Analytical Method Development and Validation of Efonidipine Hydrochloride Ethanolate in Bulk and Dosage form by UV-Visible Spectrophtometry

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***Abstract*— A simple, precise, accurate, economical and reliable UV Spectrophotometric method has been developed for the estimation of Efonidipine in tablet dosage form. The drug shows maximum absorption at 253nm in methanol and obeys Beer's law in the concentration range of 10-30 µg /mL with good correlation coefficient (R^2=0.997). The results of analysis were validated by recovery studies. The recovery was found to be 96-99%. Limit of detection (LOD) and limit of quantification(LOQ) were found to be 2.82 µg/ml and 8.57µg/ml respectively. The relative standard deviation was found to be < 2.0 % in all cases. The Proposed Spectrophotometric method was validated as per the ICH Q2 (R1) guidelines. The proposed method can be used for the reliable quantification of Efonidipine in bulk form and routine analysis of pharmaceutical formulations.**

Index Terms: **Efonidipine, UV Spectrophotometry, Absorbance maxima, Method validation.**

INTRODUCTION

Efonidipine hydrochloride is chemically 2-(N-benzylanilino) ethyl 5-(5, 5-dimethyl-2-oxo- 1, 3, 2λ5-dioxaphosphinan-2-yl)-2, 6-dimethy l-4-(3-nitrophenyl) -1, 4-dihydropyridine-3-carboxylate; hydrochloride [1,2] . Efonidipine is a dihydropyridine, calcium channel blocker with anti-hypertensive activity.

Efonidipine acts on both L-type and T-type calcium channel. Because inhibition of T-typecalcium channels in SA node attenuate reflex tachycardia, this drug favorably affect cardiac pacing. Efonidipine has slow onset and long duration of action. [3, 4]. This drug is used in the treatment of acute or chronic vascular hypertension regardless of pharmacological mechanism. Among the antihypertensive agents are diuretics; (especially diuretics, thiazide); adrenergic beta-antagonists; adrenergic alpha-antagonists; angiotensin-converting enzyme inhibitors; calcium channel blockers; ganglionic blockers; and vasodilator agents [5, 6. 7.]

Efonidipine 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). 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. 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. 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.

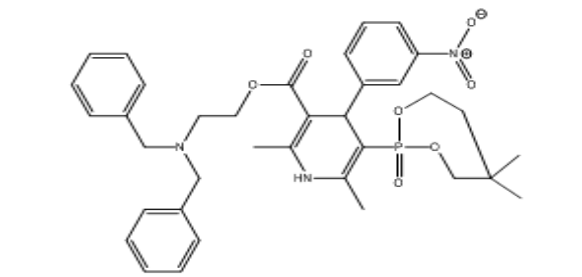

Figure 1: Chemical structure of Efonidipine

The present study is to validate UV Spectrophotometric method for quantitative analysis of Efonidipine in pharmaceutical dosage form.

MATERIAL & METHODS

Preliminary Analysis of Drug

Efonidipine

a) Description: The sample of Efonidipine was observed for its color and texture. Efonidipine is Pale yellow and crystalline powder.

b) Solubility: The sample of Efonidipine was taken in test tubes and observed for solubility in various solvents like water, methanol and acetonitrile.

c) IR Spectrum: 1mg of Efonidipine was kept on Selenide crystal and scanned in the region of 400-4000 $cm^{-1}$and infrared spectrum was obtained.

d) Melting Point: The sample of Efonidipine was taken in capillary and kept in melting point apparatus, obtained melting point was compared with the reference.

Selection of solvent: A number of trails were made to find out the ideal solvent system for dissolving the drug. Thesolvents such as water, methanol and acetonitrile, n-hexane and ethanol were tried based onthe solubility of the drug.Efonidipineis soluble in Methanol. Efonidipine was dissolved in methanol, a clear solution was obtained. Better absorption maximum was found to be 253 nm with methanol .So methanol was selected as optimized solvent in this spectrophotometric method.

Instruments used: UV-Visible Spectrophotometer (Systronic 2201model). The UV-VIS spectrophotometer achieves a resolution of 1 nm with matched quartz cells of 1 cm path length.

