

HPTLC and RP-HPTLC Method Development and Validation for the Estimation of Felodipine in Bulk and Pharmaceutical Formulation

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Abstract

The aim of this work is to establish developed and validated method for the pharmaceutical analysis of Felodipine in bulk and pharmaceutical formulation by High Performance Thin Layer Chromatography HPTLC (NP) and Reverse Phase-High Performance Thin Layer Chromatography RP-HPTLC (RP). Chromatographic separation was performed on Pre-coated aluminum plates with 250 μ m layer of Silica gel 60 F₂₅₄ and Silica gel 60 RP-18 TLC F₂₅₄S using Toluene: Methanol (8:2 v/v) and acetonitrile: water: glacial acetic acid (8:2:1 v/v/v) as a mobile phase, respectively. Scanning was carried out densitometrically at 237 nm. The R_f value of Felodipine in NP and RP were 0.40 and 0.53 and the reliability of the method was assessed by the evaluation of linearity which was found to be 300-1800 and 500-3000 ng/band with the r²=0.998 correlation coefficient along with the accuracy of the method in terms of % recovery was found to be from 98-101 \pm 1.04 % and 99-100 \pm 0.47 % and the limit of detection and quantification were 11.51, 34.90 and 29.90, 90.61, respectively. The method can be used for routine analysis of Felodipine in bulk and pharmaceutical formulation.

Keywords: Felodipine (FDP); TLC; Densitometry; Validation

Introduction

Felodipine (FDP) is chemically known as 4-(2,3-Dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acid ethyl methyl ester (Figure 1). The molecular formula is C₁₈H₁₉Cl₂NO₄. This corresponds to a molecular weight of 384.3 gm [1]. It is a dihydropyridine calcium channel blocker used mainly for the management of hypertension and angina pectoris like the other calcium channel blockers [2]. Being a dihydropyridine derivative, FDP has the advantages of being a more selective vasodilator and having less cardiac effects than non-dihydropyridine calcium antagonists [3].

In the literature survey analytical methods have been reported for the estimation of Felodipine which include capillary gas chromatography [4], RP-HPLC [5-8], Stability-Indicating RP-HPLC [9,10], Micellar liquid chromatography [11], LC-MS [12], Spectroscopy [13-15], HPLC in rabbit plasma and pig serum [16,17].

The aim of this work is to establish simple, precise, sensitive and effective RPy HPTLC and RP-HPTLC in Bulk and Pharmaceutical Formulation. The method was validated as per ICH guidelines.

Experimental

Chemical and reagents

Felodipine (Pure) were obtained as a gift sample from Glenmark Pharmaceutical Ltd., Mumbai. All chemicals and reagents were used of HPLC grade and were purchased from Merck Chemicals, India.

HPTLC instrumentation

The High Performance Thin layer Chromatography (HPTLC) system (Camag Switzerland) having Camag Linomat 5 applicator with Camag TLC Scanner 3, Camag twin trough developing chambers (20 \times 10 cm), Camag UV Cabinet with dual wavelength UV lamps (254 and 366 nm), Camag Hamilton micro syringe (100 μ L), Pre-coated aluminum plates with 250 μ m layer of Silica gel 60 F₂₅₄ and Silica gel 60 RP-18 TLC F₂₅₄S and Electronic analytical balance Shimadzu AUX-120 was used for all the weighing.

Chromatographic conditions

Chromatographic separation was performed on 20 \times 10 cm pre-coated aluminum plates with 250 μ m layer of silica gel 60 F₂₅₄ and Silica gel 60 RP-18 TLC F₂₅₄S (E. Merck, Darmstadt, Germany) for NP and RP, respectively. The plates were prewashed with methanol and activated at 100°C for 10 minutes prior to the application. Sample was spotted in the form of 6 mm width with the help of Camag Hamilton micro syringe (100 μ L) on TLC plate from the bottom edge using Linomat 5 applicator. The TLC plate was developed in twin trough developing chamber using Toluene: Methanol (4:1 v/v) for NP and acetonitrile: water: glacial acetic acid (4:1:0.3 v/v/v) for RP, as mobile phase at room temperature (25°C \pm 2°C) with the 20 minutes of chamber saturation up to the 80 mm development distance. Densitometry scanning was

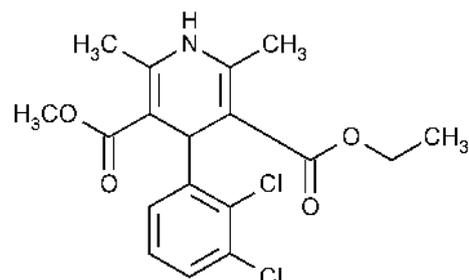


Figure 1: Structure of felodipine.

