

Development and Validation of Liquid Chromatography (RP-HPLC) Methodology for Estimation of Efonidipine HCl Ethanolate (EFD)

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Abstract

A Reversed Phase High Performance Liquid Chromatographic (RP-HPLC) method using symmetry C18, 5.0 mm column was developed for the determination of Efonidipine Hydrochloride Ethanolate (EFD). The mobile phase acetonitrile and water ratio was selected 85: 15 via flow rate were 0.8 mL/min and elution was monitored at 254 nm. Response was a linear function of concentration over the range 20-140 µg/ml (R²=0.9994) and the limits of detection was 681.83 ng/ml. The limit of quantification was 2.06 µg/ml. The coefficient of variation for intra-assay and inter-assay precision was less than or equal to 1.5% and the accuracy was 104.0-105.0% and method was validated accordance with International Conference on Harmonization (ICH) guidelines to check content uniformity. In this lieu, a simple and rapid with good accuracy precision validated method is developed which is applicable in quality-estimation, in-future.

Keywords: Efonidipine HCl Ethanolate (EFD); RP-HPLC; UV-detector; Validation

Introduction

Efonidipine hydrochloride ethanolate (NZ-105), (±)-2-[Benzyl(phenyl)amino]ethyl-1,4-dihydro-2,6-dimethyl-5-(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-4-(3-nitrophen-yl)-3-pyridine carboxylate hydrochloride ethanol, is a dihydropyridine calcium antagonist with a phosphonate backbone and that was discovered at Nissan Chemical Industries, Ltd. [1,2]. This active drug ingredient was initially studied for development as a hydrochloride salt without ethanol, obtained through the addition of hydrochloric acid to efonidipine acetone solution. It showed an excellent antihypertensive effect in patients with various kinds of hypertension (essential, severe, renal). Efonidipine has slow onset and long duration of action [3,4].

It is well known that many 1,4-dihydropyridine derivatives are subject to the first-pass effect, and that the primary metabolism step of most derivatives involves oxidation of the dihydropyridine ring to the corresponding pyridine analogue [5,6]. However, it has been suggested that efonidipine is less likely to be subject to the first-pass effect than other dihydropyridine derivatives and that its dihydropyridine ring is oxidized mainly after metabolism of the side chain [7]. Additionally, efonidipine has distinct properties when compared with other calcium channel blockers. The studies indicated that efonidipine therapy simultaneously improves blood pressure, endothelial function, and metabolic parameters without substantially altering insulin sensitivity in non-diabetic patients with hypertension [8,9]. During literature survey, till date there is no analytical liquid chromatography method are available for determination of Efonidipine individually. Therefore, the objectives of this study were to develop a simple, rapid, and validated method for estimation of efonidipine and to validate the method in accordance with International Conference on Harmonization (ICH) guidelines.

Experimental

Chemicals and reagents

Efonidipine Hydrochloride Ethanolate (EFD, 99.0% pure) was a gift sample from Ajanta Pharma Ltd. (Mumbai, India). HPLC-grade Acetonitrile was purchased from Merck, India. High-purity water was prepared using Millipore purification system. Other chemicals and reagents were of AR grade.

Instrumentation

LC was performed with an Agilent Technologies equipment 1260 infinity comprised of a G1311B/C quaternary pump VL, a G1329B auto sampler, a G1314F VL variable wavelength programmable UV-visible detector, a column oven, and a G4208A VL system controller with EZ Chrom Elite software. Analysis was carried out at 254 nm. Samples (10 µL) were injected by means of a Rheodyne injector fitted with a 10 µL loop. Compound was separated on a 250 × 4.6 mm C18 column, 5 µm particle size at ambient temperature. The mobile phase consisted of acetonitrile and water (85:15, v/v) that was set at a flow rate of 0.8 ml/min.

Method validation

System suitability: The system suitability was assessed by six replicate analyses of the drug at a concentration of 100 µg/ml. The acceptance criterion was ± 2% for the per cent coefficient of variation (% CV) for the peak area and retention times of the drug.

LOD and LOQ: In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3 σ/s and 10 σ/s criterions, respectively; where σ is the standard deviation of the peak area ratios and s is the slope of the corresponding calibration curve [10].

Linearity: A stock solution of EFD (1000 µg/ml) was prepared by dissolving 50 mg drug in 50 mL acetonitrile. Solutions of different concentration (20-140 µg/mL) for construction of calibration plots were prepared from this stock solution. The calibration curves were constructed with seven concentrations ranging 20-140 µg/ml. The prepared dilutions were inserted in series, area was calculated for each dilution and concentration was plotted against peak area. The equations of linear regression were performed using least-squares method.