Reagents and Materials: API- Efonidipine pure drug was obtained as a gift sample from Ajanta Pharmaceuticals Ltd. Mumbai, Maharashtra, India. Tablets of 40 mg strength were purchased from the local pharmacy in Solapur under commercially available brand name Efonta (Ajanta Pharmaceuticals Ltd.). Analytical grade methanol was used as solvent.

Selection of detection wavelength: Appropriate dilutions of Efonidipine were prepared from the standard stock solution (100µg). Using UV- VIS Spectrophotometer, the dilutions of Efonidipine were scanned over a range of 200-330 nm. It was observed that the drug showed maximum absorbance at 253 nm which was selected as the wavelength for detection.

Preparation of standard drug solutions: 100 mg of Efonidipine pure drug was accurately weighed and transferred into a 100 mL volumetric flask containing Methanol. The volume was made up to the mark with solvents to get the stock solution (1000µg/mL).from this solution 1 ml was pipette out and transferred to another 10 ml volumetric flask containing methanol and the volume was adjusted to 10 ml with same solvent to give the concentration of 100µg/ml. This solution was further diluted with the same to get the working standard solution.

Preparation of Calibration curve: Aliquots of standard drug (1 mL to 3 mL, 100 µg/mL) solution in methanol were transferred into a series of 10 mL volumetric flasks and the solution was made up to 10 mL with methanol. After setting the instrument for its spectral properties the solutions were scanned in the wavelength ranging from 200 nm - 330 nm. The wavelength of maximum absorption for Efonidipine was found at 253 nm. Calibration data is presented in Table 1. Calibration curve was prepared by plotting concentration of Efonidipine on x- axis and their respective absorbance's on y-axis.

Procedure for assay of pharmaceutical formulations: 20 tablets weighed and powdered. The powder equivalent to 10 mg of Efonidipine was weighed and transferred into100 ml volumetric flask and dissolved in methanol LR. This solution was sonicated for 15 minutes and final volume was made up to the mark with methanol LR (tablet solution). 1ml of solution

was transferred into 10 ml of volumetric flask and diluted up to 10ml with methanol. From this 2 ml was pipetted out and volume was adjusted to 10ml to give the conc. of 20µg/ml. and the absorbance of the solution was measured at 253 nm.

Validation of the developed method[8]:

Linearity: The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity can be assessed by performing single measurements at several analyte concentrations. A linearity correlation coefficient above 0.9995 is acceptable for most methods, especially for major components in assay methods. The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample.

Precision: The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. Precision was determined by intra-day and inter-day study. The repeatability of the method was evaluated by carrying out the assay 3 times on the same day and intermediate precision was evaluated by carrying out the assay on 3 consecutive days for the sample solution. The percent relative standard deviation (% RSD) was calculated.

Accuracy (Recovery studies): The accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted true value. Accuracy studies were performed at three different levels (80%, 100% and 120%) by standard addition method and the samples were analyzed in triplicate by the proposed method. Known amount of standard Efonidipine at 80%, 100% and 120% of predetermined sample was added to a pre quantified tablet sample. The results of accuracy are presented in Table

Ruggedness: Method ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents and so on. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method. The results of ruggedness are presented in Table.

Robustness: The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters". The most important aspect of robustness is to develop methods that allow for expected variations in the separation parameters. For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range. The absorbance was measured and assay was calculated for six times. The results of robustness are presented in Table.

LOD and LOQ: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Limit of Detection and Limit of Quantitation were calculated using following formula LOD= 3.3(SD) and LOQ= 10 (SD) / S, where SD=standard deviation of response (absorbance) and S= slope of the calibration. The results of LOD and LOQ are shown in Table.

