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Rp-hplc method development and validation for simultaneous estimation of efonidipine hydrochloride ethanolate and telmisartan in their synthetic mixture

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Abstract

A Novel, selective, accurate and rapid Reversed Phase High Performance Liquid Chromatographic (RPHPLC) method for the analysis of Efonidipine Hydrochloride Ethanolate and Telmisartan in binary mixture has been developed and validated. The chromatographic system consisted of a Phenomenex Kinetex ® 5µ C18 Size: 150 * 4.6mm column and the separation was achieved by using ambient temperature with a mobile phase containing mobile Phase Acetonitrile:25mM Phosphate Buffer pH 4.9 (45:55). The samples were monitored at 253 nm for detection at a flow rate of 1.0 mL/min and the retention time was about 7.77 and 4.10 mins for Efonidipine Hydrochloride Ehanolate and Telmisartan respectively. The calibration curve was linear over the concentration range 5-30 and 10-60 µg/mL for Efonidipine Hydrochloride Ehanolate and Telmisartan respectively. The proposed method is accurate in the range of 99.75% - 100.10% recovery and precise (%RSD of intraday variation and % RSD of inter day variation were found to be within the acceptance criteria). Therefore, this method can be used as a more convenient and efficient option for the analysis of Efonidipine Hydrochloride Ehanolate and Telmisartan in Quality control laboratory.

Keywords: RP-HPLC, Efonidipine Hydrochloride Ehanolate and Telmisartan.

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Introduction

Efonidipine Hydrochloride Ethanolate is a novel dihydropyridine derrivative calcium channel blocker. It is chemically known as 2-(*N*-benzylanilino)ethyl 5-(5,5-dimethyl-2-oxo-1,3,2λ5-dioxaphosphinan-2-yl)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro pyridine-3-carboxylate;ethanol;hydrochloride and its chemical stucture is shown in (fig 01). Telmisartan is chemically known as 2-[4-[[4-methyl-6-(1-methylbenzimidazol-2-yl)-2-propylbenzimidazol-1-yl] methyl] phenyl] benzoic acid and its chemical structure shown in (fig 02). Efonidipine is a dihydropyridine calcium channel blocker used to treat the hypertension and angina pectoris. It inhibits both L- and T- type calcium channels, thereby leading to vasodilation and decrease the automaticity of heart. Telmisartan is an angiotensisn-Ⅱ subtype AT1 receptor antagonist which lowers the high blood pressure and prevents the heart strokes, heart attacks and kidney problems [1-5]. It also improves the carbohydrate and lipid metabolism. Therefore, this combination of EHE and TEL is mainly used to treat hypertension as it provides effective control of blood pressure through synergistic mechanism, EHE cause vasodilation of arterioles and TEL counteracts the stimulation of RAS and is used to reduce the incidence of peripheral oedema. Literature survey shows that less analytical methods were reported for quantification of EHE but methods are available in biological fluids and finished products is done by using stability and physicochemical characterization, fluorescence and circular dichroism spectroscopic method , RP-HPLC and LC-MS/MS [6-10]. Many analytical methods are reported for estimation of Telmisartan individually or in combination with other drugs and some of them are RP-HPLC [14, 15], RP-

UPLC [16], HPTLC [17], LC-MS [18] and UV-spectrometric [19]. However, until now there is no any chromatographic method is reported for simultaneous estimation of Efonidipine Hydrochloride Ethanolate and Telmisartan in their combination.

