Development and Validation of Stability Indicating RP-HPLC Method for Efonidipine Hydrochloride ethanolate

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Abstract **- The paper describes development and validation of a stability indicating chromatographic assay method for Efonidipine Hydrochloride ethanolate (EFD) in solid pharmaceutical dosage form. EFD and degradant products under varied stress conditions like hydrolysis at a range of pH, temperature, oxidation and exposure to light were analysed by developed RP-HPLC method with proper separation as well as good peak shape. The developed method consisted of mobile phase Methanol: water (50:50 v/v). The flow rate was 0.8 mL/min with a run time of 10 min and detection at 270 nm. The assay was performed on marketed formulation that showed 99.52% labelled claim. During the forced degradation studies, Efonidipine showed maximum degradation (10 %) under oxidative stress followed by 8 % photodegradation. The drug showed lower degradation under acid, base and thermal stress conditions, to the extent of 4 %, 3 % and 6 % respectively. It was also observed that the retention time of the degradant under photolytic and oxidative degradation were the same probably due to the formation of the same product.**

Index Terms **- Efonidipine hydrochloride ethanolate, Stability indicating assay method, stress conditions, anti-hypertensive, photodegradation.**

1.1 INTRODUCTION

Efonidipine hydrochloride ethanolate (EFD), (±)-2-[Benzyl(phenyl) amino] ethyl-1,4-dihydro-2,6-dimethyl-5-(5,5-dimethy-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-4-(3-nitorophenyl)-3-pyridine carboxylate is a racemic antihypertensive agent. It belongs to the dihydropyridine class of calcium channel antagonists and has a phosphonate backbone. It was discovered at Nissan Chemical Industries, Ltd. [1,2] It showed excellent antihypertensive effect in patients with all types of hypertension (essential, severe, renal). Efonidipine has slow onset and long duration of action [3,4]. All 1,4-dihydropyridine derivatives are subject to the first-pass effect, and the primary metabolism step involves oxidation of the dihydropyridine ring to the corresponding pyridine analogue [5,6]. However, it has been suggested that EFD is less likely to undergo the first-pass effect and its dihydropyridine ring is oxidized mainly after metabolism of the side chain [7]. Additionally, EFD has distinct properties when compared with other calcium channel blockers. The objective of the study was to develop a specific, simple, rapid, reliable and validated stability indicating assay method for the estimation of EFD in accordance with International Conference on Harmonization (ICH) guidelines.

1.2 MATERIAL AND METHODS

1.2.1 Reagents and Materials

Efonidipine Hydrochloride Ethanolate (EFD, 99.0% pure) was received as a gift sample from Ajanta Pharma Ltd. (Mumbai, India). HPLC-grade Methanol was purchased from Merck, India.AR grade HCl, NaOH pellets and H_20_2 was obtained from SD Fine Chem Ltd. The deionized and ultra-pure water used in all experiments was obtained from the Milli-Q System (Millipore).

1.2.2 Instrumentation and chromatographic conditions

LC was performed with Shimadzu equipment LC 2010 that was equipped with auto sampler, UV visible detector, a column oven, and LC Solution software. Analysis was carried out at 270 nm, samples (10 µL) were injected automatically. The column dimensions were 250 × 4.6 mm C18 column, 5 µM particle size. The mobile phase consisted of Methanol and water (50:50, v/v) that was set at a flow rate of 0.8 mL/min.

The Shimadzu AES-220 analytical balance was used for weighing.

1.2.3 Method Development

1.2.3.1 Sample Preparation: 10 mg of working standard of EFD was accurately weighed and dissolved in 10 mL of methanol to give a stock solution of 1 mg/mL. Furthermore, standard solutions were made by diluting the stock solution with the mobile phase to give solutions in the concentration range of 10 µg/mL to 50 µg/mL.

1.2.3.2 Method Development: EFD is hydrophobic and is almost insoluble in aqueous solutions, whereas it is soluble in organic solvents like acetonitrile and methanol. During the development phase, the use of ACN and water as the mobile phase resulted in asymmetric peak with an unacceptable tailing factor (>2). Changes in the mobile phase composition by changing the solvent as well as ratio worked well to get the tailing factor within the acceptable limit (1.2) resulting in good peak symmetry and response. The flow rate was optimized at 0.8 mL/min with the retention time of the drug around 3.38 min. The peak shape and symmetry (Figure 1) were found to be good when a mobile phase composition of 50:50, v/v (methanol: water) was used for the drug as well as for force degradant samples.

