Research article

Analytical method for simultaneous estimation of efonidipine hydrochloride ethanolate and
telmisartan by validated RP-HPLC method

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ABSTRACT

This research aimed to develop and validate a specific, precise, accurate, robust and cost-effective reversed phase high performance liquid chromatographic method for simultaneous quantification of Efondipine Hydrochloride Ethanolate and Telmisartan in synthetic mixture. Efondipine Hydrochloride Ethanolate is a L- and T- type dual calcium channel blocker and Telmisartan is angiotensin receptor blocker given in patient having essential hypertension associated with diabetes and no produced reflex tachycardia. The literature survey disclosed no reported method available for simultaneous estimation method for this combination. Hence, an attempt was made to develop and validate the reversed phase high performance liquid chromatographic method. In developed RP-HPLC method, analytes separation were employing Waters C 18 Column (150 mm × 4.6 mm, 5 µm). The quantification is carried out using mobile phase Potassium Dihydrogen Orthophosphate: Acetonitrile (30:70 v/v %) containing 1 % orthophosphoric acid were mixed to adjust pH 3. Area were recorded at 254 nm for both analytes and retention time was found to be 7.933 min and 3.187 min for Efondipine Hydrochloride Ethanolate and Telmisartan at 0.8 ml/min flow rate. The estimation method validated according to the ICH Q2 (R1) guideline. The validated data represent that the develop method was linear over a concentration range of 5-30 µg/ml for Efondipine Hydrochloride Ethanolate and 10-60 µg/ml Telmisartan. The Accuracy in terms of mean percent recovery for the analytes was found in the range of 98-100.10 % and 98.31-100.10 % both drugs respectively. The method was precise and reliable with relative standard deviation of lower than 2 %. The detection limit found for Efondipine Hydrochloride Ethanolate 0.028 µg/ml and 0.032 µg/ml of Telmisartan. This simple, reliable and precise method can be used for estimation of both drug in quality control laboratories.

Keywords: Efondipine Hydrochloride Ethanolate, ICHQ2 (R1), RP-HPLC, Synthetic Mixture, Telmisartan

INTRODUCTION

Efondipine Hydrochloride Ethanolate is a 2- [Benzy1 (phenyl) amino] ethyl-1,4-dihydro-2,6-dimethyl-5-(5,5-dimethy1-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-4(3-nitropnen-yl)-3 pyridine carboxylate hydrochloride ethanol. Structure of Efondipine Hydrochloride Ethanolate (EFO) given in Figure1 (A). EFO is a 1,4 dihydropyridine derivative Calcium Channel blocker, which inhibits both L- and T-type of calcium channel. Other dihydropyridine act mainly on L–type calcium channels, causing reflex tachycardia, which negatively affects cardiac function. Because T-type calcium channels in the sinoatrial node attenuate refelxtachycardia, a dual L- and T-type calcium channel blocker EFO inhibit T-type calcium channel blocker in sinoatrial nodule prolong the late phase 4 depolarisation of SA node and suppresses heart rate.[1,2] Also increases the glomerular filtration rate without increasing intraglomerular pressure and prevents hypertension induced renal damage.[3] Telmisartan is a 4′-[(4-methyl-6-(1-methyl-1H benzimidazole-2-yl)-2-propyl-1H-benzimidazole-1-yl] methyl[1-biphenyl carboxylic acid. Telmisartan (TELMI) structure given in figure1 (B). TELMI is Benzimidazole derivative and a non peptide angiotensin II receptor antagonist with anti-hypertensive property. [4] It inhibit the renin-angiotensin system (RAS), in addition acts as a selective agonist of peroxisome proliferative activated receptor gamma (PPARγ), a regulate the insulin and glucose metabolism in skeletal muscle. That dual mode of action may provide protective benefits against the vascular and renal damage caused by diabetes and cardiovascular disease.[5, 6]

The EFO 20 mg and TELMI 40 mg fixed dose combination given in hypertensive patient with Type-2 diabetes mellitus, to maintain blood pressure and insulin resistance, also prevent calcium channel blocker side effect of peripheral edema in hypertension. This fixed dose combination approved for phase 3 clinical trial conducted by Ajntra pharma, India.[7] Various Analytical method are available to determine the EFO and TELMI Individually which include HPLC...
METHOD DEVELOPMENT
Preparation of Buffer solution
Weigh accurately and transfer about 1.36g of potassium dihydrogen orthophosphate and 2 ml of triethylamines in 800 ml of water, adjust the pH 3 with 1% orthophosphoric acid and add water sufficient to produce 1000 ml.