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performed at 237 nm on a Camag TLC scanner 3 and was operated by winCATS software version 1.3.0.

Preparation of standard stock solution

An accurately weighed 50 mg of Felodipine (FDP) was transferred into a 50 mL volumetric flask, dissolved in methanol and volume made up to the mark with the same solvent to achieve 1000 ng/ μ L.

Method Validation

Linearity

Linearity was performed using working standard stock solution of FDP in the range of 300-1800 ng/spot and 500-3000 ng/spot by applying 0.3-1.8 and 0.5-3.0 μ L, for NP and RP, respectively.

Analysis of tablet formulation

To determine the amount of Felodipine in tablets (Felogard ER-10 mg/tablet); twenty tablets were accurately weighed and finely powdered. An amount equivalent to 10 mg from tablets powder were transferred into 10 mL volumetric flask and extracted with methanol by shaking mechanically for 15 min and volume was made up to the mark and filtered using 0.41 μ m filter (Millifilter, Milford, MA). From the above solution 900 and 1500 ng/spot were applied for NP and RP, respectively.

Accuracy

Accuracy was determined in terms of % recovery. Recovery study of FDP was carried out by over spotting of the known amount of the standard FDP in the sample at 80%, 100% and 120% level for both methods. The total concentration of the drug was determined. At each level three determinations were performed.

Precision

Precision of the method was estimated as intra-day and inter-day changes by analyzing 600, 900, 1200 and 1000, 1500 and 2000 ng/spot in triplicate on the same day for three times for intra-day precision and three consecutive days for the inter-day precision for NP and RP, respectively.

Limit of detection (LOD) and Limit of quantification (LOQ)

Detection limit and Quantification limit was calculated by the RPased on the SD of the response and the slope of the calibration curve. Sensitivity of the proposed method was estimated as per ICH guidelines in terms of limit of detection $LOD=3.3\times\sigma/S$ and $LOQ=10\times\sigma/S$, where, σ is the standard deviation and S is the slope.

Specificity

The specificity of the method was determined by analyzing standard drug and tablet formulation. The spot of the FDP in the tablet formulation was confirmed by comparing the R_f value and the spectra with respect to standard drug. The peak purity was assessed at three different level i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

Robustness

Robustness of the method was performed by spotting 900 and 1500 ng/spot of FDP for NP and B on TLC plate by making small deliberate changes in the chromatographic conditions to examine the effects on the results by performing the parameters include mobile phase composition, development distance, activation of plate and duration of chamber saturation.

Result and Discussion

Development of optimum mobile phase

Different ratio of toluene: Methanol for NP and acetonitrile: water for RP was tried as a mobile phase but different errors was observed such as tailing of spot, less persistence and spreading of spots. In order to overcome the problems, toluene: methanol (8:2 v/v) and acetonitrile: water: glacial acetic acid (8:2:1 v/v/v) was tried and results observed in good resolution, sharp and symmetrical peak with R_f 0.40 (Figure 2) NP and 0.53 (Figure 3) RP. It was observed after prewashing of TLC with methanol (followed by drying and activation) and pre-saturation of chamber with mobile phases for 20 min.

Linearity

The linear regression data for the calibration curve of FDP by NP and B was determined in the concentration range of 300-1800 and 500-3000 ng/spot with the Linear regression equation $Y=4.4713x+813$ ($r^2=0.998$) and $Y=3.2239x+968$ ($r^2=0.998$) correlation coefficient. The calibration curve shown in Figures 4 and 5 and the 3-D linearity chromatogram is shown in Figures 6 and 7, respectively.