RESULTS AND DISCUSSION

Preliminary analysis of Efonidipine such as description, solubility, melting point, and broad infrared spectrum at about 3406.16 cm^{-1} indicate presence of (N-H) Amine stretch and at 2942.51(C-H) Alkyl stretch, and at 2624.73 Carboxylic Acid OH Stretch respectively. This confirms the identification of Efonidipine

The absorption spectra were recorded in the wavelength region of 200-400 nm in UV method, the absorption maxima curve was shown in Figure 2 and3.The proposed method obeyed Beer's law in the concentration range of 10-30 µg/mL with good

correlation coefficient of R2 =0.997. Calibration data is presented in Table 1. Beer's law range was confirmed by the linearity of the calibration curve of Efonidipine was shown in Figure 4. Precision of the method was reported in terms of relative standard deviation and it should be evaluated by using a minimum of 3 determinations over which shows % RSD less than 2 indicates that the method was precise and the results are presented in Table 3-7.Recovery studies were carried out for the developed method by addition of known amount of standard drug solution of Efonidipine to pre-analyzed tablet sample solution at three different concentration levels. The resulting solutions were analyzed by the proposed methods. The recovery (Table 10) was in the range of 96.875to 99.16percentages. The limit of detection and limit of quantitation for estimation of Efonidipine were 2.82µg/mL,8.57µg/mL respectively. Ruggedness was performed by two different analysts under same experimental condition. The % RSD was calculated. The results were reported to be within the limits. It reveals that the proposed method was found to be rugged and the results are tabulated in Table 13 for the determination of Efonidipine. For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influences of the variables were determined. The absorbance was measured and assay was calculated. The results of robustness are presented in Table 12. The results are within the specified limits which states that this method is robust. The developed method was applied to the analysis of tablet formulations found to be within the proposed limits and the mean % assay value was found to be 98 %. The assay results are given in Table 9. The developed method has good linearity, accuracy and precision results indicates that the high quality of the method.

Preliminary Analysis of Efonidipine

Table.1. Results of Preliminary Analysis of Efonidipine

Sr.no	Tests	Observations	Results
1.	Description	Pale yellow and crystalline powder	Complied
2.	Solubility	Soluble in methanol and in acetonitrile, Insoluble in water	Complied
3.	Identification test	Infrared spectrum peaks at 3406.16, 3219.57, 2942.51, 2624.73 cm^{-1}	complied
4.	Melting point	170^0C	complied

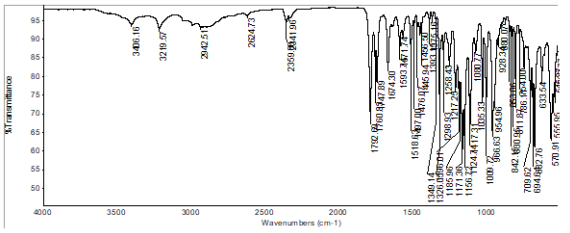

Figure 2: IR Spectrum of Efonidipine

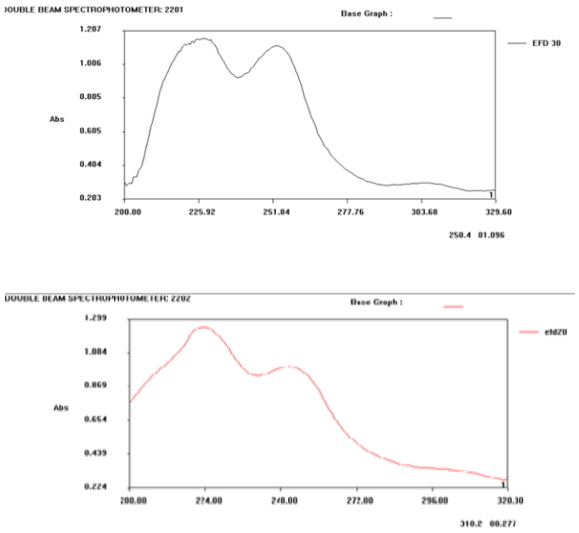

Figure 2 and 3: UV spectrum of Efonidipine between 200-330nm in mobile phase

Table.2. Linearity data for Efonidipine:

Concentration(µg/mL)	Absorbance
10	0.467
15	0.807
20	1.05
25	1.308
30	1.603

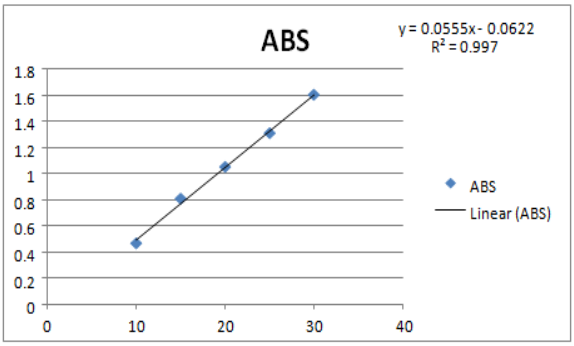

Figure.4 .Calibration curve of Efonidipine by UV method

Table 3: Intraday morning precision

Sr. no	Conc. (µg/ml)	Absorbance	SD	%RSD
1	20	1.127		
2	20	1.134	0.010693	0.3169
3	20	1.113		
		y= 1.1246		