Preparation of Mobile Phase
Prepare a mixture of buffer and acetonitrile in the volume ratio 30:70 % v/v. Mix well and sonicate to degas the mixture.

Preparation of standard stock solutions
The standard stock solution of analytes was prepared by accurately weighed 20 mg of EFO and 40 mg TELMI in to 100 ml of volumetric flask, 50 ml of methanol was added and sonicated to dissolve. Volume was making up to the mark with methanol to get a concentration of EFO 200 μg/ml and TELMI 400 μg/ml. Appropriate volume from this solution was further diluted up to 50 ml volumetric flask with methanol to obtained working concentration for EFO was 20 μg/ml and 40 μg/ml of TELMI.

Chromatographic Condition
High performance liquid Chromatographic separation was performed on Dionex sparation module with UV detector. The various chromatographic conditions were used for separation of analytes, as shown in Table 1.

Table 1: Reverse Phase Chromatographic condition

| Parameter            | Descriptions                        |
|----------------------|-------------------------------------|
| Column               | C18 (15 cm x 4.6 mm, 5μm)           |
| Mobile Phase         | Potassium Dihydrogen Orthophosphate Buffer pH 3: Acetonitrile (30:70 % v/v) |
| Flow Rate            | 0.8 ml/min                          |
| Wavelength           | 254 nm                              |
| Temperature          | 30°C                                |

System suitability
The suitability of the system was assessed by injecting five replicate injections of a standard solution of EFO and TELMI. The system suitability parameters were then evaluated for Tailing factor, Retention time, Resolution and Theoretical plates of a standard chromatogram.

Method validation
Analytical validation parameters for the analysis of the proposed method were determined according to ICH Q2 (R1) guideline. [24]

Linearity
From the working stock solution aliquote 2.5-15 ml solution diluted with methanol up to 100 ml to obtained concentration range 5-30 μg/ml of EFO and 10-60 μg/ml of TELMI with phosphate buffer: acetonitrile mobile phase and injection volume of 20 μl.

Specificity
Specificity was performed under 6 replicates at a concentration of 10 μg/ml EFO and 20 μg/ml of TELMI by injecting sample solution with and without the addition of excipient, diluent and blank to check the interference of excipients. The specificity of the method was evaluated by calculating percentage interference.

MATERIAL AND METHOD
Chemical and Reagent
Efonidipine Hydrochloride Ethanolate and Telmisartan were provided by as a gift sample from Zuventus Healthcare Ltd., Mumbai, India. HPLC grade Methanol, Acetonitrile and Water were used of Finar Pvt. Ltd., Mumbai. All chemicals and excipient used were of analytical grade provided by B.K. Mody Government Pharmacy College, Rajkot.

Instrument
The HPLC system used for chromatographic development was Dionex separation module with a UV detector. HPLC system consist of an Ultimate 3000 pump (Binary) and Rheodyne injector valve with a fixed loop of 20 μl. Chromelone © Dionex corporation,6.80 SR15 Build 4656 (243203) software was used for estimation. All weighing was done on electronic balance (MAB 220 Wensar, Simadzu-AUW2200).

Selection of Solvent
Based on the solubility study, Methanol was selected as the solvent for dissolving EFO and TELMI.

Figure 1: (A) Chemical structure of efonidine hydrochloride ethanolate (B) Chemical structure of telmisartan

(B)

(A)
Accuracy
The accuracy study was determined by the standard drug-drug addition method. Previously analyzed sample (10 µg/ml of EFO and 20 µg/ml of TELMI) were spiked with concentration levels of 80%, 100% and 120% of extra standard respectively at three replicates. The accuracy of the method was evaluated by calculating percentage recovery.

