calculated by plotting the area under the curve *versus* concentration for diltiazem hydrochloride and impurity F.

The limit of detection (LOD) and limit of quantification (LOQ) were confirmed by analyzing samples with known concentrations and finding the minimum level at which the analyte can be reliably detected. Here LOD is determined as the signal-to-noise ratio of 3:1, whereas LOQ is determined as the signal-to-noise ratio of 10:1.

The precision of the method was calculated by studying the intraday and interday precisions. For intraday precision, six sets of solutions (3000 ng/zone for diltiazem hydrochloride and 150 ng/zone for impurity F) were prepared individually and measured twice, whereas for interday precision, the same protocol was performed under the same conditions on different days.

The accuracy of the method was calculated by performing the same experimental condition for three different concentrations of sample solution. The % mean recovery was calculated for 80%, 100%, and 120% (corresponding to 2400, 3000, and 3600 ng/zone) concentration level by adding known quantity of sample. For each set, three samples were separately prepared and measured twice.

The robustness of the method was confirmed by making small but deliberate changes in the analytical parameters of the developed method.

3 Results and discussion

3.1 Method development and optimization

The method was optimized for the simultaneous quantification of diltiazem hydrochloride and its impurity F. In the development trails, different proportions of solvents were employed to obtain the best separation over HPTLC plate for quantification. The mobile phase containing chloroform−methanol−formic acid (7.5:1.5:0.2, *V/V*) provided idle resolution with an R_F value of 0.68 ± 0.02 for diltiazem hydrochloride and 0.52 ± 0.02 for impurity F. The presence of impurity F was confirmed by comparing the R_F values of the impurity present in sample with the impurity standard, and it was further confirmed by mass spectroscopy (Figs. 2, 3). The selection of the wavelength was confirmed by taking UV−Vis spectrum of the samples. From the UV−Vis spectrum, the wavelength of the detection was fixed at 254 nm. Earlier, diltiazem and its impurity F were determined by HPTLC in raw material and in tablet formulation [18]. The present study is an advancement of the previously reported method in terms of robustness and confirmation of the impurity F over HPTLC and mass spectrometry.

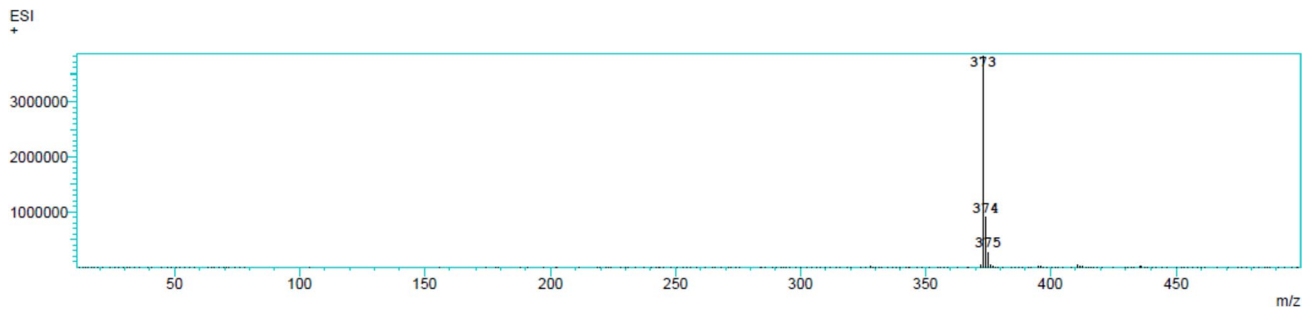

Fig. 2 Mass spectrum of sample of impurity F (desacetyl diltiazem)