Table 4: Intraday afternoon precision

Sr. no	Conc. (µg/ml)	Absorbance	SD	%RSD
1	20	1.126		
2	20	1.115	0.01253	0.3749
3	20	1.101		
		y=1.114		

Table 5: Intraday evening precision

Sr. no	Conc. (µg/ml)	Absorbance	SD	%RSD
1	20	1.103		
2	20	1.097	0.004163	0.1263
3	20	1.095		
		y=1.098		

Table 6: Interday morning precision

Sr. no	Conc. (µg/ml)	Absorbance	SD	%RSD
1	20	1.105		
2	20	1.106	0.001528	0.0461
3	20	1.103		
		y=1.10		

Table 7: Interday Afternoon precision

Sr. no	Conc. (µg/ml)	Absorbance	SD	%RSD
1	20	1.08		
2	20	1.1	0.009428	0.009428
3	20	1.1		
		y=1.10		

Table 8: repeatability

Sr. no	Conc. (µg/ml)	Absorbance	SD	%RSD
1	20	1.081		
2	20	1.077		
3	20	1.078	0.002582	0.0398
4	20	1.082		
5	20	1.083		
6	20	1.083		
		y=1.080667		

Table 9: Assay study

Tablet formulation	Label claim	Amount taken	Amount found	Assay%
Efonta	40 mg	20µg/ml	19.6	98

Table 10: accuracy study

Sr. no	Level of % recovery	Amount of tablet sample(ml)	Amount of std drug added(µg/ml)	Amount added(µg/ml)	Amount found(µg/ml)	% recovery
1	Blank	4	0	0	0	-
2	80	1	0.6	16	15.5	96.875
3	100	1	1	20	19.6	98
4	120	1	1.4	24	23.8	99.16

Table 11: limit of detection and limit of quantitation

LOD (µg/ml)	2.82 µg/ml
LOQ (µg/ml)	8.57µg/ml

Table 12: Robustness

Sr. no	Wavelength(nm)	Absorbance	SD	%RSD
1	264	0.177		
2	265	0.180		
3	266	0.1841	0.002645	1.46337
4	267	0.1832		
5	268	0.181		
6	269	0.179		

Table 13: Ruggedness

Analyst- 1		
Conc (µg/ml)	absorbance	Average, Standard deviation and %RSD
1	0.218	Average= 0.220833
2	0.219	Standard deviation =0.002137
3	0.222	%RSD =0.967687
4	0.221	
5	0.224	
6	0.221	
Analyst-2		
1	0.224	
2	0.226	Average= 0.223167
3	0.221	Standard deviation =0.002639
4	0.224	%RSD= 1.182723
5	0.225	
6	0.219	

SUMMARY AND CONCLUSION

Summary of UV spectroscopic Methods of Efonidipine

Table 14: for summary

Sr. no	parameters	Values
1	Beer's law limit((µg/ml)	10-30
2	Absorption maxima(nm)	253
3	Standard regression equation	y = 0.0555x-0.0622
4	Correlation coefficient	0.997
5	Accuracy	96.8-99.1%
6	Precision(%RSD) repeatability	0.0398%
7	LOD	2.82 µg/ml
8	LOQ	8.57µg/ml
9	Robustness(%RSD)	1.46337
10	Ruggedness(RSD)	0.967687 and 1.182723
11	Assay (%)	98

CONCLUSIONS

The UV-spectrophotometric method was developed and it is found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of Efonidipine in API and its pharmaceutical dosage form without any interference from the excipients. This method can be effectively applied to the routine analysis of Efonidipine in API. Its advantages are the low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

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Conflict of Interest

We have no conflicts of interest to disclose.

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