Original Article

Development and Validation of Three Novel UV Spectrophotometric Methods for Simultaneous Estimation of Efonidipine Hydrochloride Ethanolate and Telmisartan in Their Synthetic Mixture and Its Comparison Using ANOVA

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ABSTRACT

Efonidipine hydrochloride ethanolate and telmisartan combination is used for to treat hypertension treatment and under clinical phase 4 study. It is necessary to develop suitable quality control methods for rapid and accurate determination of these drugs. Three simple, accurate, sensitive, precise and economical UV spectrophotometric methods (A, B &, and C) have been developed for simultaneous estimation of efonidipine hydrochloride ethanolate and telmisartan in their synthetic mixture. Method (A) is based on the first order derivative spectrophotometric method at zero crossing wavelength. In this method the zero crossing zero-crossing point of efonidipine hydrochloride ethanolate is 326 nm and for telmisartan is 272 nm. The linearity was obtained in the concentration range of 8-20 µg/ml for efonidipine hydrochloride ethanolate and 16-40 µg/ml for telmisartan using methanol as a solvent. Method (B) is based on absorbance correction method, method; it was performed at 347 nm for efonidipine hHydrochloride ethanolate and at 296 nm for telmisartan. Method (C) is based on dual wavelength method developed using absorbance difference at 242.5 nm and 257.5 nm for efonidipine hydrochloride ethanolate and 244.5 nm and 287 nm for telmisartan. The accuracy and precision of the methods were determined assessed and validated statistically. All the methods showed revealed good reproducibility and recovery. The three methods were compared using one way ANOVA. All methods were found to be rapid, specific, precise and accurate and these methods require no preliminary separation and found no interferences from the tablet excipients so it can be used for routine analysis of both drugs in quality control laboratories.

GRAPHICAL ABSTRACT
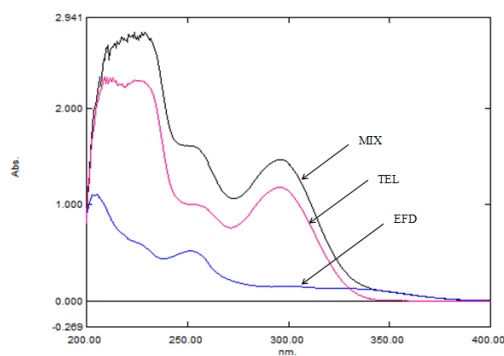
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Introduction

Efonidipine hydrochloride ethanolate (EFD) chemically known as 2-(*N*-benzylanilino)ethyl 5-(5,5-dimethyl-2-oxo-1,3,2λ^5-dioxaphosphinan-2-yl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylate;ethanol; hydrochloride (Figure 1). It is 1, 4-dihyropyridine calcium channel blocker and it is used as anti-hypertensive and anti-angina drug. It inhibits both the L and T type calcium channel, increasing the vasodilation and decreasing the automaticity of the heart [1, 2]. Telmisartan (TEL) chemically known as 2-4-[[4-methyl-6-(1-methylbenzimidazol-2-yl)-2-propylbenzimidazol-1-yl] methyl] phenyl] benzoic acid (Figure 2). It is Angiotensin type 1 receptor blocker and it is used as anti-hypertensive and anti-cardiac arrhythmias drug [3-5]. Therefore, this combination of EFD and TEL is primarily utilized to treat hypertension as it provides effective control of blood pressure through synergistic mechanism, EFD cause vasodilation of arterioles and TEL counteracts the stimulation of renin angiotensin system (RAS) and is used to reduce the incidence of peripheral oedema [6-8]. It was reported that the analytical methods were reported for quantification of efonidipine hydrochloride ethanolate and telmisartan alone and in combination with other drugs but not a single method is reported for the simultaneous estimation of both EFD and TEL [9-19]. Therefore, simple, rapid, accurate and reliable method for simultaneous estimation of these drugs seemed to be necessary. The purpose of this study was to determine and validate both the drugs concurrently by simple, accurate, rapid and precise spectrophotometric method for routine analysis.
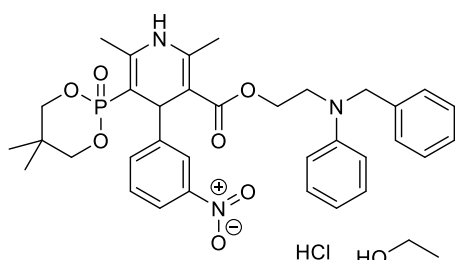

