Development and validation of a RP-HPLC-PDA method for simultaneous estimation of Drotaverine and Etoricoxib in tablet and its application for dissolution studies

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ABSTRACT

A simple, precise, rapid and accurate RP-HPLC-PDA method has been developed for the simultaneous estimation of Drotaverine HCl (DRT) and Etoricoxib (ETR) in tablet formulations. The chromatographic separation was achieved on Waters Kromosil C18 column (250 mm x 4.6 mm, 5.0 µ particle size) using methanol: THF: acetatebuffer (51:09:40 v/v) pH adjusted to 6.0 with acetic acid, flow rate was 0.9ml/min and column was maintained at 55 °C. Quantification and linearity was achieved at 244 nm over the concentration range of 1.6 – 80µg/ml for Drotaverine HCl and 1.8 – 90µg/ml for Etoricoxib. The method was validated for specificity, linearity, accuracy, precision, LOD, LOQ and robustness. The proposed method was optimized and validated as per the ICH guidelines. Method was used to test dissolution sample successfully. Thicocolchicoside and Hydrochlorothiazide were used as internal standard.

Key words: Drotaverine, Etoricoxib, RP-HPLC-PDA, method validation, column liquid chromatography, dissolution.

INTRODUCTION

Drotaverine hydrochloride (DRT), 1-[(3,4-diethoxy phenyl) methylene]-6,7-diethoxy-1,2,3,4-tetra hydro isoquinolene is an analogue of papaverine[1]. It acts as an antispasmodic agent by inhibiting phosphodiesterase IV enzyme, specific for smooth muscle spasm and pain, used to reduce excessive labor pain [2]. Literature survey reveals that few UV spectrophotometric [3-8] and HPLC [9-13] methods have been reported for estimation of drotaverine hydrochloride individually or in combination with other drugs.
Etoricoxib (ETR), a newer cyclo-oxygenase-2 inhibitor, is mainly used in the management of osteoarthritis, rheumatoid arthritis and acute gouty arthritis. Chemically, Etoricoxib is a 5-chloro-6’-methyl-3-[4-(methylsulfonyl) phenyl]-2, 3’-bipyridine, and is not yet official in any pharmacopoeia [14]. Its impurity studies and HPLC/MS-MS methods in matrix have been reported [15-18].

Authors have developed Area under curve & First Derivative spectroscopy methods and Absorption corrected & Derivative spectroscopy methods for these combinations in the same laboratory.

To the best of our knowledge there is no HPLC method reported that can simultaneously determine both drugs. Hence there was the need to develop a new column chromatographic method for the analysis of both drugs simultaneously. Therefore the aim of the present study was to develop a sensitive, precise, accurate and specific HPLC method for the determination of DRT and ETR simultaneously in formulation and application of the method for dissolution studies.

MATERIALS AND METHODS

Instrumentation
The HPLC system consisted of a binary pump (model Waters 515 HPLC pump), auto sampler (model 717 plus Auto sampler), column heater, and PDA detector (Waters 2998). Data collection and analysis were performed using Empower- version 2 software. Separation was achieved on Kromosil C18 (250 mm x 4.6 mm, 5.0 µ) column. The column was supported with C18, (20 x 3.9 mm, 5 µ) guard column. A calibrated dissolution apparatus (USP II) was used with paddles for dissolution studies. Shimadzu analytical weighing balance - Model AUW220D and Equip-Tronics micro controller pH Meter Model EQ-621 was used for study.

Materials and Reagents
Two Batches of Tablet formulations (Batch No.JT901 and JT902) were supplied by JPLC Pharma ltd. (Jalgaon) and used for analysis containing DRT 80 mg and ETR 90 mg per tablet. Drug sample of DRT (% purity 98.5%) was kindly supplied as a gift sample by Alkem Laboratories Ltd., Mumbai and drug sample of ETR (% purity 99.8%) was gifted by Mapro Pharmaceuticals, Vapi, Gujarat. These samples were used without further purification. HPLC grade Methanol and Tetrahydrofuran supplied by LOBA Chemie Pvt. Ltd., Mumbai, India were used. Double distilled water was used throughout the study. Mobile phase was degassed by filtration (0.45µm) and sonicaton.

Chromatographic Conditions
Waters Kromosil C18, (waters C18, 250mm x 4.6 mm, 5µ) column was used as a stationary phase. The isocratic elution with Methanol: THF: Acetate buffer (pH 6.0) (51: 09: 40 v/v) mobile phase at the flow rate of 0.9ml/min was carried out. The run time was set at 8 min and temperature was maintained at 55°C. The volume of injection was 20 µl, prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase. Detector signal was monitored at a wavelength of 244 nm.
Preparation of Standard Solutions and Calibration Curve

Stock solution of DRT and ETR (1µg/ml) were separately prepared in methanol. To study the linearity range of each component, serial dilutions of DRT and ETR each were made from 1.6 to 80µg/ml and 1.8 to 90µg/ml, respectively in mobile phase and injected on to column. Calibration curves were plotted as concentration of drugs versus peak area response. From the standard stock solutions, a mixed standard solution was prepared containing the analytes in the given ratio and injected on to column. The system suitability test was performed from six replicate injections of mixed standard solution.

Analysis of Tablet Formulations

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 80 mg of DRT (90 mg of ETR) was weighed and dissolved in the 80 ml of methanol with the aid of ultrasonication for 10 min and solution was filtered through Whatman paper No. 41 into a 100 ml volumetric flask. Filter paper was washed with the solvent, adding washings to the volumetric flask and volume was made up to mark. The solution was suitably diluted with methanol to get of 40µg/ml of DRT (45µg/ml of ETR), filtered through 5 micron, nylon66 membrane filter and injected on to column. Structures of analytes and internal standards are shown in Fig 1.