Precision
Precision was calculated at the repeatability and intermediate precision levels. Repeatability was performed under 6 replicates at a concentration 10 µg/ml and 20 µg/ml of EFO and TELMI respectively. Intra-day and Inter-day precision of EFO and TELMI were performed in triplicate at three different concentration levels 80%, 100%, 120%. The result is express in term of relative standard deviation (RSD).

Robustness
The robustness of the method was evaluated by assaying the test solutions after slight but deliberate variation of the chromatographic condition. In this study the condition was change like a wavelength, Flow rate and Mobile phase composition. The variation made for Wavelength ± 1 nm, Flow Rate ± 1 ml/min and Mobile phase composition ratio ± 1ml.

LOD and LOQ
The limit of detection (LOD) and limit of quantitation (LOQ) were calculating according to formula as given in ICH Q2(R1).[24] The calibration curve was constructed; five standard deviation of the intercepts and the slope were calculated.

\[
LOD = 3.3 \times \text{Standard deviation} / \text{Slope} \\
LOQ = 10 \times \text{Standard deviation} / \text{Slope}
\]

Assay of synthetic mixture
Synthetic mixture was prepared by equivalent to take 20 mg for EFO and 40 mg of TELMI with common tablet excipient (Starch, PVP, HPMC, Lactose, Talc, Mg Stearate) in adequate amount. This synthetic mixture was diluted with methanol to make concentration of EFO 10 µg/ml and 20 µg/ml of TELMI.

RESULT AND DISCUSSION
Optimized Method
The objective of the method development was to obtained well-resolved chromatographic peaks of both drugs. Various composition and different proportion of solvents comprising buffer, methanol, water and acetonitrile were tried as a mobile phase. The mobile phase of different composition and ratio used but it was a poor resolution, merged peak and tailing were observed. The well resolved, symmetrical peak was observed with Potassium Dihydrogen orthophosphate: Acetonitrile (30:70 % v/v) at pH 3 adjusted with Orthophosphoric acid at 254 nm uv detection with flow rate 0.8 ml/min. The elution time of EFO 7.933 min and TELMI 3.187 min with optimum retention time. The peak area of the drug estimate using Chromeleon6.80 SR15 Build4656 software. These conditions were identified to achieve an easy to use, cost-effective, and better peak resolution, tailing factor and less total runtime. Optimized chromatogram shown in Figure 2.

System suitability parameter
The efficiency of the column was expressed by number of theoretical plates and tailing of peak was obtained in acceptance. The optimized method is acceptable as system suitability parameters are valid as it passed the criteria of acceptability, as shown in Table 2.

| Parameter | Telmisartan | Efonidipine Hydrochloride Ethanolate |
|-----------|-------------|-------------------------------------|
| Retention Time | 3.187 min | 7.933 min |
| Theoretical Plate | 4248 | 6154 |
| Resolution | 0.00 | 18.17 |
| Tailing Factor | 1.21 | 1.14 |
| Area | 2366017.270 µAU | 844892.252 µAU |
Linearity
The linear regression analysis data for the calibration plot represent linear relationship with respect to peak area in concentration range of 5-30 µg/ml for EFO and 10-60µg/ml for TELMI. The correlation coefficient of EFO and TELMI was found to be 0.9997 and 0.9993 respectively. The calibration graph for EFO and TELMI are shown in Figure.3.

Specificity
Excipient interference was not observed at the working wavelength and any co elute peak of excipient were not observed near the analyte peak. The method presented in this study is specific for EFO and TELMI.

Accuracy
Recovery of the method was found within a range of 98.00-100.10 % and 98.31-100.10 % for EFO and TELMI respectively after spiking a previously analyzed sample with standard drug. The recovery value is shown in Table 3 indicating method is accurate.

Precision
Repeatability and intermediate precision expressed in terms of RSD. The proposed method showed less than 2 % RSD, confirming that the method was sufficiently precise. The data obtained from precision experiment is given in Table 4 and Table 5.