1.2.3.3 Degradation Studies: All degradation experiments were performed at a concentration of 100 µg/mL. The degradation study was initiated with minimal concentration of degradant and exposure for the shortest duration. Final degradation pattern was concluded by changing one parameter and keeping other constants. The degradation under hydrolytic conditions was studied using 20 mL of 0.1 M methanolic HCl and 0.1 M methanolic NaOH at 70°C for 1 h. The samples were neutralized by adjusting the pH to 7.0. Oxidative stress condition was studied at 3% H_2O_2 at 70°C for 1 h. Thermal decomposition experiments were carried out by keeping the drug at 70°C for 2 h in hot air oven. [13] Photodegradation was studied using 10 µg/mL solution of EFD kept in photostability chamber and exposed at 1.2 million Lux hours of visible light and 200 Watt hours /sq.m. of UV energy.[14] These exposed degraded sample was then analysed by means of the developed RP-HPLC method.

1.2.4 Method validation

1.2.4.1 System suitability

The system suitability was assessed by five replicate analyses of the drug at a concentration of 100 µg/mL. The acceptance criterion was ± 2% for the percent coefficient of variation (% CV) for the peak area and retention times of the drug.

1.2.4.2 Linearity

A stock solution of EFD (1000 µg/mL) was prepared by dissolving 25 mg EFD in 25 mL methanol. Solutions of different concentration was prepared from the stock solution for construction of calibration plots. The calibration curves were obtained with the concentrations ranging from 10-50 µg/mL. The prepared dilutions were inserted in series from 10-50 µg/mL, area was calculated for each dilution and concentration was plotted against peak area. The equations of linear regression was derived using least-squares method.

1.2.4.3 LOD and LOQ

In the present study, the LOD and LOQ were calculated using the formulae 3.3 σ/s and 10 σ/s criteria, respectively; where σ is the standard deviation of the peak area ratios and s is the slope of the corresponding calibration curve [10].

1.2.4.4 Accuracy

The accuracy of an analytical method is defined as the similarity of the results obtained to the true value while the precision is a measure of the closeness of result. [11] Accuracy was determined by the standard addition method. Previously analysed marketed samples of EFD (30 µg/mL) were spiked with 80, 100, and 120% EFD standard and the mixtures were analysed by the projected method. The experiment was performed in triplicate.

1.2.4.5 Precision

Precision was calculated as the repeatability and intermediate precision levels. Repeatability was calculated by the determination of system precision for six replicate injections of the mixed standard solutions in groups of three. [12].

1.2.4.6 Robustness

The robustness of the method was determined to assess the effect of slight but deliberate variations

among the chromatographic conditions on the determination of EFD [15]. In this study the chromatographic conditions selected were flow rate (0.6, 0.8, and 1.0 mL/min), mobile phase ratio (45:55; 50:50; and 55:45 v/v), column temperature (33, 35 and 37 °C) and the wavelength for detection (268, 270, and 272 nm).

1.3 RESULTS AND DISCUSSION

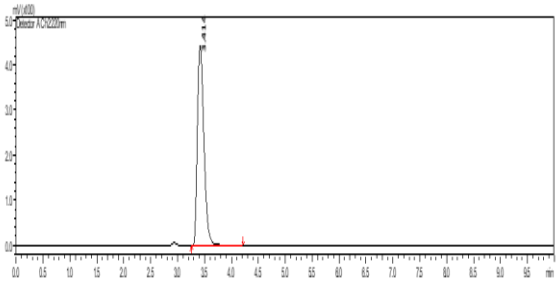
1.3.1 Degradation Studies

High-performance liquid chromatographic method was developed to analyse the samples of EFD. The stressed samples were then analyzed using the developed chromatographic method. The representative chromatograms of the stress degradations are shown in Figures1a–f while the degradation profile is given in Table-1. During acid degradation one additional degradant peak was observed in the chromatogram at 4.24 min with 4 % degradation while basic degradation showed a degradant peak prior to the standard drug peak at 2.83 min with a degradation of 3%. Oxidative degradation showed a degradant peak at 4.25 min with 10% degradation while photodegradation also showed degradant peak at 4.25 min with 8% degradation. In thermal condition 10% drug was degraded and the degradant peak was observed at 4.42 min.

Table-1: Degradation Profile of EFD

Degradant	Retention Time (min)	Area Percent
Standard	3.38	100
Acidic	4.24	4
Basic	2.83	3
Oxidative	4.25	10
Thermal	4.42	6
Photodegradation	4.25	8

Figure-2: Chromatogram showing symmertic peak of EFD (30 µg/mL)



1.3.2. Method Validation

1.3.2.1 System suitability

The %CV of peak area and retention time for drug was within 2% acceptance range (Figure-2) indicating the suitability of the system (Table-2). The efficiency of the column was estimated by Peak area, number of theoretical plates and tailing factor.

