International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

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STABILITY INDICATING HPTLC METHOD FOR EFONIDIPINE

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ABSTRACT

Efonidipine is a drug used in the treatment of Hypertention. The literature survey did not reveal any stability-indicating method for Efonidipine by high performance thin layer chromatographic technique. Chromatographic separation was optimised for the Efonidipine by using Ethyl acetate: Dichloromethane as a mobile phase in the ratio of 6:1(v/v). The retardation factor was found to be 0.45 ±0.03. Detection wavelength was selected as 250 nm. The linear range for analysis was 500-2500 ng/band which gives good linear relationship with regression coefficient 0.999. The accuracy of the method was determined by the recovery studies. The LOD and LOQ were 73.23 and 221.90 ng/band respectively. Stress degradation conditions like hydrolysis under different pH conditions, photolytic degradation, thermal degradation and oxidative degradation was performed as per ICH Q1A(R2) and Q1B guidelines. This method can conveniently be used for quantitative analysis of Efonidipine on routine basis.

Keywords: Efonidipine, Antihypertensive, Stability indicating, HPTLC

INTRODUCTION

Efonidipine HCL Ethanolate (EFO) is a new calcium channel blocker with dihydropyridine and phosphinane derivative. It blocks both T-type and L-type calcium channels. It has a slow onset and longer duration of action. In a patient with essential hypertension, it causes an increase in renal blood flow, a decrease in renal vascular resistance, and an increase in glomerular filtration rate. Chemically it is 2-(N-benzylanilino) ethyl 5-(5, 5-dimethyl-2-oxo- 1, 3, 2λ5}-dioxaphosphinan-2-yl)-2, 6-dimethyl-4-(3-nitrophenyl)-1, 4-dihydropyridine-3- carboxylate, ethanol, hydrochloride.

As per literature search methods like LC-MS [1], RP-HPLC [2-4], UV [5, 6], HPTLC [7], were reported but to the best of our knowledge there is no stability indicating HPTLC methodin literature.

This present work describes the simple and rapid stability indicating method for determination of Efonidipine as per ICH guidelines. HPTLC (High Performance Thin Layer Chromatography) is a sensitive sophisticated technique. It is easy to learn and operate. This work is done on CAMAG instrument with WINCATS software.

MATERIALS AND METHODS

Chemicals and Reagents

Efonidipine was received as gift sample from Zuventus Healthcare Limited, Pune. Other chemicals and reagents like Methanol (AR grade), Ethyl Acetate (AR grade), Dichloromethane (HPLC grade), Hydrochloric acid (AR grade), Sodium Hydroxide (AR grade), Hydrogen Peroxide (AR grade) are purchased from LOBA CHEMIE PVT. LTD, Mumbai.

Instrument

The sample application was performed with help of CAMAG HPTLC system equipped with Linomat 5 sample applicator operated under a gentle stream of nitrogen, coupled with a Hamilton microliter syringe (100 μl). CAMAG TLC SCANNER 3 was used for detection and densitometry scanning. Data acquisition was done by WINCATS software (version 1.4.3). UV-spectral analysis was performed on UV- Visible spectrophotometer (Make-JASCO, Model-V730). Photostability study was performed in photostability chamber (Make-NEWTRONIC, Model- NEC103RSPI).

Preparation of standard stock solution

Accurately weighed 10 mg of Efonidipine was taken into 10 ml volumetric flask and add 1 ml DMSO and shaken well till it gets dissolved. After that volume was made up with methanol, to get separate standard stock solutions of Efonidipine (1000 μg/ml) respectively. It was then appropriately diluted with methanol to get 100 μg/ml as a working solution.