Analysis of tablet formulation

A single spot at R_f 0.40 and 0.53 was observed for NP and B in the chromatogram of FDP. There was no interference from the excipients commonly present in the tablet. The %drug content and %RSD were calculated. The low %RSD value indicated the suitability of this method for the routine analysis of Felodipine in pharmaceutical dosage forms. Results are discussed in Table 1.

Recovery study

The proposed methods when used for extraction and subsequent estimation of FDP from the pharmaceutical dosage form after over spotting with 80,100 and 120% of additional drug for both methods mean recovery is within acceptable limit. The % recovery listed in Table 2.

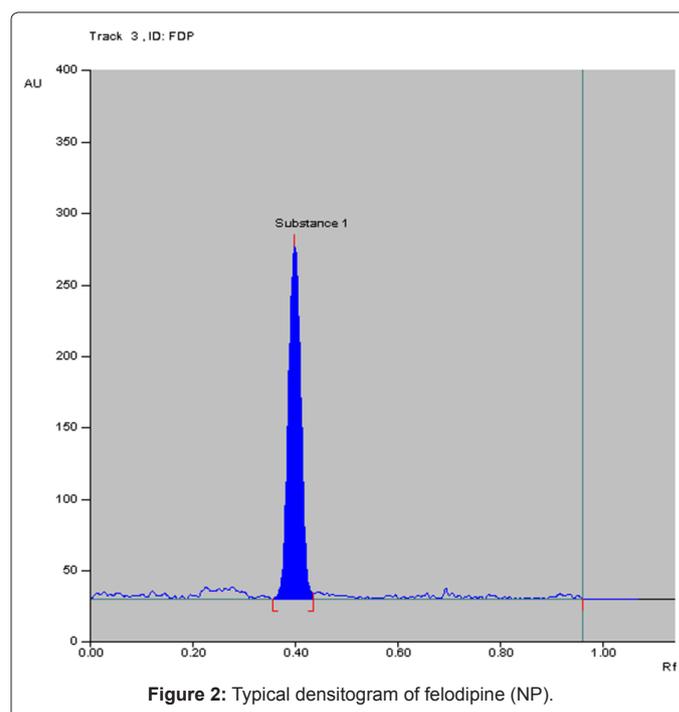
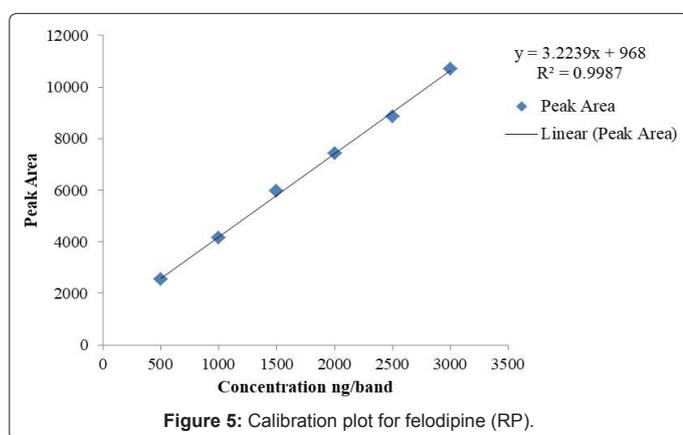
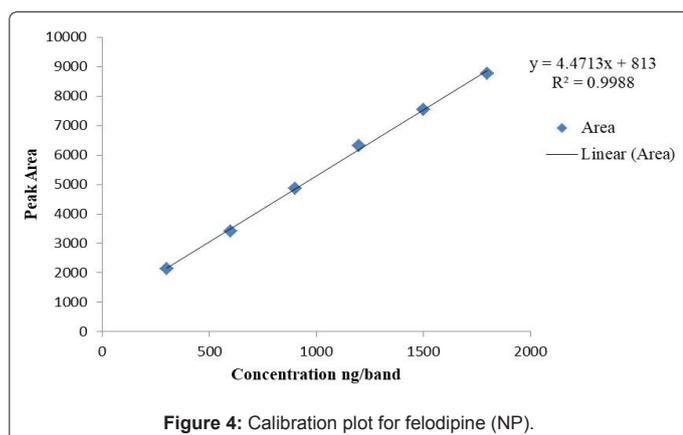
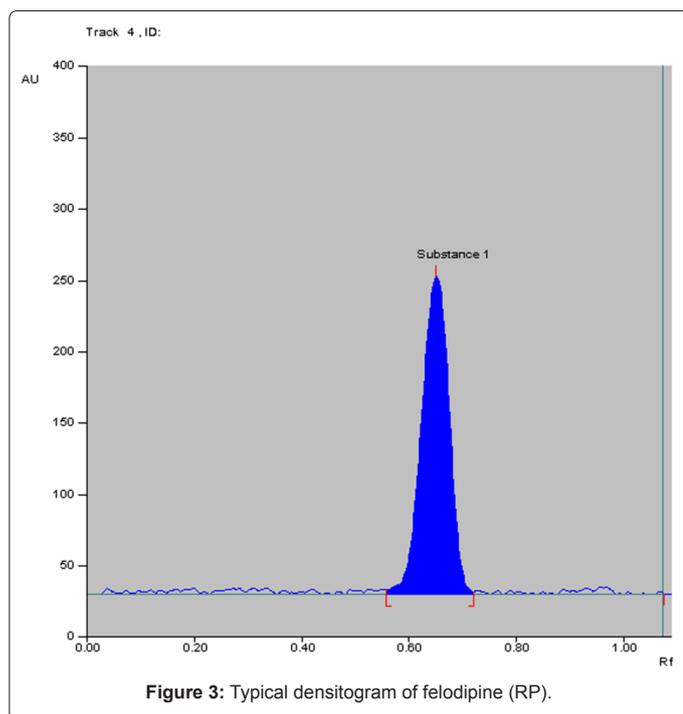