Accuracy: The accuracy of an analytical method is defined as the similarity of the results obtained to the true value and precision is defined as the degree of that similarity [11]. Accuracy was determined by the standard addition method. Previously analysed samples of EFD (100 µg/mL) were spiked with 50, 100, and 150% extra EFD standard and the mixtures were analysed by the projected method. The experiment was performed in triplicate.

Precision: Precision was calculated at the repeatability and intermediate precision levels. Repeatability was calculated by the determination of system precision for nine replicate injections of the mixed standard solutions in groups of three, at three different levels [12].

Degradation studies: All degradation experiments in solution were performed at a drug concentration of 100 µg/ml. For acid, basic and oxidation degradation, 5 mg of EFD was heated with 5 ml of 1 N HCl, 1 N NaOH and 10% H₂O₂ at 80°C for 1 h, and then neutralized by

adjusting the pH to 7.0. For thermal decomposition experiments, the drug was kept at 80°C for 24 h in vacuum oven [13].

Robustness: The robustness of the method was determined to assess the effect of slight but deliberate variation of the chromatographic conditions on the determination of EFD [14]. In this study the chromatographic conditions selected were flow rate (0.6, 0.8, and 1.0 mL min⁻¹), mobile phase ratio (80:20; 85:15; and 90:10), and the wavelength for the detection of DDEA (249,254, and 259 nm).

Results and Discussion

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 acetonitrile and water as the mobile phase resulted in asymmetric peak with a greater tailing factor (>2). The tailing factor was within the acceptable limit (1.2) resulting in good peak symmetry and response. A flow rate of 0.6 ml/min resulted in drug retention time beyond 08 min that was more time consuming. Also, the low flow rate and less run time consumes comparatively less mobile phase solvents that phase was optimized at 0.8 ml/min with the retention time of the drug around 6.3 min and that of the will prove cost-effective during routine analysis of drug samples. The peak shape and symmetry (Figure 1) were found to be good when a mobile phase composition of 85:15, v/v (acetonitrile: water) was used of the drug.

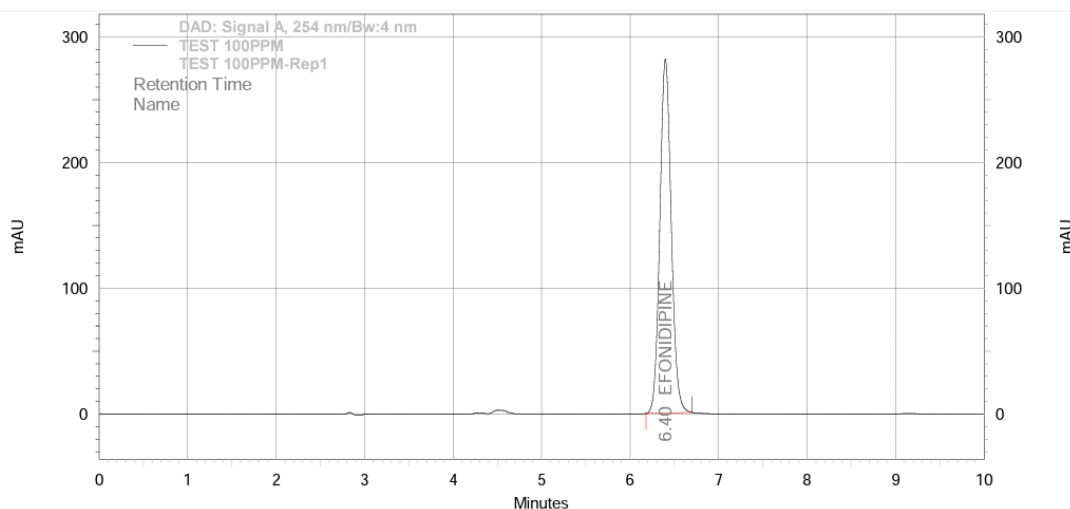


Figure 1: Sharp and symmetry peak of EED.

Method Validation

System suitability

The %CV of peak area and retention time for drug was within 2% indicating the suitability of the system (Table 1). The efficiency of the column was expressed by number of theoretical plates and Asymmetry for the six replicate injections.

Linearity: The calibration plot was linear over the wide concentration range examined (20-140 µg/ml). The mean correlation coefficients R²=0.9994 and regression equations was y= 51370x+48713

EFD (100 µg/ml)	Retention time (min)	Peak area	Asymmetry	Theoretical plates (USP)
Mean (n=6)	6.39	5236573	1.16	11932.5
S.D.	0.01	10613.94	0.02	107.24
%CV	0.15	0.2	1.38	0.89

Table 1: System suitability study.