Selection of analytical wavelength

Stock solution of Efonidipine of strength 25

µg/ml was prepared using methanol and UV spectrum was recorded. It showed maximum absorbance at 250 nm. Spectrum is shown in **Figure 1**.
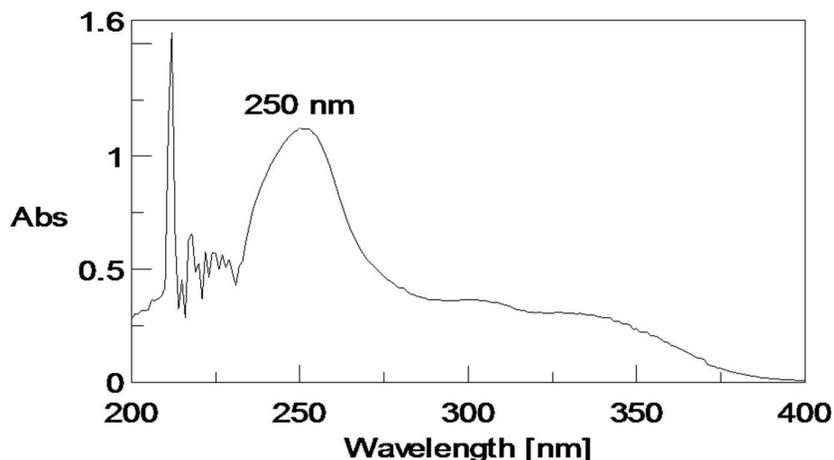

Figure 1: UV Spectrum of Efonidipine (25 µg/ml)

Optimization of chromatographic conditions

Chromatographic separation of Efonidipine drug was performed on Aluminium plates precoated with silica gel 60 F$_{254}$, (10 cm × 10 cm with 250 µm layer thickness). Sampleswere applied on the plate as a band of 8 mm width using 100 µl syringe with Linomat applicator. The mobile phase was composed of Ethyl acetate:Dichloromethane (6:1 v/v). 10 cm × 10 cm twin trough glass chamber was used for linear ascending development of TLC plate with 10 min saturation conditions. The migration distance was 80 mm. Densitometric scanning was performed 250 nm, operated by software, slit dimensions were 6 x 0.45 mm.

Chromatographic conditions like Saturation time, Band length, Detection wavelength, Stationary phase and Mobile phase were optimized and summarized in **Table 1**. The standard densitogram of Efonidipine (2000 ng/band) is shown in **Figure 2**.

Table 1: Optimized chromatographic parameter

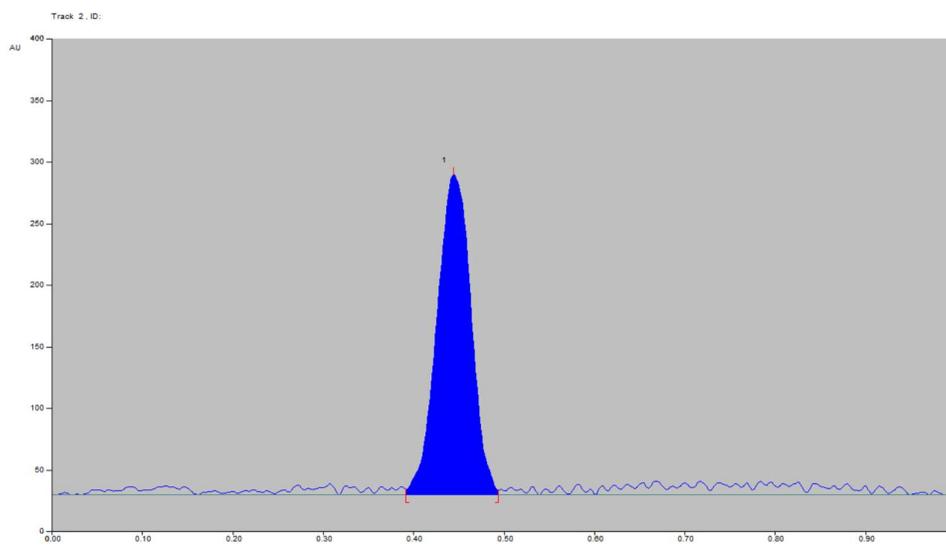| Parameter	Condition used for analysis
Stationary Phase	Merck's TLC aluminium plates pre-coated with silica gel G 60 F254
Mobile Phase	Ethyl acetate: Dichloromethane (6:1 v/v)
Band Length	8 mm
Saturation time	10 min
Detection wavelength	250nm
Rf Value	0.45 ±0.03