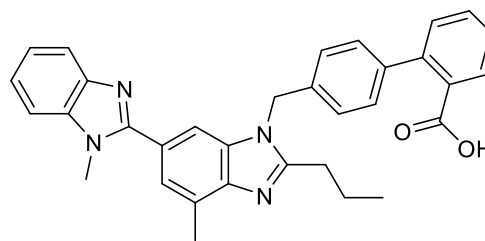**Figure 1:** Chemical structure of efonidipine hydrochloride ethanolate

Figure 2: Chemical structure of telmisartan

Material and methods

Instrument

A UV Probe type UV-VIS double beam spectrophotometer (Shimadzu 1800) with 1 cm Quartz cells was used in this experiment. Analysis was performed using direct mode over a wavelength range from 200–400 nm. The instrument settings were zero order and first derivative mode and band width of 2 nm in the range of 200-400 nm. All weights were taken on electronic balance.

Reagents and materials

Efonidipine hydrochloride ethanolate gift sample was provided by Pure Chem Pvt. Ltd., Ankleshwar, Gujarat. Telmisartan gift sample was provided by Cadila Healthcare Limited, Ankleshwar, Gujarat.

Selection of solvent

Solubility of both the drugs was performed by using various solvents such as distilled water, methanol, ethanol and acetonitrile. It was found that both the drugs were soluble in methanol so it was selected as solvent.

Preparation of stock sStock solutionsSolution

25 mg of EFD and TEL were weighed accurately and transferred to separate 25 ml volumetric flask. Then 10 ml ml of methanol was added to both the flask and sonication was done. Makeup was done by filling methanol up to up to the mark to obtain the primary stock of 1000 µg/ml. By diluting primary stock solution of EFD (5 µg/ml) and TEL (10 µg/ml) in different 25 ml volumetric

flask, secondary stock EFD (200 μg/ml) and TEL (400 μg/ml) was obtained. From secondary stock solution various working solutions were prepared.

Method A: First Order Derivative Spectroscopic Method

For first order derivative method, working solution of both the drugs was prepared. For EFD working solutions from 8-20 μg/ml mlL was prepared by transferring the mentioned amount (0.4 ml, 0.5 ml, 0.6 ml, 0.7 ml, 0.8 ml, 0.9 ml ml, and 1 ml) of secondary stock solution to 10 ml volumetric flask and the dilution of this solution was done by filling the methanol up to the mark. In the same way TEL working solutions from 16-40 μg/ml was prepared by transferring the mentioned amount (0.4 ml, 0.5 ml, 0.6 ml, 0.7 ml, 0.8 ml, 0.9 ml and 1 ml) of secondary stock solution to 10 ml volumetric flask and the dilution of this solution was done by filling the methanol up to the mark. Then zero order spectra of the above solutions were recorded. Then these spectra were derivatised to first order spectra. From overlain first order spectra of EFD (14 μg/ml) and TEL (28 μg/ml) zero crossing points (ZCP) of EFD and TEL were obtained. Wavelength selected as the ZCP for EFD was 326 nm where TEL gives the substantial absorbance while the ZCP for TEL was 272 nm where EFD was giving substantial absorbance. From the dertivatised spectra of mixtures estimation of EFD was done at 272 nm (ZCP of TEL) and estimation of TEL was done on 326 nm (ZCP of EFD). Then the calibration curve of both the drugs was obtained by plotting the graph between absorbance vs concentration and the concentration of both the drugs was assessed.

Method B: Absorbance Correction Method

In this method two wavelengths were selected from which one wavelength was λ_{max} of one drug at which the other drug will not give any substantial absorbance. On other wavelength both the drug and mixture all three were give absorbance, in which interfering drug absorbance

subtracted from the mixture absorbance to obtain the corrected absorbance of another drug. Hence, this method is modification of simultaneous equation method. In this method, it was observed that EFD was giving substantial absorbance at 347 nm (λ_{max} of EFD) while TEL was practically nil. Therefore, estimation of EFD can be done at 347 nm without interference of TEL. At 296 nm TEL, EFD and Mixture all three were giving substantial absorbance. So, for estimation of TEL absorbance of EFD was subtracted from mixture absorbance so that the absorbance of TEL was obtained. Obtained absorbance of TEL is known as corrected absorbance of TEL. The concentration of TEL was calculated from calibration curve at 296 nm by using corrected absorbance.