Table-2: System Suitability Parameters

No. of Injection	Peak Area	Theoretical Plates (N)	Tailing Factor
1	192213	5965.59	1.084
2	199241	5975.58	1.053
3	199275	5920.34	1.056
4	199241	5899.36	1.067
5	199223	5947.21	1.085
Mean	198379.33	5941.61	1.069
SD	1328.85	31.614	0.0134
%RSD	0.66	-	-

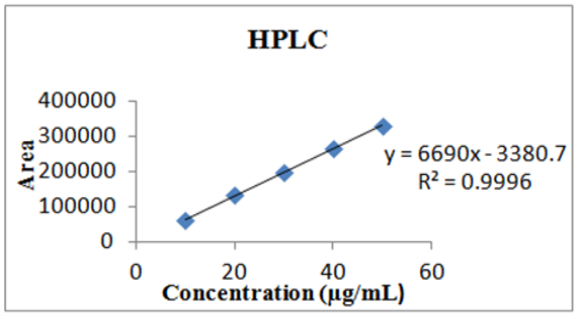
1.3.2.2 Linearity

The calibration plot was linear over the concentration range examined (10-50 µg/mL). The value of mean correlation coefficient was R^2=0.9996 and regression equation was y = 6690x-3380.7 (Table-3 and Figure-3). The Standard Error (SE) of the slope and intercept were 6690 and 3380.7 respectively. There were no significant differences between the slopes of calibration plots constructed on three different days.

Table-3: Data for Linearity

Sr. No.	Concentration (µg/mL)	Area Mean ± SD (n=5)	%RSD
1	10	61021 ± 425.944	0.69
2	20	132968.2 ±1103.18	0.82
3	30	199196.8 ± 1895.68	0.65
4	40	268665.6 ± 20146.61	0.76
5	50	328776 ± 1104.77	0.33

Figure-3: Calibration Curve



1.3.2.3 LOD and LOQ

LOD and LOQ, determined by the standard deviation method as described in the experimental section, was 0.788 µg/mL and 0.236 µg/mL respectively, indicating the method can be used for detection and quantification of EFD in a very low concentration of EFD. (Table-4).

Table-4: Data of LOD and LOQ (n=5)

Sr. No.	Parameters	Observed values
1	Average of slope	3036.7
2	Standard deviation of intercept	718.627
3	Limit of detection (µg/mL)	0.788
4	Limit of quantification (µg/mL)	0.236

1.3.2.4 Accuracy

The recovery of the method was 99.2-99.5% after spiking a previously analysed test solution with additional drug standard. The values of recovery and RSD are shown in Table-5; % RSD is less than 1% that indicates that the proposed method is accurate.

Table-5: Data for Recovery Study

Sr. No.	% EFD	Quantity Added (mg)	% Recovery	
			Mean Quantity Obtained (mg)	% Recovery ± %RSD (n=3)
1.	0%	-	9.99	-
2.	80%	8	7.96	99.5 ± 0.891
3.	100%	10	9.92	99.2 ± 0.963
4.	120%	12	11.92	99.33 ± 0.947

1.3.2.5 Precision

The data obtained from precision experiments are given in Table-6 for intra-day and inter-day precision studies. The % RSD values for both intraday and interday precision studies were well within the accepted limits < 2% confirming that the method is precise.

Table-6: Data for Intra-day and Inter-day precision studies

Concentration (µg/mL)	Intraday Precision		Interday Precision	
	Peak Area ± SD (n=3)	%RSD	Peak Area ± SD (n=3)	%RSD
10	60769.3 ±1105.94	0.59	60798 ± 1108.8	0.711
30	132336 ± 1246.3	0.67	133454 ± 1247.24	0.847
50	199375 ± 1411.29	0.92	199245 ± 1397.62	0.934

1.3.2.6 Robustness

The method was found to be robust, as slight but deliberate changes in the method parameters had no detrimental effect on the performance of the method as shown in Table-7.

Table-7: Data pertaining to Robustness Study

Parameter	Change in Parameter Mean peak area (n=3)			Mean Peak Area ± SD (n=3)	%RSD
Flow Rate (mL/min)	0.6	0.8(Optimized)	1.0	198468 ± 1257.276	0.633
	198639	197133	199631		
Temperature (º C)	33	35(Optimized)	37	197833 ± 608.276	0.3074
	198233	197133	198133		
Wavelength (nm)	268	270(Optimized)	272	197433 ± 435.89	0.220
	197933	197133	197233		
Mobile phase composition (v/v)	45:55	50:50(Optimized)	55:45	197317 ± 502.585	0.254
	197289	197833	196829		