Figure 2: Densitogram of Efonidipine (2000 ng/band, Rf =0.45)

Forced degradation studies

The degradation conditions were as per ICH guidelines Q1A (R2) **[8]**. The strength of reagent and the time of exposure were optimized to obtain 10-30 % degradation. We have reported the optimized conditions which are as follows:

Acid catalyzed hydrolytic degradation

5 ml of stock solution (1000 µg/ml) was mixed with 5 ml 1 M HCl and the volume was made up with methanol upto 50 ml. The solution was refluxed for 30 min at 80^0C, cooled to room temperature. The resultant solution (100 µg/ml) was applied to TLC plate and developed using optimized mobile phase.

Base catalyzed hydrolytic degradation

1 ml of Efonidipine working solution of 1000 µg/ml was mixed with 1 ml of 0.5 M NaOHand made up the volume to 10 ml. After 2 h at room temperature, the resultant solution of 100µg/ml was applied to TLC plate and developed using optimized mobile phase.

Oxidation degradation

1 ml of Efonidipine working solution of 1000 µg/ml mixed with 1 ml of 30% H_2O_2 v/v and made up the volume of 10 ml. After 24 h at room temperature, the resultant solution of 100 µg/ml was applied on TLC plate and developed using optimized mobile phase.

Thermal degradation

The thermal degradation was carried out by placing the drug in solid state in an oven at 80 °C for 4 h. A sample was taken out from oven, cooled to room temperature, weighed and diluted in methanol to provide a final concentration of 100 µg/ml of Efonidipine, which was then applied to HPTLC and evaluated under optimized chromatographic conditions.

Photolytic degradation [9]

Accurately weighed 50 mg Efonidipine and transferred it into a clean petri dish and exposedto UV light till the exposure of 200 watt-h/sq. mt. and white cool fluorescent light upto exposure of 1.2 million Lux h. After completion of required illumination the sample was removed. Appropriately weighed and diluted to get 100 µg/ml. The resultant solution was applied to TLC and analyzed under optimized chromatographic conditions.

Validation of analytical method

Validation of developed HPTLC method was carried out as per the ICH Q2 (R1) guideline [10].

Specificity

Peak purity profiling studies and assay was carried out for evaluating the specificity of the method. Peak purity for the peaks of all degradation conditions were carried out with the helpof spectral detection by WINCAT software.

Linearity

Appropriate volumes of working solution of Efonidipine (100 µg/ml) were applied on the TLC plate (volume 5, 10, 15, 20 and 25 µl) thus leading to spotted amounts in the range of 500-2500 ng/band. The plate was developed and this procedure was repeated for 5 times.

Precision

The precision method was demonstrated by intraday (repeatability) & inter-day (intermediate) precision studies. For intraday precision, six replicates (500 ng/band) of standard solution (100 µg /ml) were spotted on TLC plate on the same day after some time interval. In inter-day precision study, application of six replicates (500 ng/band) of standard solution (100 µg/ml) was spotted on TLC plate on three consecutive days. The % RSD was calculated and values was found less than 2 %.

Assay

Marketed product of Efonidipine tablet was used for assay. 2 replicates of sample solution (100 µg/ml) were prepared from 1000 µg/ml stock solution. After sonication and filtration, 10µl volume of each sample solution was applied on TLC plate. The plate development was done with mobile phase and scanned at 250 nm. Peak area was recorded and % recovery was calculated.

Accuracy

Accuracy of the method was determined by standard addition method. Marketed product ofEfonidipine tablet (assay solution) was analyzed by adding known amount of pure drug at 80, 100, 120 % level. 2 replicates of 3 concentrations (1800 ng/band, 2000 ng/band, and 2200 ng/band) were evaluated and % recovery was calculated.