Corrected absorbance = Total absorbance – Interfering absorbance.

The concentration of two drugs (X and Y) in the mixture can be calculated using following equations:

$$Cy = A2 / ay2 \qquad (1)$$
$$Cx = A1\text{-}ay1* Cy/ax1 \qquad (2)$$

Where, A1 and A2 are the absorbance of mixture at $\lambda 1$ and $\lambda 2$ respectively, ay1 and ay2 are absorptivities of y at $\lambda 1$ and $\lambda 2$ respectively, ax1 is absorptivity of X at $\lambda 2$, C_X is concentration of X, C_y is concentration of Y.

Method C: Dual Wavelength Method

The utility of dual wavelength data processing program is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. This is directly proportional to the concentration of the component of interest, independent of the interfering components. The pre-requisite for dual wavelength method is the selection of two such wavelengths where the interfering

component shows same absorbance whereas the component of interest shows significant difference in absorbance with concentration. Working solution of EFD and TEL was scanned in UV between 200-400 nm. Overlain spectra of both the drugs were obtained from which two wavelengths for EFD and TEL was selected. For EFD two wavelengths that are 242.5 nm and 257.5 nm was selected. The absorbance difference of EFD was zero but TEL and mixture has shown some significant absorbance difference at 242.5 nm and 257.5 nm. However, the difference obtained from absorbance of TEL and mixture at 242.5 nm and 257.5 nm was same. For TEL two wavelengths that are 244.5 nm and 287 nm was selected. The absorbance difference of TEL was zero but EFD and mixture had shown some significant difference at 244.5 nm and 287 nm. However, the difference obtained from absorbance of EFD and mixture at 244.5 nm and 287 nm was same. Hence, the estimation of EFD was done by calculating the absorbance difference at 244.5 nm and 287 nm while estimation of TEL was done by calculating the absorbance difference at 242.5 nm and 257.5 nm.

Analysis of EFD and TEL in their synthetic mixture
For estimation of both the drugs in their synthetic mixture, tablets were prepared synthetically in lab using common pharmaceutical ingredients. From that, twenty tablets were weighed and average weight was calculated. The powder equivalent to 20 mg of efonidipine Hydrochloride ethanolate and 40 mg of telmisartan were transferred to 100 ml volumetric flask. 25 ml methanol was transferred to volumetric flask and sonicated for 10 min. Then methanol was filled up to the mark of volumetric flask. Concentrations obtained are 14 μg/ml (EFD) and 28 μg/ml (TEL). These solutions were scanned according to the wavelength selected in different methods. In method 1 EFD solution is scanned at 272 nm and TEL solution is scanned at 326 nm. In method 2 EFD solution is scanned at 347 nm (λ max of EFD) and TEL solution is scanned at 296 nm (λ max of TEL). In method 3, other concentrations

12 μg/ml (EFD) and 24 μg/ml (TEL) were selected and absorbance difference of EFD is calculated at 244.5 nm and 287 nm while TEL is calculated at 242.5 nm and 257.5 nm. Absorbance obtained from three methods was put into their respective calibration curve equations and concentration is obtained.and % label claim was found.

Validation parameters
According to ICH guideline (Q2 R1) these three methods were validated.

Accuracy
By using standard addition method interference of the excipients was checked by calculating the % recovery of drug. In this method standard solution of EFD and TEL were added to sample solution and the standard drug recovered was calculated in terms of mean recovery with upper and lower limits with its % RSD.

Precision/Repeatability
By keeping the parameter of proposed methods constant solutions of EFD and TEL was scanned (n=6) and absorbance were recorded.

Intermediate precision
In this intraday and interday precision is measured. Three concentrations of EFD and TEL was scanned on the thrice a day for intraday and for interday same concentrations was scanned on three different days. The results of intraday and interday precision were calculated in terms of % RSD.

Limit of detection (LOD) and Limit of Quantification (LOQ)
By using 3 s/m and 10 s/m LOD and LOQ was calculated respectively where, S is the standard deviation of intercept (n=6) of the sample and m is the slope of the corresponding calibration curve.

Analysis of variance (ANOVA)

This statistical tool is used to check the variation between the three developed methods used for the simultaneous estimation of EFD and TEL in their synthetic mixture.