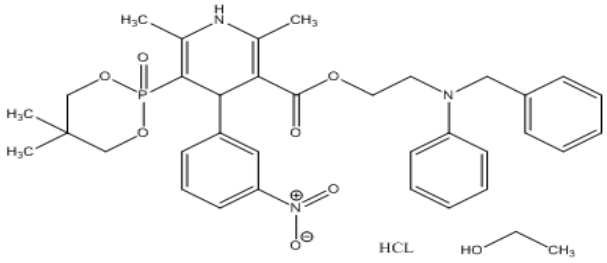

Fig 01: Chemical structure of Efonidipine Hydrochloride Ethanolate

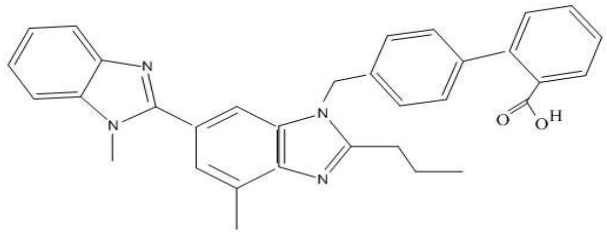

Fig 02: Chemical structure of Telmisartan

Material and Methods

Instrumentation and Chemicals

The liquid chromatographic system comprise of the following components was used for analysis.HPLC system (Shimadzu-LC 20AD binary pump system) equipped with UV detector manual injector and operated by LC Solution Software. C18 column (250 mm × 4.6 mm i.d., particle size 5 μm) was used for separation. Mobile phase used for separation was mixture of Acetonitrile and 25 mM Phosphate buffer pH 4.9 (45:55 v/v). The flow rate was kept at 1.0 ml/min, column temperature was ambient (25°C), eluents were detected by UV detector SPD - 20A at 253 nm, and the injection volume was 20 μl. 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.

Preparation of Phosphate buffer pH 4.9

About 3.40 g potassium dihydrogen phosphate and 3.55 g anhydrous disodium hydrogen phosphate were weighed and dissolved in water to produce 1000 ml. The pH was adjusted by using 1 M sulphuric acid or 1 M sodium hydroxide solution.

Preparation of Mobile Phase

Mobile phase was prepared by mixing 45 mL of Acetonitrile with 55 mL of 25 mM Phosphate buffer pH 4.9. The mobile phase was filtered through 0.45 µm membrane filter paper.

Preparation of Standard Stock Solution

About 25 mg of EHE and TEL were weighed accurately and transferred to separate 25 ml volumetric flask. Then 10 ml of methanol was added to both the flask and sonication was done. Makeup was done by filling methanol upto the mark to obtain the primary stock of 1000 µg/ml. By diluting primary stock solution of EHE (12.5 ml) and TEL (12.5 ml) in different 25 ml volumetric flask, secondary stock EHE (500 µg/ml) and TEL (500 µg/ml) was obtained. From secondary stock solution various working solutions were prepared.

Method Development

To develop a suitable (specific) and robust HPLC method for the determination of EHE and TEL, different mobile phases were employed to achieve the best separation and resolution. The method development was started with C18 column (250 mm × 4.6 mm i.d., particle size 5 μm) with the Flow rate of 1.0 mL/min and the column temperature was monitored at 25ºC and the injection was 20 µL. UV detection was performed at 253 nm and the sample temperature was maintained at 25ºC.

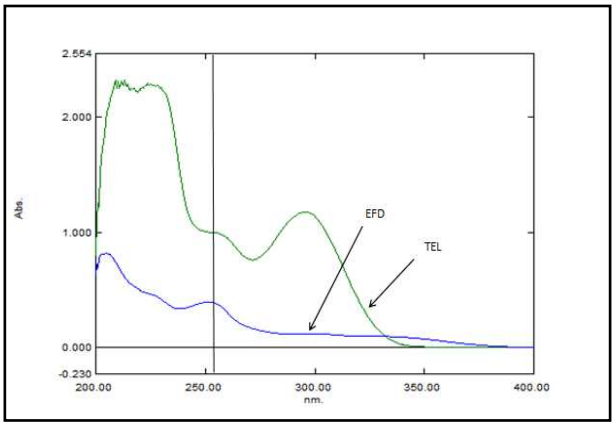

Fig 03: Overlain spectra of EFD (12 µg/mL) and TEL (24 µg/mL)