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

The LOD and LOQ were calculated using

equations, LOD= 3.3 x σ/S; LOQ =10 x σ/S, respectively where σ is the standard deviation of the y-intercepts and S is the slope of the calibration curve.

Robustness

Robustness of developed method was evaluated by small but deliberate changes in mobile phase ratio saturation time, effect of time from spotting to development and time from development to scanning, detection wavelentgh. Mobile phase volume was changed by ± 0.2 ml, saturation time was varied by ±5 min i.e., 5 min and 15 min. Detection wavelength was varied by ±2 nm. One factor at a time was varied at conc. of 1000 ng/band for Efonidipine to study effect of each factor on peak area of drug.

RESULTS AND DISCUSSION

Forced Degradation Studies

In order to evaluate the stability indicating property of the developed method, forced degradation studies were carried out in accordance with ICH guidelines Q1A (R2). See **Table 2**.

Pandya CP *et al* [1] it was observed that Efonidipine was labile to alkaline and photolytic conditions in solution form, whereas it was stable in acidic, oxidative, and photolytic conditions in solid state. No degradation was observed when Efonidipine was subjected to 1 M HCl at 80°C for 5 hours. We have observed 11.54 % degradation under acidic condition, 17.44 % degradation under basic condition, 18.14

% degradation under thermal condition, 21.85 % degradation under oxidation conditions respectively. The drug was found to be susceptible to all stress conditions except photolytic condition. Only in acid catalyzed hydrolytic condition we got a degradation product. The representative densitogram is shown in **Figure 3**. The spectral scanning is shown in **Figure 4**.

Method validation

The summary of the validation parameters with their results are shown in **Table 6**.

Specificity

Specificity was monitored by peak purity studies for both standard and sample and it wasfound to be as shown in the **Table 3**.

Linearity

Linearity was determined by plotting amount spotted vs peak area. Linearity range was found to be 500-2500 ng/band. The correlation coefficient was found 0.999 with equation of y = 4.2763x + 575.78. The densitogram was shown in the **Figure 5**. for linearity. The calibration curve shown in **Figure 6**.

Precision

Repeatability and Intermediate precision was performed. % RSD for was found to be 0.71 % & 0.65% respectively. The representative densitogram is shown in **Figure 7**.