(Table 2 and Figure 2). The Standard Error (SE) of the slope and intercept were 31.086 and 10750.122 respectively. There were no significant differences between the slopes of calibration plots constructed on three different days.

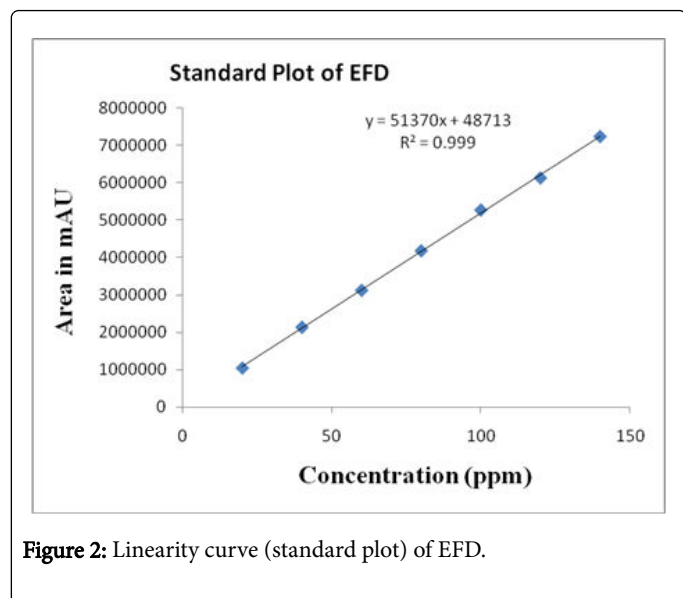


Figure 2: Linearity curve (standard plot) of EFD.

Validation parameters	Efonidipine HCl ehanolate (at 254 nm)
Range (µg/ml)	20-140
Regression equation	y=51370x+48713
%RSD Slope	0.06
Correlation coefficient (R2)	0.9994

Table 2: Validation parameters of the HPLC method of EFD.

Detection and quantization limits (sensitivity): LOD and LOQ, determined by the standard deviation method as described in the experimental section, was 681.83 ng/ml and 2.06 µg/ml respectively, indicating the method can be used for detection and quantification of EFD in a very wide concentration range (Table 3).

S. No.	Parameters	Efonidipine HCl Ethanolate (EFD)	
1	Accuracy	Recovery%	104.0-105.0%
		% RSD	0.32%
2	Limit of detection (µg/ml)	681.83 ng/ml	
3	Limit of quantification (µg/ml)	2.06 µg/ml	

Table 3: Results of the parameters.

Accuracy: The recovery of the method was 104.0-105.0% after spiking a previously analysed test solution with additional drug standard. The values of recovery and RSD are shown in the Table 3; RSD was always less than 1%, which indicates the proposed method is accurate.

Precision: The data obtained from precision experiments are given in Table 4 for intra- and inter-day precision studies. The % R.S.D. values for intra-day precision study were <1.0% and for inter-day study were <2.0%, confirming that the method was sufficiently precise.

Nominal Concentration		80%	100%	120%
Intra-day (n=9)	Mean	4223660.7	5235441	6254252.33
	S.D.	5546.87	26272.57	7809.39
	%CV	0.13	0.5	0.12
Inter-day (n=3)	Mean	4152266.8	5214807.5	6208978.9
	S.D.	61571.02	73808.84	66555.28
	%CV	1.48	1.42	1.07

Table 4: Intra- and inter-day accuracy and precision of EFD HPLC assay for nominal concentration.

Degradation studies: The degradation studies involving Thermal, acid and base exposed that EFD were not fully degraded (Table 5). However, in oxidation conditions (10% H₂O₂), the drug was unstable and the degradation peak eluted at different time interval (Figure 3).

Sample Name	Retention time	Area percent	Peak name
Efonidipine standard	6.36	100	Efonidipine
Efonidipine acidic	4.99	0.1	Unknown 1
Efonidipine basic	5	0.17	Unknown 1
Efonidipine oxidation	3.25	-	Blank
	4.93	0.15	Unknown 1
	5.19	0.14	Unknown 2
	5.64	0.33	Unknown 3
	7.3	0.03	Unknown 4
Efonidipine thermal	No degradation	-	-

Table 5: Degradation profile of EFD.

Robustness: The method was found to be robust, as slight but deliberate changes in the method parameters have no detrimental effect on the method performance as shown in Table 6.