Result and Dissection

Method A: First order derivative spectroscopic method

First order spectra show more resolution than zero order spectra in terms of zero crossing points. Figure 3 and 4 shows the overlain first order spectra of EFD and TEL respectively. At 326 nm EFD has zero crossing point and TEL can be estimated. At 272 nm TEL has zero crossing point and EFD can be estimated.
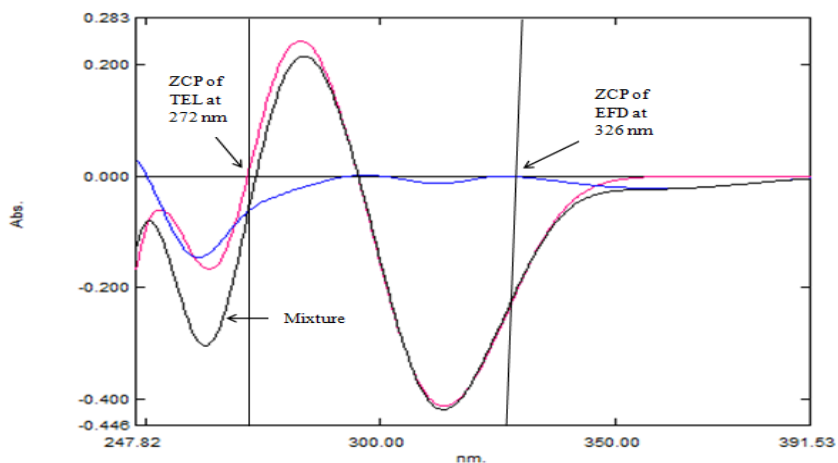

Figure 3: Overlain first order derivative spectra of EFD (14 µg/ml) and TEL (28 µg/ml)
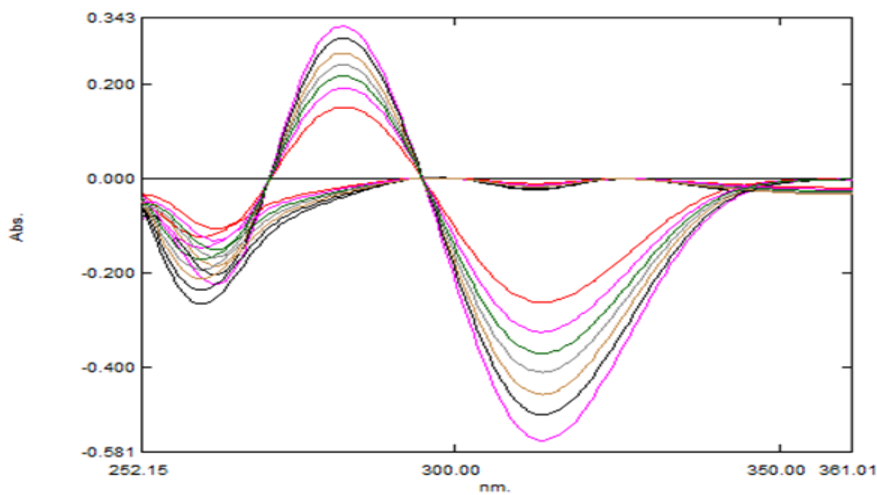

Figure 4: Overlain first order derivative spectra of standard EFD (8-20 µg/ml) and TEL (16-40 µg/ml)

Method C: Dual wavelength method

From Figure 7, four wavelengths were selected where one drug gives zero absorbance difference. On each other wavelength absorbance difference was measured and the calibration curve was plotted for both the drugs. Figure 8 demonstrates the overlain spectra of EFD and TEL.