Table 2: Summary of forced degradation studies for Efonidipine

| Sr. No.	Degradation Conditions	% Recovery	Peak Purity	
			Purity Front	Purity Tail
1	Acidic Condition (1 M HCl for 30 min at 80 °C)	88.46	0.998	0.996
2	Alkali Condition (0.5 M NaOH for 2 h at RT)	82.56	0.999	0.997
3	Photo stability: 1) UV (200 watt hmeter square)	97.11	0.997	0.996
	2) cool white Fluorescent light (1.2 million lux h)	96.32	0.998	0.997
4	Oxidative Condition (30 % H_2O_2 for 24 h at RT)	78.15	0.996	0.998
5	Thermal Condition (80 °C for 4 h)	81.86	0.998	0.999
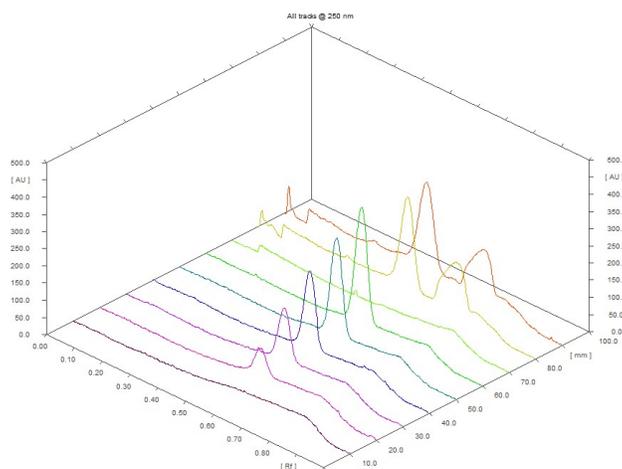
Figure 3: 3D Densitogram of Acid catalyzed hydrolytic Degradation (track1-blank, track2,3,4,5,6 –standard 500-2500 ng/band, track 7-blank, track 8,9- stress solution)
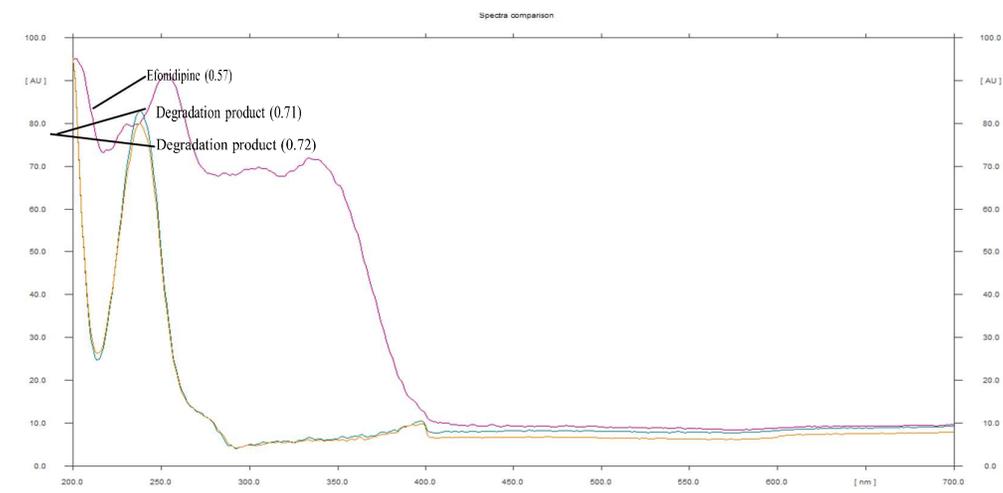

Figure 4: Spectral overlay for acid degradation having degradation product

Table 3: Specificity studies

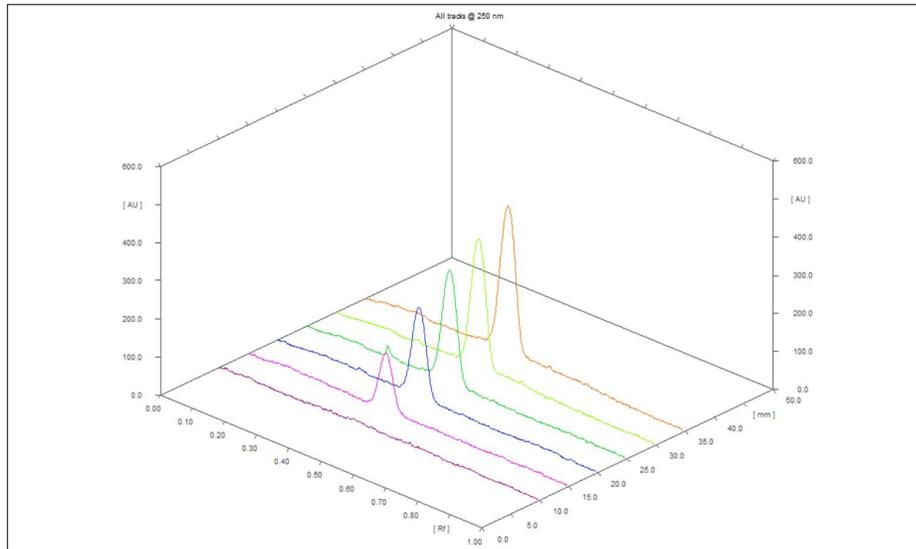| Name	Retention factor	Peak purity r(s,m)	Peak purity r(m,e)
Standard	0.45	0.999	0.998
Sample	0.45	0.999	0.997