Figure 2: Typical densitogram of felodipine (NP).



Precision

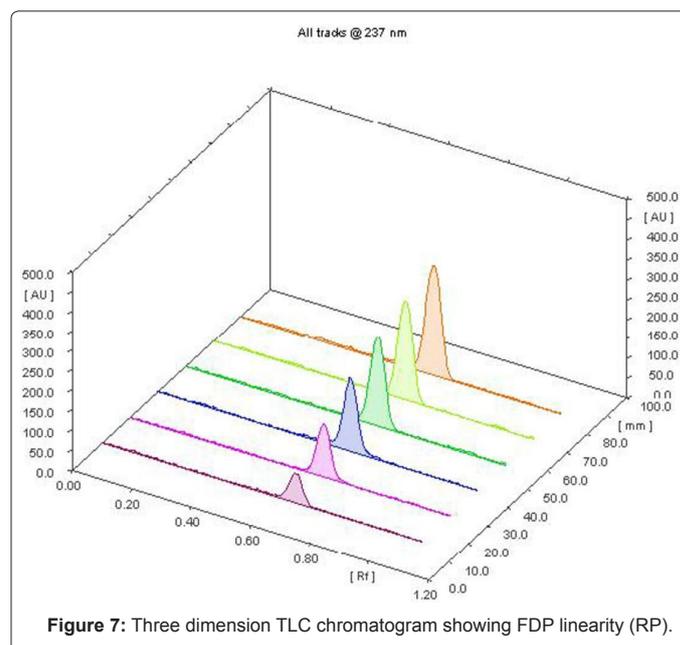
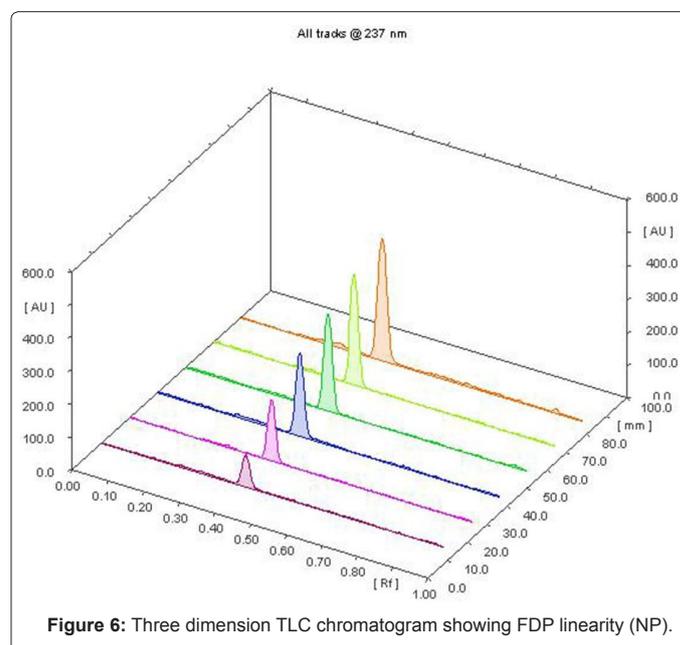
The precision of the proposed method was estimated in terms of % relative standard deviation (%RSD). The results depicted revealed high precision of the method is presented in Table 3.