Table 4: Repeatability data of efonidipine hydrochloride ethanolate and telmisartan

| Drug   | Concentration (µg/ml) | Area Mean ± SD (n=6) (µAU) | RSD |
|--------|-----------------------|----------------------------|-----|
| EFO    | 10                    | 44660.107 ± 2405.848       | 0.54|
| TELMI  | 20                    | 1242465.355 ± 3947.657     | 0.31|

LOD and LOQ
LOD and LOQ of EFO and TELMI were determined by equation according to ICH Q2 (R1) guideline. LOD and LOQ found to be 0.028 µg/ml and 0.086 µg/ml respectively, indicating the method can be used for detection and quantification of drugs in wide concentration range.

Table 5: Intra-day and Inter-day precision of efonidipine hydrochloride ethanolate and telmisartan

| Drug   | % Level  | Intra-day Mean Area ± SD (µAU) | Inter-day Mean Area ± SD (µAU) | RSD |
|--------|----------|--------------------------------|--------------------------------|-----|
| EFO    | 80 %     | 365152.888 ± 3069.572          | 367003.037 ± 2213.972          | 0.65|
| TELMI  | 100 %    | 442145.546 ± 2838.603          | 441411.131 ± 2101.560          | 0.47|
|        | 120 %    | 516333.962 ± 6465.431         | 513070.428 ± 6820.630          | 1.32|
|        | 80 %     | 1035618.497 ± 2470.984        | 1035529.609 ± 4815.596        | 0.46|
| TELMI  | 100 %    | 1243724.043 ± 4626.230        | 1243474.286 ± 4047.887        | 0.32|
|        | 120 %    | 1494555.364 ± 4605.540        | 1496816.042 ± 5226.140        | 0.34|

Robustness study
The method was found robust, as slight but deliberate changes in the parameter have no marked effect on the method performance as shown in Table 6.

Table 6: Robustness study data of Efonidipine hydrochloride ethanolate and telmisartan

| Drug   | low rate (ml/min) | Mean Area ± SD (µAU) | RSD |
|--------|-------------------|----------------------|-----|
| EFO    | 0.7               | 448103.552 ± 3991.696| 0.89|
| TELMI  | 0.8               | 446248.387 ± 5468.397| 1.22|
|        | 0.9               | 439039.517 ± 2891.112| 0.65|
|        | 0.7               | 1252406.443 ± 5777.062| 0.46|
|        | 0.8               | 1251693.471 ± 4083.132| 0.32|
|        | 0.9               | 1246566.545 ± 4989.681| 0.40|
| EFO    | 255               | 437825.741 ± 3043.214| 0.69|
| TELMI  | 254               | 447836.852 ± 2522.946| 0.56|
|        | 255               | 443727.763 ± 5578.599| 1.25|
|        | 253               | 1241599.715 ± 3023.257| 0.24|
|        | 254               | 1248500.411 ± 8888.594| 0.71|
|        | 255               | 1240286.385 ± 2238.923| 0.18|

Assay of Synthetic mixture
Experimental results of the amount of EFO and TELMI in the selected normally present common tablet excipients was good agreement with the label claims thereby suggesting that there was no interference from any of the excipients. The % Assay of synthetic...
mixture of EFO and TELMI are shown in Table 7.

| Drug    | Conc. (µg/ml) | Area(µAU) Mean ± SD (n=3) | % Content |
|---------|--------------|--------------------------|-----------|
| EFO     | 10           | 446526.589 ± 1584.180     | 98.53 %   |
| TELMI   | 20           | 1237093.896 ± 1536.112    | 98.68 %   |

**CONCLUSION**

From these research findings, it can be concluded that the proposed method is simple, sensitive, specific and reliable with good accuracy and precision. Both analytes peak was well resolved. The major advantage of this technique is that it is developed for synthetic mixture and helpful for researcher to developed for its combined dosage form. Has, potential benefit for the quantification of both drug in commercially available dosage form by the pharmaceutical industry and analytical laboratories in Future.

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**CONFLICTS OF INTEREST**

The authors declare no conflict of interest.

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