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Development and Validation of RP-HPLC for Estimation of Efonidipine Hydrochloride Ethaolate in Pharmaceutical Formulation

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ABSTRACT
Efonidipine hydrochloride ethanolate is a dihydropyridine calcium channel blocker used to treat hypertension. It's a third-generation calcium channel blocker, also known as NZ-105, that blocks both T-type and L-type calcium channels. It's known for its slow onset and long duration of action. Efonidipine hydrochloride ethanolate is a solvate, meaning it's a compound formed by the combination of efonidipine hydrochloride and ethanol in an equimolar ratio. Its molecular formula is C36H45ClN3O8P, and its molecular weight is 714.19 g/mole. The aim of the present study is to develop and validate the RP-HPLC method for estimating Efonidipine hydrochloride ethanolate in Pharmaceutical Dosage Form, focusing on the application of suitable analytical techniques, optimization, and validation in accordance with ICH guidelines, while selecting the appropriate drug and developing an analytical methodology. In this study, we found that the pharmaceutical dose tablet formulations containing Efonidipine hydrochloride ethanolate (EHE) may be accurately measured using the RP-HPLC method. The RP-HPLC technique is sensitive, accurate, precise, and repeatable; it also demonstrates high repeatability. Efonidipine hydrochloride ethanolate (EHE) tablet dosage formulation analysis may also be conducted with success. These techniques do not experience any influence from additives, matrices, etc. To further understand these trials, additional research on other medication formulations is needed.

Keywords: Efonidipine hydrochloride ethanolate; RP-HPLC; Validation; ICH guidelines; cyclooxygenase.

I. INTRODUCTION

Efonidipine hydrochloride ethanolate is a third-generation calcium channel blocker used to treat hypertension, blocking both T-type and L-type calcium channels. It is a solvate, formed by the combination of efonidipine hydrochloride and ethanol in an equimolar ratio. Its chemical properties include its molecular formula C36H45ClN3O8P and its molecular weight 714.19 g/mole. Its primary action is to block calcium channels, leading to vasodilation and reduced blood pressure. In hypertension, it increases renal blood flow, decreases renal vascular resistance, and increases glomerular filtration rate (1-5). It is marketed under the brand name Landel and has been studied for its potential in atherosclerosis and acute renal failure. However, it has low aqueous solubility and oral bioavailability, which can limit its effectiveness. Studies have focused on improving solubility through co-crystallization and solid dispersions. Efonidipine's solid state properties involve its interaction with chloride ions and efonidipine molecules. It is advised to avoid alcohol consumption due to potential side effects and consult a doctor before use during pregnancy, breastfeeding, or for individuals with liver or kidney problems (6-10). The aim of the present study is to develop and validate the RP-HPLC method for estimating Efonidipine hydrochloride ethanolate in Pharmaceutical Dosage Form, focusing on the application of suitable analytical techniques, optimization, and validation in accordance with ICH guidelines, while selecting the appropriate drug and developing an analytical methodology.

II. MATERIALS AND METHODS

2.1 Procurement of the Drug

Efonidipine hydrochloride ethanolate, a medication from Arch Pharma labs Ltd Thane, is available in a 10g package with a purity of 99.8 to be used as Reference drug while Muvera 15, Sun Pharma Lab. Ltd India which contains 15 mg dosage of Efonidipine hydrochloride ethanolate to be used as test drug.

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2.2 Method and Procedure

2.2.1 Selection of Mobile Phase

The mobile phases tested include methanol: water (90:10), methanol: water (80:20), acetonitrile: water (90:10), acetonitrile: phosphate buffer 10mm (90:10), acetonitrile: phosphate buffer 10mm (80:20), and acetonitrile: phosphate buffer (75:25) with pH 4.5.

2.2.2 Chromatographic Conditions

The chromatographic conditions were established through trial and error, maintaining constant consistency throughout the method. The column was Inertsil 4.6 x 250 mm, with a particle size of 5 µm, stationary phases of C18 Inertsil, mobile phase of Acetonitrile: Phosphate Buffer (75:25), pH 4.5, and a sample size of 20 µL.

2.2.3 Validation of the Method

Adjusting several UFLC settings (FDA, 1995, 1997, 2000, 1994, 1987; USP, 2000) confirmed the reliability of the UFLC approach (16). Calibration plot least-squares linear regression analysis verified the UFLC method's linearity (17), the limits of detection and quantification for the medicines mentioned were determined to be three and five epochs, respectively, above and below the baseline noise, The process adhered to the guidelines established by the United States Pharmacopoeia (USP, 2000), specificity (17), precision (18) accuracy (19), robustness (20) and ruggedness (21) were determined.

III. RESULTS AND DISCUSSION

3.1 Selection of the Mobile Phase

From various mobile phases tried, mobile phase containing Acetonitrile: Phosphate Buffer (75:25) pH 4.5 was selected, since it gives sharp reproducible retention time for EHE (Figure 1).

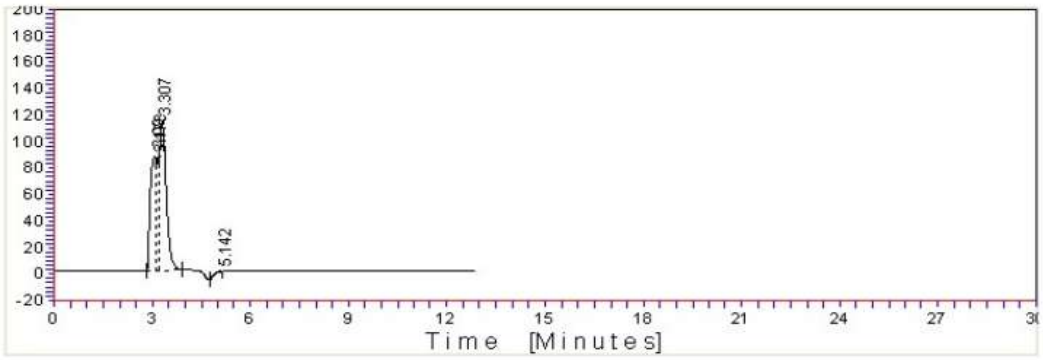

Figure 1: Trial Chromatogram obtained by using Acetonitrile: water (80:20) as mobile phase.