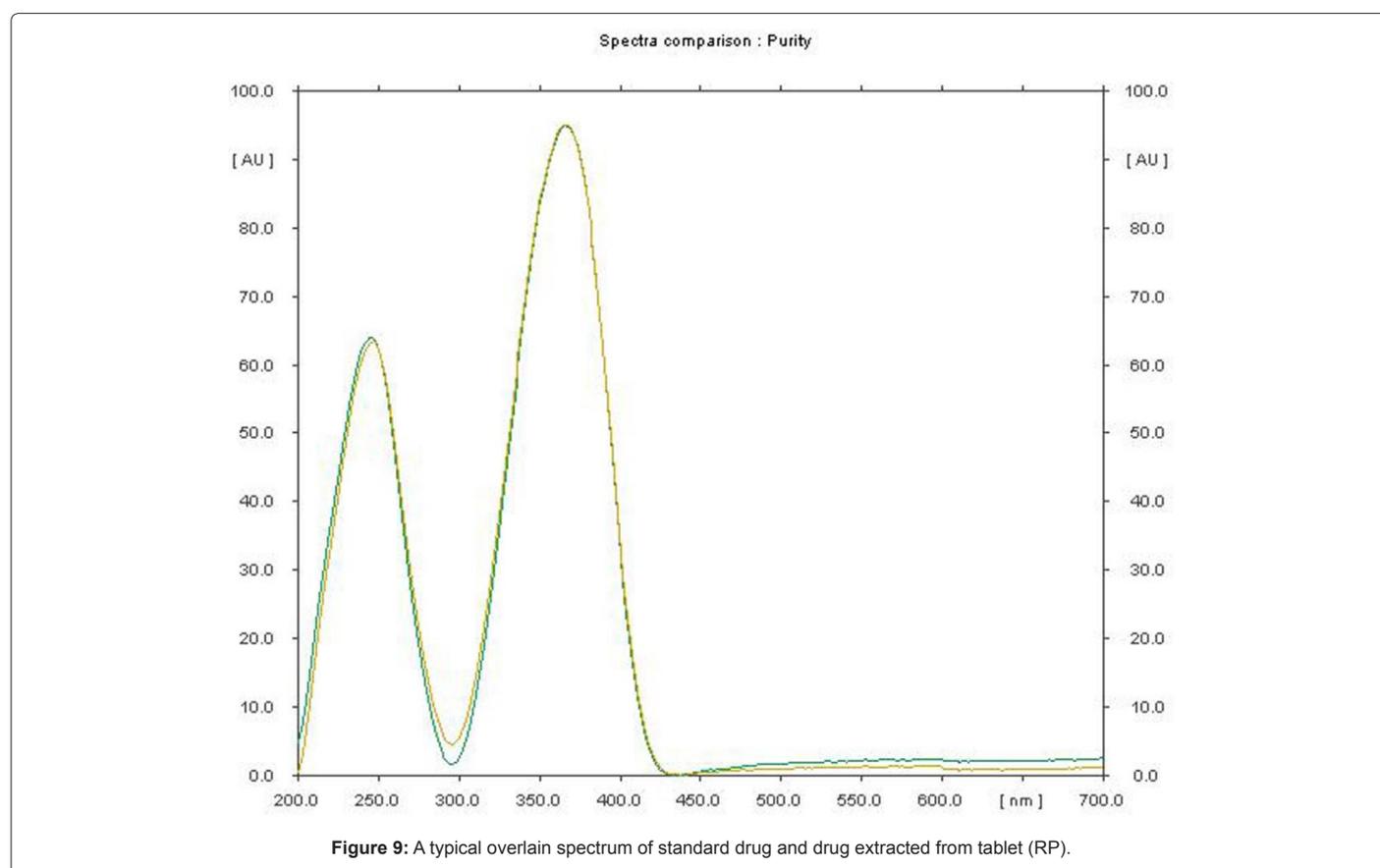
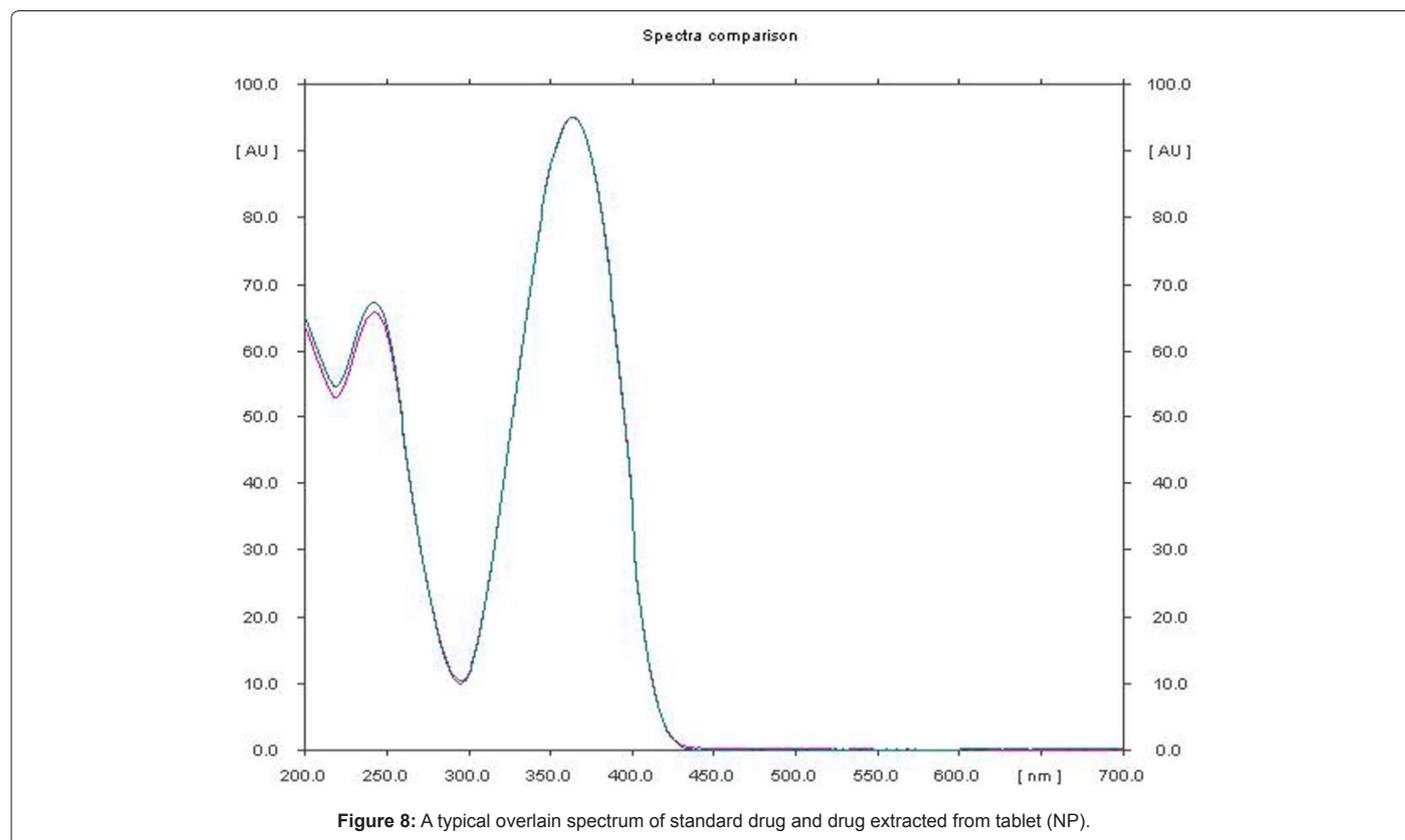
Limit of detection (LOD) and Limit of quantification (LOQ)