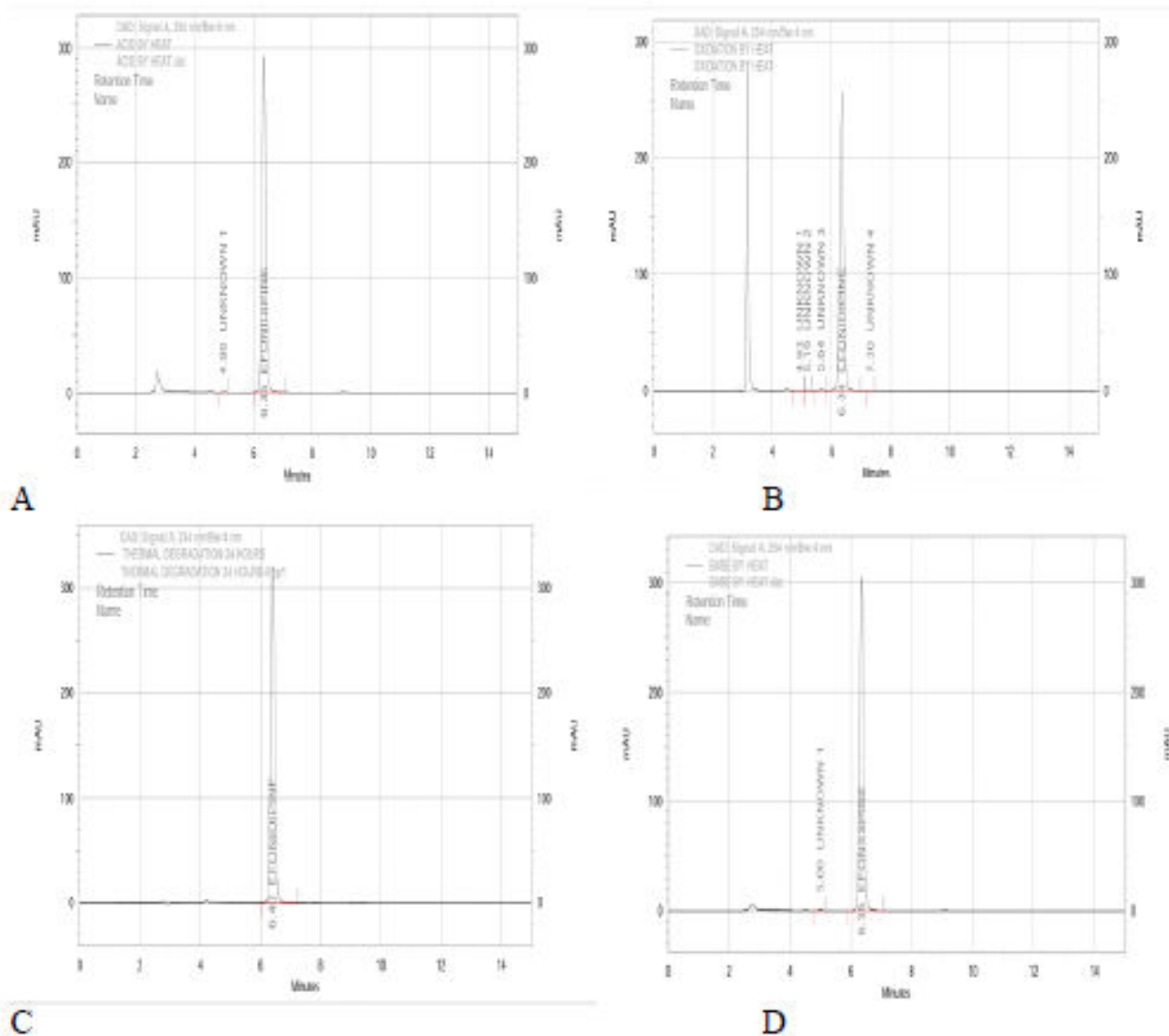


Figure 3: The different degradation profiles by: A) Acid; B) Base; C) Oxidation; and D) Thermal.

Parameters		Peak area	Retention time	Theoretical plates
Flow rate (Figure 4)	0.6	7028695	8.5	13814.3
	0.8	5308011	6.3	11860.7
	1	4219923.7	5.1	10673.7
Mobile phase ratio	80:20:00	5265232.3	7.8	12000.3
	85:15:00	5247418.7	6.3	11747

	90:10:00	5249634.3	5.4	11876
Wavelength	249	5108420.3	6.3	11885.3
	254	5282163.3	6.3	11848.3
	259	4828787.3	6.3	11806.3

Table 6: Parameters of robustness at different flow rate for mobile phase and wavelength.