Optimized chromatographic conditions:

The chromatographic estimation was performed using following conditions:

1. Stationary phase: C18 column (250 mm × 4.6 mm i.d., particle size 5 μm)
2. Column temperature: 25ºC
3. Mobile phase: Acetonitrile : 25 mM Phosphate buffer pH 4.9 (45:55 % v/v)
4. Flow rate: 1 mL/ min
5. Injection volume: 20 µL

6. Detection: UV detector SPD-20A at 253 nm
7. Run time: 9 min

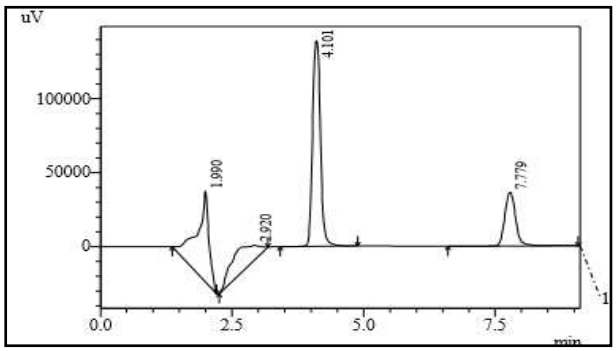

Fig 04: Chromatogram of EHE and TEL by using Acetonitrile : 25 mM Phosphate Buffer pH 4.9 (45:55 % v/v)

Method Validation [11-13]

The developed HPLC method extensively validated for assay of synthetic mixture of EHE and TEL using the following parameters.

System Suitability

System suitability tests are an integral part of chromatographic method validation. The tests were used to verify that the reproducibility of the chromatographic system is adequate for analysis. To ascertain its effectiveness system suitability tests were carried out on freshly prepared standard stock solution and 6 replicates of working standard samples were injected into the optimized chromatographic system, parameters like retention time (RT), plate number (N), peak area and peak asymmetry of sample were calculated these results are presented in the Table 01 for EHE and TEL respectively.

Table 01: System suitability parameters of EHE and TEL

Parameters	Data obtained	
	EHE	TEL
Retention time (R_t) ± SD	7.76 ± 0.03	4.12 ± 0.05
Area ± SD	601699 ± 552.83	2191405 ± 922.62
Theoretical plates per column (N) ± SD	7363 ± 21.51	3857 ± 14.52
Symmetry factor/Tailing factor (As) ± SD	1.14 ± 0.02	1.15 ± 0.04
Resolution	11	

1 Linearity:

Linearity was determined by scanning different standards concentrations at 6 levels in triplicates. The solution of increasing concentration in the range of 5 - 30 µg/mL of Efonidipine Hydrochloride Ethanolate and 10 – 60 µg/mL of Telmisaratn were prepared from the 2º stock solution of EHE (500 µg/ mL) and TEL (500 µg/ mL). A graph of Concentration vs. Absorbance was plotted and regression equation was obtained. Linearity data of EHE and TEL are summarized in Table 02 and Table 3. Calibration curve of EHE and TEL are shown in Fig 05 and Fig 06.

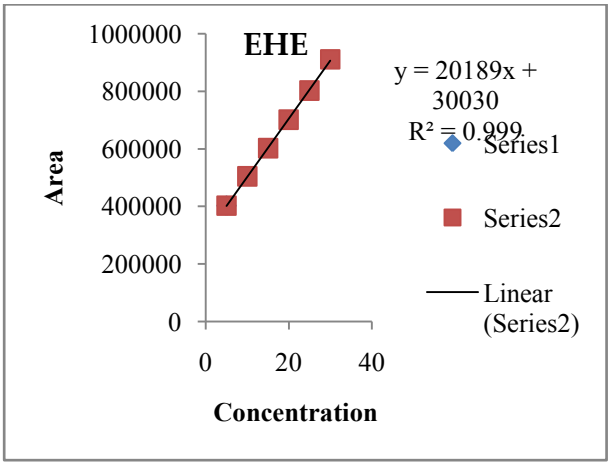

Fig 05: Calibration curve of EHE (5-30 µg/mL)