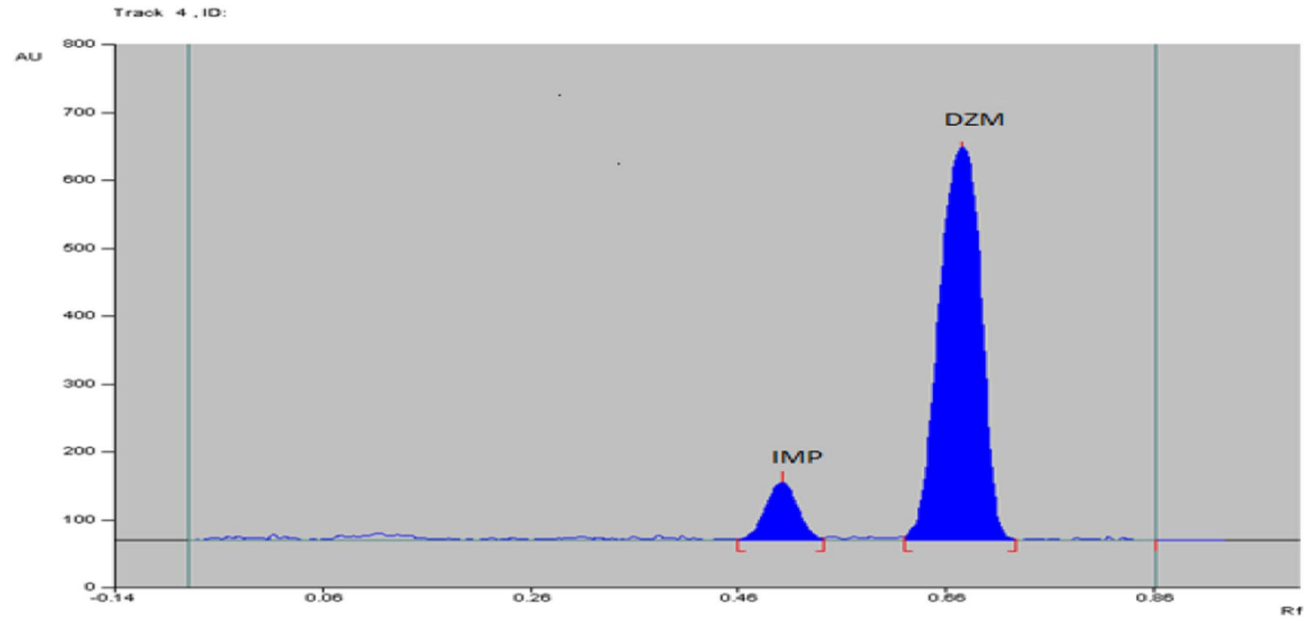

Fig. 3 TLC densitogram of diltiazem hydrochloride (3000 ng/zone) and its impurity F (150 ng/zone)

3.2 Method validation

3.2.1 Linearity

The linearity of the method was confirmed by calculating the correlation coefficient value of different concentrated test preparations ranging from 1800 to 4200 ng/zone for diltiazem hydrochloride and 90 to 210 ng/zone for impurity F. The chamber was saturated for 20 min with mobile phase at ambient temperature. The linear regression data of the calibration curve, listed in Table 1, showed good linear relationship over the calibration range (Fig. 4). Moreover, the correlation coefficient values of diltiazem hydrochloride and its impurity F were 0.9998 and 0.9975, respectively (Figs. 5, 6).

3.2.2 Precision

The method's precision was confirmed by calculating % relative standard deviation (%RSD) in terms of intraday and interday precision. It was further found that, for intraday precision, the %RSD value was 1.49 for diltiazem hydrochloride and 1.70 for impurity F, whereas, for interday precision, the %RSD value was 0.62 for diltiazem hydrochloride and 0.55 for impurity F (Table 2).

Table 1 Linearity study data

Set	Concentration (ng/zone)	Average area under the curve	R^2 value	Regression equation	LOD & LOQ
Diltiazem hydrochloride					
1	1800	8160.3	0.9998	$y = 4.4078x + 285.74$	76.81 ng/zone
2	2400	10,900.5			
3	3000	13,600.5			232.77 ng/zone
3	3600	16,100.1			
5	4200	18,783.8			
Impurity F					
1	90	1489.6	0.9975	$y = 17.027x + 1.84$	29.21 ng/zone
2	120	2111.1			
3	150	2549.6			87.05 ng/zone
4	180	3059.5			
5	210	3569.4			