Figure-1: Chromatograms for Degradation Studies

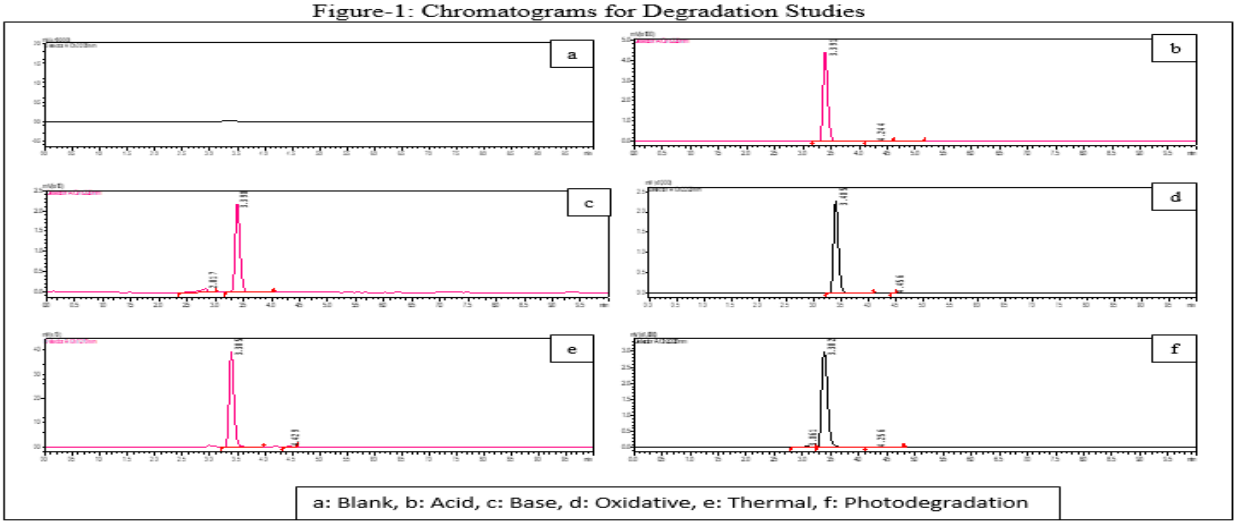
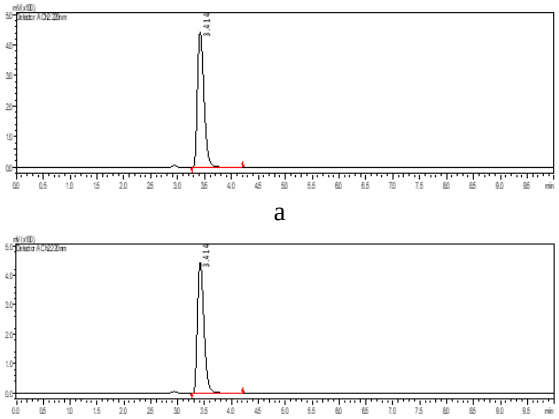


Figure-4: Comparison of Standard EFD with Assay of Marketed Formulation



a

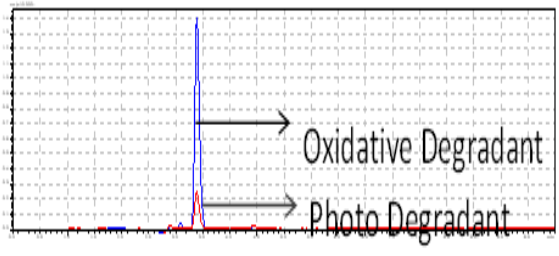
b

Figure-4: a: Standard; b: Test (Marketed Formulation; Efnocar 20mg)

1.4 CONCLUSION

The Stability Indicating RP-HPLC assay method was developed and is sensitive and specific for the quantitative determination of EFD. Also, the low flow rate and less run time consumes comparatively less mobile phase solvents. The shortening of run time would prove to be cost-effective during routine analysis of drug samples. During the Comparison of chromatographic behaviour of degradant under oxidative stress and photodegradation it was observed that the retention time of both the degradant sample was at 4.25 min (Figure-5). Hence, it can be concluded that the degradant formed under the oxidative and photo-degradation conditions is the same; (however, further experiments can prove it beyond any doubt it is by photo-oxidation and not by photolytic pathway). Also, the method is validated for various parameters, hence has been applied for the estimation of drug in pharmaceutical dosage forms. EFD tablets of 10 mg strength were evaluated for the amount of EFD present in the formulation. Each drug sample was analysed in triplicate as mentioned in the sample preparation of the experimental section. The amount of EFD was 99.52%. The chromatogram of EFD from the tablets matched with that of standard EFD as shown in Figure-4 (a). The degradation and robustness data during method validation showed that the developed process in robust to change in conditions. The developed validated method is suitable for regular analysis and worth assessment of EFD in pharmaceutical products or dosage forms.

Figure-5: Comparison of Oxidative degradation and Photodegradation



1.5 ACKNOWLEDGEMENT

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