Table 02: Calibration curve of EHE

Conc.(µg/mL)	Mean of Area ± SD (n=3)	% RSD
5	402456 ± 771.31	0.19
10	503959 ± 662.88	0.13
15	601752 ± 865.61	0.14
20	701046 ± 906.39	0.12
25	802143 ± 963.05	0.12
30	910301 ± 916.54	0.10

Table 03: Calibration curve of TEL

Conc.(µg/mL)	Mean of Area ± SD (n=3)	% RSD
10	1285476 ± 1500.36	0.11
20	1735328 ± 1419.42	0.08
30	2191541 ± 2132.98	0.09
40	2665408 ± 2691.38	0.10
50	3113989 ± 1887.52	0.06
60	3545213 ± 2547.29	0.07

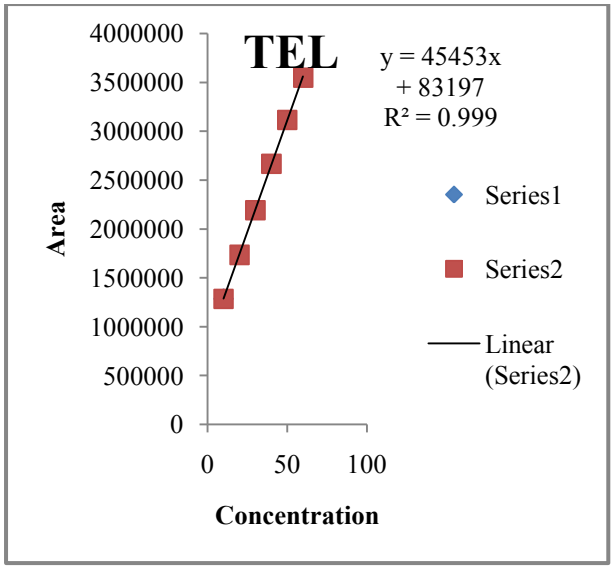

Fig 06: Calibration curve of TEL (10-60 µg/mL)

2 Precision

a. Repeatability

Precision of the method was checked by scanning the solutions containing 15 µg/ mL of Efonidipine Hydrochloride Ethanolate and 30 µg/ mL of Telmisartan for six different times under same experimental condition. The result of repeatability shown in Table 03.

b. Intraday precision

Intra-day precision of the proposed method was carried out by analyzing three different concentrations (i.e. low, medium and high from range) of drug for three times on the same day (with 1 hour of interval). Solutions of Efonidipine Hydrochloride Ethanolate of 5, 15 and 30 µg/ mL and Telmisartan of 10, 30 and 60 µg/ mL were analyzed for three times on the same day. % RSD was calculated for each. The result of Intraday shown in Table 04.

c. Interday Precision:

It was carried out by analyzing three different concentrations (i.e. low, medium and high from range) of drug on three different days. Solutions of Efonidipine Hydrochloride Ethanolate of 5, 15 and 30 µg/ mL and Telmisartan of 10, 30 and 60 µg/ mL were analyzed on three different days. % RSD was calculated for each. The result of Interday shown in Table 05.

3 Accuracy (Recovery study)

Accuracy of the method is to check the closeness of the true value with the obtained result. Accuracy of the method was performed by standard addition method. The recovery study was performed by calculating the spiked concentration of standards at 80%, 100% and 120% of EHE and TEL to preanalyzed mixture containing EHE and TEL. The experiment was performed in triplicates. The result was evaluated in terms of % Recovery. The results of the accuracy studies are described in Table 4 to 5.