Figure 5: 3D Densitogram of Efonidipine linearity (500-2500 ng/band)
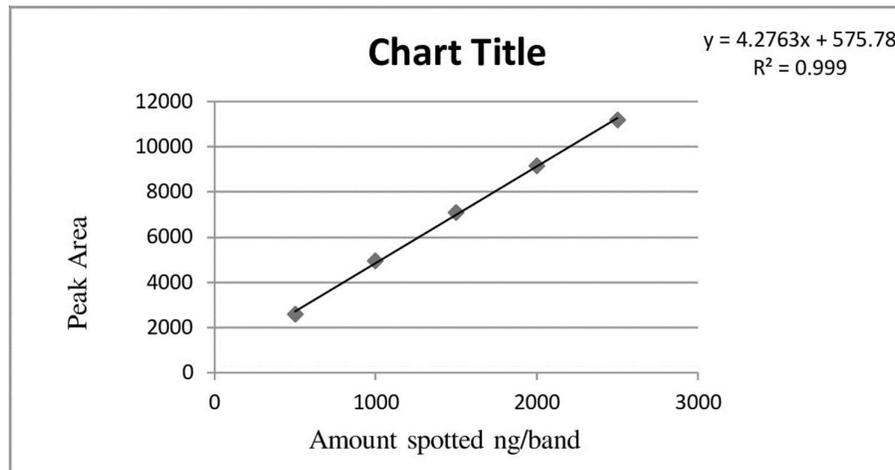

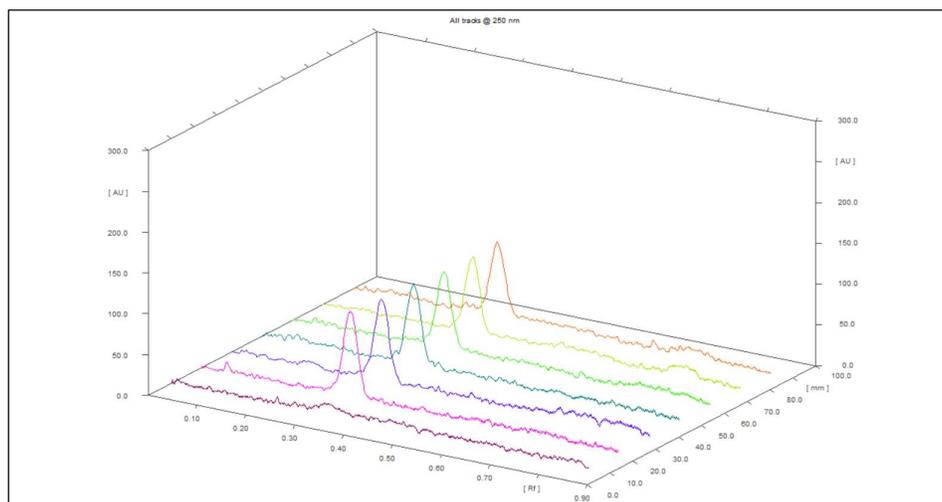**Figure 6: Calibration curve of Efonidipine**

Figure 7: 3D Densitogram of precision of Efonidipine (6 replicate of 500 ng/band)

Assay

Assay was carried out using marketed formulation (tablet). The drug content of tablet has been accurately weighed and final dilution was made using methanol and spotted on TLC plate, the densitogram was recorded and the % drug content was found to be 116.74%.

Accuracy

The % mean recovery was found to be 100.65% for Efonidipine which indicated that the proposed method is accurate for estimation of drug in tablet dosage form. The percent recovery for Efonidipine was found to be in range as shown in **Table 4**. The representative densitogram is shown in **Figure 8**.

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

LOD and LOQ were calculated by y-intercept method. LOD and LOQ was found to be inrange i.e., 73.23 and 221.90 ng/band respectively.

Robustness

It was observed that there were no marked changes in the peak areas, which confirmed thatthe developed method was robust. For results of robustness see **Table 5**.