Table 1, Table 2 and Table 3 exhibit the results of assay, results of accuracy studies and summary of various validation parameters of all methods, respectively.
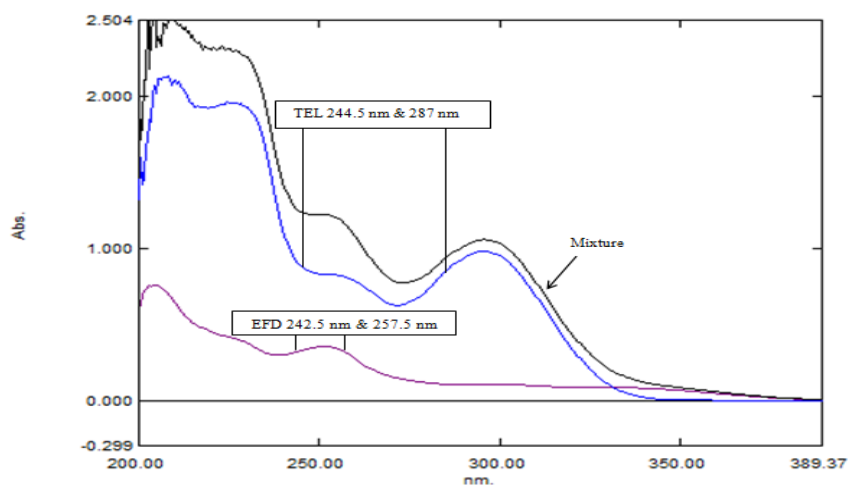
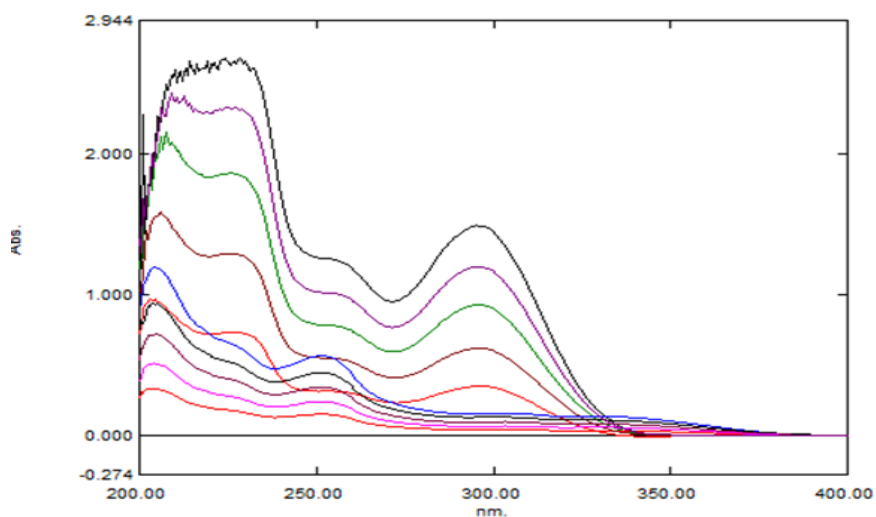**Figure 7:** Overlain spectra of EFD (12 µg/mL) and TEL (24 µg/mL)

Figure 8. Overlain spectra of EFD (4-20 µg/ml) and TEL (8-40 µg/ml)

Table 1. Assay results for tablets using the proposed methods

Synthetic Mixture	Proposed Methods	Label claim (mg)		Amount found (mg)		% Label claim Assay (n=3) ± SD	
		EFD	TEL	EFD	TEL	EFD	TEL
Tablet	METHOD A	20	40	19.95	39.97	99.79± 1.41	99.93± 0.46
	METHOD B	20	40	19.92	40.05	99.61± 1.17	100.13± 0.33
	METHOD C	20	40	19.81	39.79	99.07 ± 0.75	99.47 ± 1.20

Table 2: Application of the standard addition technique to the analysis of EFD and TEL in their synthetic mixture by the proposed methods

Method	Drug	Level	Conc. preset (μg/ml)	Spiked conc. (μg/ml)	Total conc. taken (μg/ml)	Mean of total Conc. found (μg/ml)	Amt. recovered (μg/ml)	% Recovery ± SD (n=3)	% RSD
Method A	EFD	80%	8	6.4	14.4	14.42	6.45	100.76 ± 1.78	1.76
		100%		8	16	16.00	8.03	100.35 ± 1.42	1.41
		120%		9.6	17.6	17.58	9.61	100.08 ± 1.18	1.18
	TEL	80%	16	12.8	28.8	28.67	12.78	99.85 ± 1.55	1.55
		100%		16	32	31.79	15.90	99.38 ± 0.46	0.47
		120%		19.2	35.2	35.17	19.28	100.42 ± 1.03	1.02
Method B	EFD	80%	8	6.4	14.4	14.33	6.39	99.80 ± 1.49	1.49
		100%		8	16	16.04	8.09	101.18 ± 1.19	1.17
		120%		9.6	17.6	17.47	9.53	99.23 ± 0.99	1.00
	TEL	80%	16	12.8	28.8	28.72	12.80	100.02 ± 0.36	0.36
		100%		16	32	31.86	15.95	99.67 ± 0.38	0.38
		120%		19.2	35.2	35.28	19.37	100.87 ± 0.32	0.32
Method C	EFD	80%	8	6.4	14.4	14.39	6.43	100.60 ± 1.42	1.41
		100%		8	16	15.94	7.98	99.85 ± 1.13	1.13
		120%		9.6	17.6	17.49	9.53	99.35 ± 0.94	0.95
	TEL	80%	16	12.8	28.8	28.68	12.78	99.88 ± 0.85	0.85
		100%		16	32	31.78	15.89	99.29 ± 1.18	1.19
		120%		19.2	35.2	35.20	19.30	100.50 ± 0.98	0.98