Sensitivity of the developed method was determined in terms of limit of detection (LOD) and limit of quantification (LOQ) for FDP. The LOD and LOQ of NP is 11.51 and 34.90 and RP was found to be 29.90 and 90.61, respectively. This indicates the adequate sensitivity of the method.

Specificity

The peak purity of FDP was assessed in NP and B by comparing the spectra at three levels, i.e. peak start (S), peak apex (M) and peak end (E) position of the spot and the result obtained for NP as $r^2(S,M)=0.999$, $r^2(M,E)=0.998$ and for RP as $r^2(S,M)=0.999$, $r^2(M,E)=0.998$. Good correlation was obtained between standard and sample spectra of FDP (Figures 8 and 9).





Method	NP		RP	
	% Drug Content \pm SD	% RSD	% Drug Content \pm SD	% RSD
Felogard ER 10mg/tab.	99.07 \pm 1.52	1.53	99.74 \pm 1.25	1.25

Table 1: Analysis of tablet formulation.

Label Claim (mg/tablet)	Level of recovery	*Drug Recovered \pm SD		% RSD	
		NP	RP	NP	RP
10	80	98.95 \pm 97.08	99.92 \pm 5.51	1.7276	0.0814
	100	98.46 \pm 55.29	98.79 \pm 57.13	0.9009	0.7745
	120	100.23 \pm 34.36	99.75 \pm 45.67	0.5111	0.5673

*Mean of three estimations at each level

Table 2: Recovery study.

Conc. (ng/spot)		Intra-day*				Inter-day*			
		% Amount found \pm SD		%RSD		% Amount found \pm SD		%RSD	
NP	RP	NP	RP	NP	RP	NP	RP	NP	RP
600	1000	99.0589 \pm 2.0616	99.9627 \pm 6.0976	0.3468	0.6099	99.5087 \pm 1.1692	99.6479 \pm 5.4186	0.1958	0.543
900	1500	100.3312 \pm 4.0753	99.6450 \pm 2.8135	0.4513	0.1882	100.5756 \pm 7.1513	99.9155 \pm 1.1020	0.7900	0.0735
1200	2000	100.0778 \pm 3.3297	99.9976 \pm 19.3978	0.2772	0.9699	100.5670 \pm 8.1933	100.0980 \pm 12.2309	0.6789	0.6109

*Mean of three estimates

Table 3: Precision.

Parameters		\pm SD of Peak area*		%RSD	
		NP	RP	NP	RP
Mobile Phase composition	-1.0 mL	93.01	97.15	2.00	1.74
	+1.0 mL	69.05	93.84	1.61	1.47
Activation of Plate	-2 min	79.02	115.97	1.99	1.97
	+2 min	92.56	108.33	1.97	1.88
Duration of saturation	-5 min	72.86	74.61	1.91	1.40
	+5 min	71.59	69.35	1.68	1.28
Development distance	-10 mm	44.01	62.71	0.86	1.17
	+10 mm	34.51	85.60	0.84	1.63

Table 4: Robustness.

Robustness

Robustness of the method was studied by calculated the standard deviation of peak area for each parameter and % relative standard deviation was found to be less than 2%. Values of %RSD are indicated in Table 4.

Conclusion

NP and B have been developed for the identification and quantification of Felodipine in bulk and pharmaceutical formulation. The method for the estimation of Felodipine was simple, accurate, precise, specific and selective. The methods were found to be linear in the concentration range of 300-1800 and 500-3000 ng/spot, respectively. The method was validated as per ICH guidelines.

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