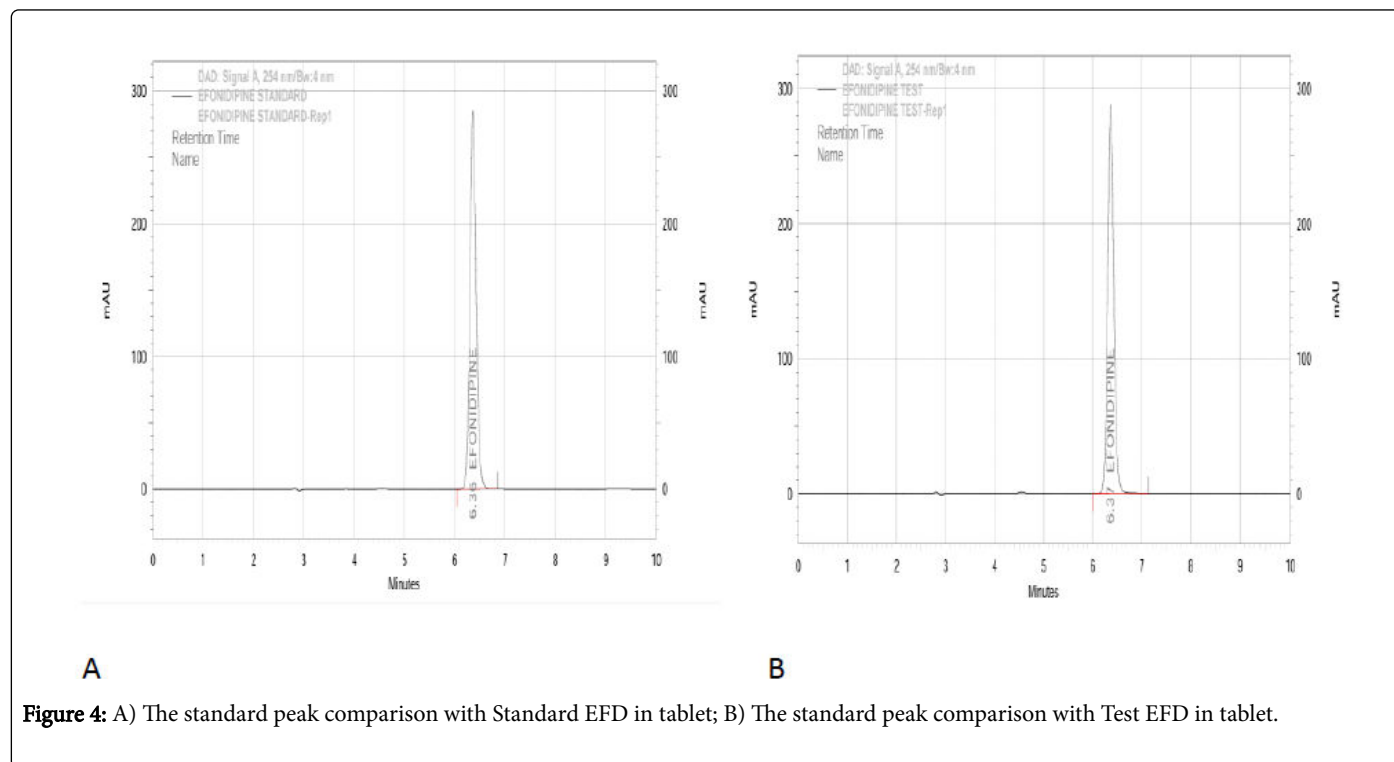


Figure 4: A) The standard peak comparison with Standard EFD in tablet; B) The standard peak comparison with Test EFD in tablet.

Conclusion

The RP-HPLC method developed is sensitive and specific for the quantitative determination of EFD. Also, the method is validated for different parameters, hence has been applied for the estimation of drug in pharmaceutical dosage forms. EFD tablets of 20 mg strength were evaluated for the amount of EFD present in the formulation. Each sample was analysed in triplicate after extracting the drug as mentioned in the sample preparation of the experimental section. The amount of EFD was 100.82%. None of the tablet ingredients interfered with the analyse peak as seen in Figure 4b. The spectrum of EFD extracted from the tablets was matching with that of standard EFD (Figure 4a) showing the purity of peak of EFD in tablets. The degradation & robustness data during method validation showed the process is not subjected to changes in conditions. The developed validated method was suitable for regular analysis and worth assessment of EFD in pharmaceutical products or dosage forms.

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