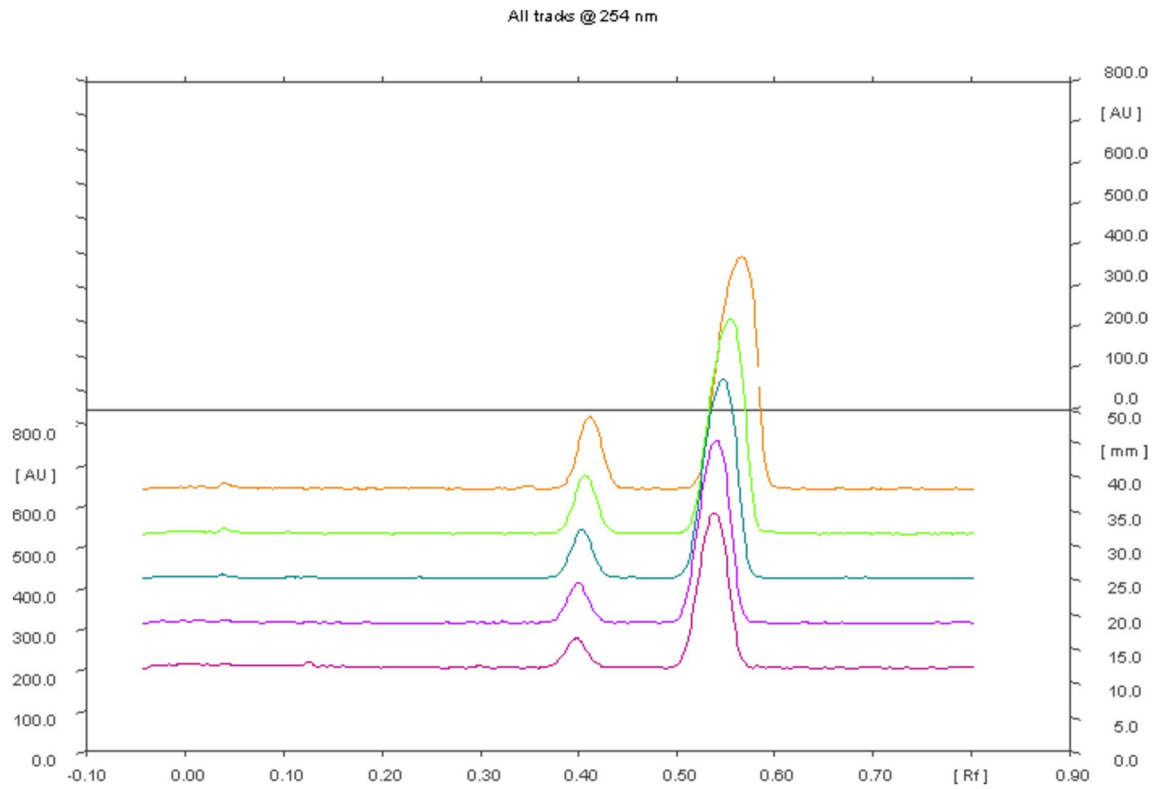

Fig. 4 Chromatogram of calibration curve

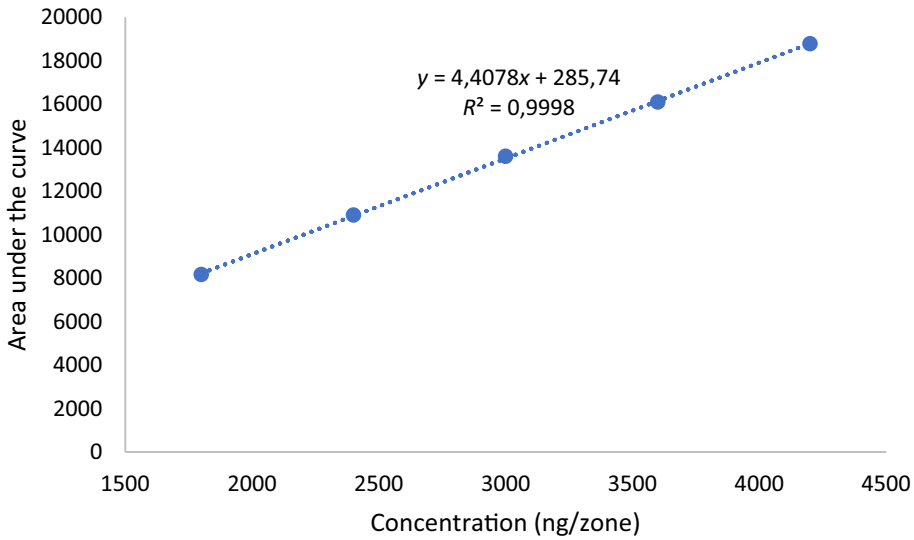

Fig. 5 Linearity study of diltiazem hydrochloride

3.2.3 Accuracy

The accuracy of the method was demonstrated by % mean recovery of three different concentrated test preparations. The mean recoveries of diltiazem hydrochloride and its impurity F were measured at 1500, 3000, and 4500 ng/zone and 75, 150, and 225 ng/zone, respectively, which corresponds to 50, 100, and 150% of the standard preparation, respectively. The mean recovery value of diltiazem hydrochloride was between 98.23% and 100.29%, whereas for impurity F it was 100.02−100.23% (Table 3).