Table 3: Summary of validation parameter by developed method

Parameters		First order derivative method		Absorbance correction method		DUAL WAVELENGTH METHOD	
		EFD	TEL	EFD	TEL	EFD	TEL
Working wavelength (nm)		326	272	347	296	Abs. Diff. at 242.5 & 257.5	Abs. Diff. at 244.5 & 287
Concentration range (μg/mL)		8-20	16-40	8-20	16-40	4-20	8-40
Slope		-0.005	-0.008	0.006	0.033	0.017	0.005
Intercept		-0.013	-0.051	-0.002	0.262	0.022	0.017
Correlation coefficient (r^2)		0.9993	0.9990	0.9989	0.9993	0.9999	0.9996
LOD (μg/mL)		0.678	0.270	0.903	0.252	0.314	0.723
LOQ (μg/mL)		2.056	0.818	2.735	0.764	0.951	2.190
Accuracy (% recovery, n = 3) ± RSD	80 %	100.76 ± 1.76	99.85 ± 1.55	99.80 ± 1.49	100.02 ± 0.36	100.60 ± 1.41	99.88 ± 0.85
	100 %	100.35 ± 1.41	99.38 ± 0.47	101.18 ± 1.17	99.69 ± 0.38	99.85 ± 1.13	99.29 ± 1.19
	120 %	100.08 ± 1.18	100.42 ± 1.02	99.23 ± 1.00	100.87 ± 0.32	99.35 ± 0.95	100.50 ± 0.98
Precision (% RSD)	Repeatability (n=6)	1.22	0.85	1.79	0.14	0.87	1.41
	Intraday (n=3)	1.06 - 1.29	0.57- 0.87	1.22- 1.26	0.13- 0.26	0.42-0.77	0.44-1.05
	Interday (n=3)	1.31- 1.85	0.74- 1.13	1.23- 1.81	0.17- 0.32	0.69-1.08	0.67-1.69
% Label claim Assay ± SD (n=6)		99.79 ± 1.41	99.93 ± 0.46	99.61 ± 1.17	100.13 ± 0.33	99.07 ± 0.75	99.47 ± 1.20

Statistical comparison of the results of the developed three methods

By using one way ANOVA variation between the three developed methods were checked and no significant variation was observed because F_{cal} is less than F_{tab}. Results of one way ANOVA are manifested in table 4 and 5.

Table 4: One way anova for EFD

Source of variation	Sum of squares	Degree of freedom	Mean of square	F_{cal}	P-Value	F_{tab}
Between Groups	0.053511	2	0.026756	0.130702	0.878468	3.68232
Within Groups	3.0706	15	0.204707	-	-	-
Total	3.124111	17	-	-	-	-

Table 5: One way anova for TEL

Source of variation	Sum of squares	Degree of freedom	Mean of square	F_{cal}	P-Value	F_{tab}
Between Groups	1.580678	2	0.790339	1.943318	0.177633	3.68232
Within Groups	6.100433	15	0.406696	-	-	-
Total	7.681111	17	-	-	-	-

Conclusion

In this research study, three UV methods (first order derivative, absorbance correction and Dual wavelength) were developed for the simultaneous estimation of efonidipine hydrochloride ethanolate and telmisartan in their synthetic mixture. During estimation of both the drugs other excipients present in the synthetic mixture had not shown any interference. Developed methods were also successfully applied to synthetic mixture and assay were found to be 99.07- 99.79 % for efonidipine hydrochloride ehtanolate and 99.47 - 100.13% for telmisartan. Result of the validation parameters were found within limits. Comparison of three methods was done using the ANOVA, and results revealed no significant difference between the methods. These methods are simple, accurate, precise and cost effective and can be used for routine analysis of efonidipine hydrochloride ehtanolate and telmisartan in their synthetic mixture.

Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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