Table 4: Accuracy (% recovery)

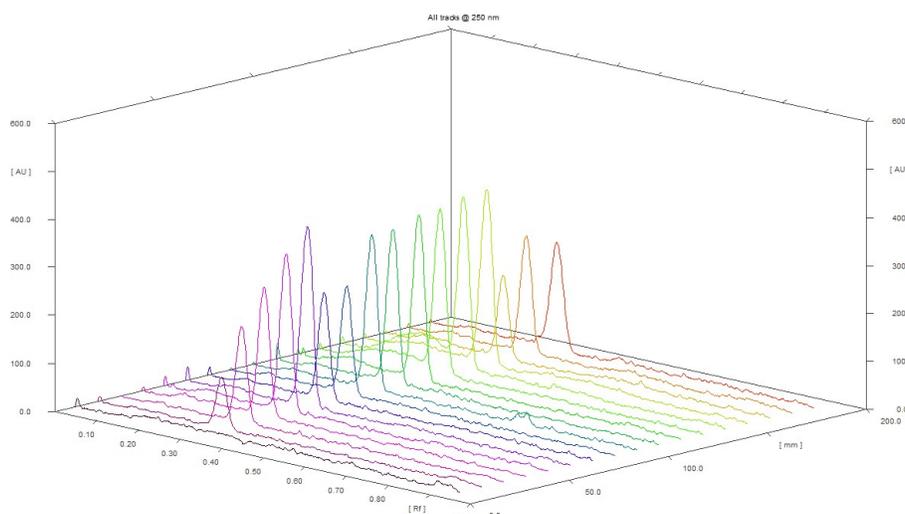| Sr.No	Amount of marketed formulation added (ng/band)	Amount of API added(ng/band)	Total amount of the drug(ng/band)	Amount recovered	% recovery
1	1000	800	1800	1951.79	99.54 %
2	1000	1000	2000	2172.00	100.55 %
3	1000	1200	2200	2404.16	101.87 %

Figure 8: 3D Densitogram of accuracy study (track1-blank, track 2,3,4,5,6 –standard 500- 2500 ng/band, track 7,8-1000 ng/band track 9,10- 80% level, track 11,12-100% level, track 13,14 – 120% level, track 15-standard 800 ng/band, track 16,17-standard 1200 ng/band)

Table 5: Robustness for Efonidipine

Parameter	Robust condition	% RSD
Mobile phase composition (Ethyl acetate:Dichloromethane 6:1v/v, ±0.2 ml)	5.8:1.2 v/v	0.50
	6.2:0.8 v/v	0.97
Saturation time (10 ± 5 min)	5 min	1.46
	15min	0.55
Time from application to development	Immediately afterapplication	0.91
	After 2 h	1.14
Time from development to scanning	Immediately after development	1.05
	After 2 h	0.74
Change in wavelength (250 ±2 nm)	248nm	1.59
	252nm	0.85

Table 6: Summary of validation parameters

Sr. No.	Validation parameters		Result
1	Specificity		Specific
2	Linearity		$y = 4.2763x + 575.78 R^2 = 0.999$
3		Intra-day Precision (%RSD)	0.71 %
		Inter-day precision (%RSD)	0.65%
4	Assay		116.74%
5	Accuracy	80 % level	99.54 %
		100 % level	100.55 %
		120 % level	101.87 %
6	LOD		73.23 ng/band
7	LOQ		221.90 ng/band
8	Robustness		Robust

CONCLUSION

This developed HPTLC method is economic, simple, rapid and stability indicating for routinequantitative analysis of Efonidipine as bulk drug and in the dosage form without interference of commonly used excipients. The developed method was validated as per ICH guidelines. Efonidipine was found to be sensitive to all stress conditions except photolytic condition and degradation product peak was found in only Acid hydrolytic condition. The peak purity value was found within limit confirming stability indicating nature of developed method. Thus, thismethod can conveniently be used for quantitative analysis of Efonidipine on routine basis.

ACKNOWLEDGEMENTS

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