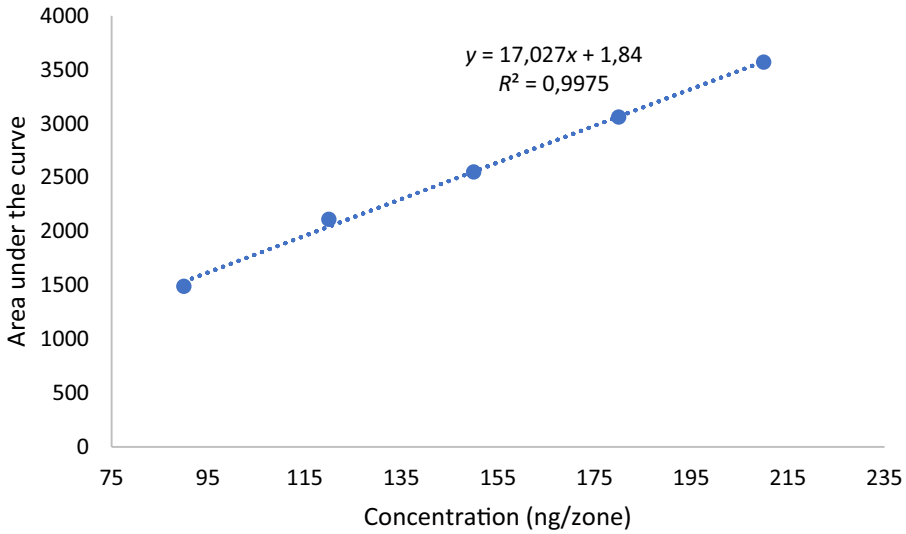

Fig. 6 Linearity study of impurity F

Table 2 Precision study data

Set	Diltiazem hydrochloride		Impurity F	
	Area under the curve		Area under the curve	
	Intraday	Interday	Intraday	Interday
1	13,321.6	13,254.2	2468.4	2500.3
2	13,588.7	13,255.6	2549.7	2506.7
3	13,872.9	13,356.9	2419.4	2480.5
4	13,682.3	13,321.6	2483.9	2477.1
5	13,410.5	13,450.2	2490.9	2499.8
6	13,450.6	13,229.3	2500.1	2510.3
Avg.	13,554.4	13,311.3	2485.4	2495.7
%RSD	1.49	0.62	1.70	0.55

3.2.4 Robustness

The robustness of the method was confirmed by checking the assay of diltiazem hydrochloride. The results of the robustness assays were 99.84% for chloroform−methanol−formic acid (7.3:1.5:0.2, *V/V*), 101.24% for chloroform−methanol−formic acid (7.7:1.5:0.2, *V/V*) and 100.23% for chloroform−methanol−formic acid (7.3:1.5:0.15, *V/V*). The observed data also confirm the robust nature of the method in terms of pH as the results were unaffected during small but deliberate changes in the amount of formic acid (Table 4).

Table 3 Accuracy study data

% Level	Set	Area	Amount found	Amount added	% Recovery	% Mean recovery	SD	%RSD
Diltiazem hydrochloride								
80	1	10,657.2	2358.7	2400.0	98.28	98.23	0.04	0.04
	2	10,647.5	2356.6	2400.0	98.19			
	3	10,651.2	2357.4	2400.0	98.22			
100	1	13,321.6	2948.4	3000.0	98.28	100.29	2.03	2.02
	2	13,588.7	3007.5	3000.0	100.25			
	3	13,872.9	3070.4	3000.0	102.34			
120	1	15,985.9	3538.1	3600	98.28	98.30	0.01	0.01
	2	15,990.2	3539.1	3600	98.30			
	3	15,991.2	3539.3	3600	98.31			
Impurity F								
80	1	1990.0	119.6	120.0	99.67	100.09	0.53	0.53
	2	1995.1	119.9	120.0	99.92			
	3	2010.5	120.8	120.0	100.69			
100	1	2501.3	150.3	150.0	100.22	100.02	0.31	0.31
	2	2500.4	150.2	150.0	100.18			
	3	2487.3	149.4	150.0	99.66			
120	1	3000.4	180.3	180.0	100.18	100.29	0.20	0.20
	2	3010.9	180.9	180.0	100.53			
	3	2999.8	180.2	180.0	100.16			

Table 4 Robustness of study data

Parameter	Initial condition	Changed condition	% assay
Mobile phase composition	Chloroform−methanol−formic acid (7.5:1.5:0.2, *V/V*)	Chloroform−methanol−formic acid (7.3:1.5:0.2, *V/V*)	99.84
		Chloroform−methanol−formic acid (7.7:1.5:0.2, *V/V*)	101.24
	Chloroform−methanol−formic acid (7.5:1.5:0.2, *V/V*)	Chloroform−methanol−formic acid (7.5:1.5:0.15, *V/V*)	100.23

4 Conclusions

A simple, sensitive, and robust HPTLC method has been developed and validated for the simultaneous quantification of diltiazem hydrochloride and its impurity F. The chromatograms of the developed method showed good separation between drug and impurity. The method was carefully validated as per recommendations of regulatory guidelines, and it was found to be linear, precise, accurate, and robust. This method is directly applicable for the quantification of diltiazem hydrochloride and its impurity F.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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