Table 04: Accuracy of EHE

Level	Amount Present	Spiked conc. (µg/mL)	Total taken (µg/mL)	Mean of Total conc. found (µg/mL)	Mean AmtRecovered (µg/mL)	Mean % Recovery ± SD	% RSD
80 %	10	8	18	18.02	7.98	99.75 ± 0.64	0.64
100 %		10	20	20.05	10.01	100.10 ± 0.44	0.43
120 %		12	22	22.05	12.01	100.08 ± 0.36	0.36

Table 05: Accuracy of TEL

Level	AmountPresent	Spiked conc. (µg/mL)	Total taken (µg/mL)	Mean of Total conc. found (µg/mL)	Mean AmtRecovered (µg/mL)	Mean % Recovery ± SD	% RSD
80 %	20	16	36	35.86	15.98	99.87 ± 0.13	0.13
100		20	40	39.85	19.97	99.85 ± 0.21	0.21

%							
120%		24	44	43.87	23.99	99.95 ± 0.14	0.14

4 Limit of Detection and Limit of Quantification (LOD and LOQ)

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the replicate, y intercept was calculated and the standard deviation of the y intercept was computed. From these values, the parameters Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined on the basis of response and slope of the regression equation. The result of LOD and LOQ shown in Table 8.

LOD and LOQ were calculated by application of following formula:

$LOD = 3.3\sigma / S$

$LOQ = 10\sigma / S$

Where, σ = standard deviation of response

S = slope of calibration curve

5 Robustness

Robustness determines the slight changes in the chromatographic conditions. It was determined by altering the parameters such as flow rate, mobile phase ratio. To study the effect of flow rate, it was changed to 0.2 units from 1.0 mL/min to 0.8 mL/min and 1.2 mL/min. The effect of ratio of mobile phase was studied by changing 2 units from 45:55 to 40:60 and 50:50. The result of robustness shown in Table 6.

6 Assay

Twenty tablets were (prepared in Lab scale with a Label Claim of 20 mg EHE and 40 mg TEL) weighed and triturated. Powder Equivalent to 20 mg EHE and 40 mg TEL (i.e. 200 mg) was weighed accurately and transferred to 100 mL volumetric flask. 25 mL methanol was transferred to volumetric flask and sonicated for 10 minutes. Volume was made up to mark with methanol. This solution was used as 1º stock solution (200 µg/mL of EHE and 400 µg/mL of TEL).

Appropriate volume (5 mL) was pipetted out accurately from 1º stock solution and was diluted up to 10 mL with methanol, to produce 2º stock solution (100 µg/mL solution of EHE and 200 µg/mL solution of TEL).

Appropriate volume (1.5 mL) from above solution was pipette out accurately and transferred up to 10 mL with Methanol, to prepare test concentration (15 µg/mL solution for EHE and 30 µg/mL solution for TEL). Concentrations were found out and % purity was calculated for both EHE and TEL. The results of assay are summarised in Table 06.

Result and Discussion

To optimize the mobile phase, various proportions of buffer with acetonitrile and methanol were tested. Mobile phase containing a gradient mixture Acetonitrile:25mM Phosphate Buffer pH 4.9 (45:55) resulted in sharp peaks with good shape and resolution. A flow rate of 1.0 mL/min was found to be optimum in the 0.8-1.2 mL/min range resulting in the short retention time, baseline stability and minimum noise. By applying the proposed method, the retention times of EHE and TEL were found to be 7.77 min and 4.10 min respectively. Quantitative linearity was obeyed in the concentration range of 5-30 and 10-60 µg/mL for EHE and TEL respectively. The limit of detection and limit of quantitation were found to be 0.15 and 0.07 µg/mL, 0.45 and 0.23 µg/mL for EHE and TEL respectively, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.

Conclusion

A simple, specific, accurate, precise, stability indicating reverse phase high performance liquid chromatography method has been developed which can be used for accurately quantitative determination of EHE and TEL for routine analysis of individual and combination of drugs. Method was validated as per ICH Q2 (R1) so it can be used by pharmaceutical industries.

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