3.2 Application of proposed method for estimation of EHE in formulation

Equal volume (20µL) of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The content EHE was calculated by comparing a sample peak with that of standard (Figure 2).

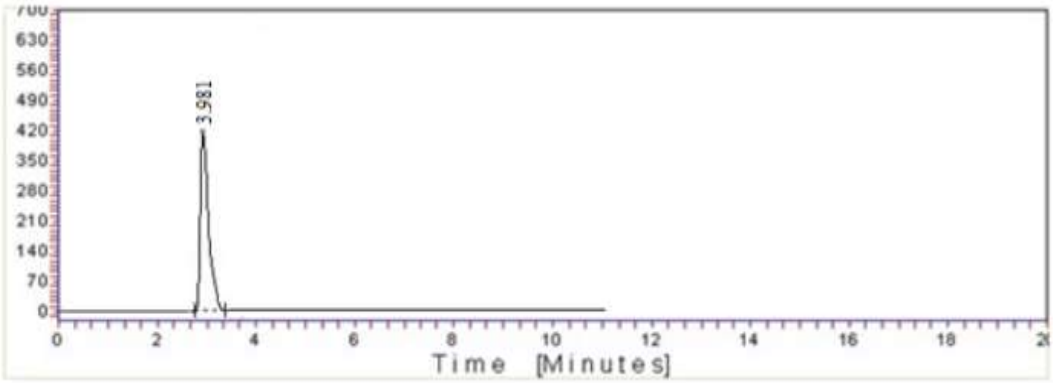

Figure 2: Chromatogram obtained by formulation of EHE

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3.3 Validation of the Method

Accuracy was ascertained on the basis of recovery studies performed by standard addition method (Table 1). Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method (Table 2). Specificity was measured as ability of the proposed method to obtain well separated peak for EHE without any interference from component of matrix. Mean retention time for – EHE – 3.981 The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix. Linearity and range: According to USP tablet powder equivalent to 80, 90, 100, 110, 120 % of label claim was taken and dissolved & diluted appropriately with mobile phase to obtain a concentration in the range of 80%-120% of the test concentration. The chromatograms of the resulting solutions was recorded. EHE marketed formulation was found to be linear in the range ± 20% of the test concentration of the respective drug (Table 3). The robustness study indicated that the factors selected remained unaffected by small variation of organic composition of mobile phase, wavelength and the flow rate. The system suitability results should lie within the limit. Hence the method was robust (Table 4). Limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Limit of quantitation is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision accuracy (Table 5). After establishing the chromatographic conditions, standard laboratory mixture was prepared and analysed by following procedure described under experimental and results. It gave accurate, reliable results and was extended for estimation of drugs in marketed tablet formulation.

Table 1: Results and statistical data for Recovery study of EHE

Sr. No	wt. of formulation	Amount of Drug Added in (µg/ml)	Peak Area of stand.	Peak Area of sample	% Recovery
	Efonidipine hydrochloride ethanolate (EHE)				
1	126	1	438956.1	437200.3	99.6
2		1		439395.1	100.1
3		1		439834.0	100.2
4		2		438517.1	99.9
5		2		443784.6	101.1
6		2		435883.4	99.3
7		3		441589.8	100.6
8		3		442467.7	100.8
9		3		442906.7	100.9

Table 2: Results and statistical data of Precision Study

Sr. No.	Weight of Standard (mg)	Weight of Sample (mg)	Peak Area of Stand.	Peak Area of Sample	% Label claim
	Efonidipine hydrochloride ethanolate (EHE)				
1	10	126	438956.1	440273.0	100.3
2		125.9		440711.9	100.4
3		126		441150.9	100.5

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Table 3: Observations of Linearity and range study for EHE

Sr. No.	%Label claim	Peak area
1	80	351164.9
2	90	395060.5
3	100	438956.1
4	110	488851.7
5	120	526847.3

Table 4: Result of Robustness study of EHE

Sr. No.	Condition	Parameter	Peak Area	RT
01	Change of wavelength	348 nm	438956.1	3.984
02		350nm	438956.1	3.981
03		352 nm	438956.1	3.980
04	Change in Temperature	30 °C	438854.2	3.983
05		25 °C	438956.1	3.981
06		20 °C	438456.2	3.979
07	Change in Flow rate	0.8 ml/min	438898.3	3.985
08		1ml/min	438956.1	3.981
09		1.2 ml/min	438987.6	3.978
10	Change in Mobile Phase	70:30	438901.5	3.986
11		75:25	438956.1	3.981
12		80:20	438974.2	3.979

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Table 5: Limit of detection (LOD) and Limit of quantitation (LOQ)

Sr. No.	Drug Name	LOD µg/ml	LOQ µg/ml
1	(EHE)	0.81	1.97

IV. CONCLUSIONS

From the studies it can be concluded that RP-HPLC technique can be successfully used for the estimation of the Efonidipine Hydrochloride Ethanolate in their pharmaceutical dosage tablet formulations. The method shows good reproducibility, the RP-HPLC method is accurate, precise, specific, reproducible and sensitive. The analysis of tablet dosage formulation of Efonidipine Hydrochloride Ethanolate can also be successfully performed. No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies.

Conflict of Interest

None

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