Validation Procedure

The HPLC method was validated in terms of precision, accuracy and linearity according to ICH guidelines. Assay method precision was determined using nine-independent test solutions. The intermediate precision of the assay method was also evaluated using different analyst on three different days. The accuracy of the assay method was evaluated with the recovery of the standards from preanalysed tablet formulation. Three different quantities (low, medium and high) of the authentic standards were added to the tablets. The mixtures were extracted and analyzed using the proposed HPLC method. Linearity test solutions were prepared as described in Formulation analysis. The LOD and LOQ for analytes were calculated using the formula LOD = (3.3 x σ)/ b and LOQ = (10 x σ)/ b respectively, where σ (standard Deviation of response), b (Slope of the calibration curve). To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The flow rate was varied by (±) 0.05 ml/min, the column Temperature was varied by (±) 2°C, Mobile phase was varied by (±) 3%, pH of mobile phase was varied by (±) 0.2, the column was changed from different manufacturer and wavelength of measurement was changed by (±) 2nm, the organic
modifier was varied by (±) 5%. The stability of the drug solution was determined using the samples for short-term stability by keeping at room temperature for 12 h and then analyzing. The long-term stability was determined by storing drug solution at 4°C for 30 days. Auto-sampler stability was determined by storing the sample solution for 24 h in the auto-sampler. For method development and optimization, retention factor ($k$) was calculated using the equation: $k = (t_R - t_M)/ t_M$. Where, $t_R =$ retention time, $t_M =$ is the elution time of the solvent front.

**Dissolution study**

A calibrated dissolution apparatus (USP II) was used with paddles at 50 RPM and bath temperature maintained at 37±1°C. Nine hundred milliliter freshly prepared and degassed 0.1 N HCl solution was used as the dissolution medium. Nine tablets were evaluated for each drug product tested. Dissolution samples were collected at 5, 10, 15, 20, 25, 30, 35, 40 and 45 mins. At each time point, a 5 ml sample was removed from each vessel sample, filtered through a nylon filter (0.45µm, 25 mm), 2.0 ml of filtrate was diluted to 10 ml with mobile phase and analyzed by HPLC. The amount of DRT and ETR in the test samples was calculated, as percentage dissolved, from the measured peak area for the test samples by using equation 1 and alternatively by using peak areas of sample ($S_1$) and Standard ($S_2$) by using equation 2.

\[
\text{Dissolved} \% = \left( \frac{\text{Conc. estimated by the method} \times 900 \times 5 \times 100}{1000 \times \text{DL}} \right) \ldots (1)
\]

\[
\text{Dissolved} \% = \left( \frac{900 \times \text{DL} \times (\text{Peak Area}(S_1))/\text{Peak Area}(S_2) \times \text{Conc. (std.)} \times 100}{\ldots (2)} \right)
\]

Where, DL- is drug load, which is 80 mg and 90 mg for drotaverine and Etoricoxib, respectively.

**RESULT AND DISCUSSION**

1. Optimization of HPLC method

In order to achieve simultaneous elution of the two components, different chromatographic conditions were attempted. Stationary phases like C8 (Qualisil), C18 (Kromosil and symmetry) were used, experimental studies revealed that the Kromosil column was the most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity. Several modifications in the mobile phase composition were performed in order to study the possibilities of changing the selectivity of the chromatographic system. These modifications included the change of the type and ratio of the organic modifier, the pH, the strength of the Acetate buffer and the flow rate. Acetonitrile and methanol individually and Methanol, Acetonitrile, THF in mixture are used for the study but it did not give good resolved peaks. The effect of changing the ratio of organic modifier on the selectivity and retention times of the test solutes was investigated using mobile phases containing concentrations of 60-40% methanol and 5%-20% THF. To minimize the peak tailing, THF (15%) was added as an organic modifier. Methanol and THF were the organic modifier of choice giving symmetrical narrow peaks. Ratio less than 70% of organic resulted in peaks with more tailing, whereas ratios higher than 70% resulted in decreased resolution. The effect of changing the pH of the mobile phase on the selectivity and retention times of the test solutes was investigated using mobile phases of pH ranging from 4.0-7.0. Thus pH of 6 was the most appropriate one giving well resolved symmetric peaks and highest theoretical plates. The effect of changing the concentration of acetate buffer on the selectivity
and retention times of the test solutes was investigated using mobile phases containing concentration of 10, 20, 30, 40 and 50 milimoles of acetate buffer (Fig 2). Thus 25 milimoles acetate buffer was found to be the most suitable giving best resolution and highest theoretical plates. The effect of flow rate on the formation and separation of peaks of the studied compounds was studied and a flow rate of 0.9 ml/ min was optional for good separation in a reasonable time. The tailing factors were <1.5 for both the peaks. The elution order was DRT ($t_r = 5.46$ min) and ETR ($t_r = 6.24$ min). The chromatogram was recorded at 244 nm as the overlaid PDA spectrum of Drotaverine and Etoricoxib showed maximum response at this wavelength (Fig 3).

Fig. 2. A. Effect of pH of Acetate Buffer in mobile phase on Retention times of DRT and ETR  
B. Effect of Ionic strength of Acetate Buffer in mobile phase on Retention times of DRT and ETR

Fig. 3. Specificity Chromatogram consists of A) Mobile Phase, B) Placebo, C) formulation, D-I) system suitability standards of DRT (80 µg/ml) and ETR (90 µg/ ml)  and online overlain PDA spectra of analytes

Choice of Internal Standard
Different drugs were investigated as internal standard. These drugs include; Lornoxicam, Thiocolchicoside, Hydrochlorthiazide, Telmisartan. Most suitable internal standards producing a well resolved peak from the drug were Hydrochlorthiazide and Thiocolchicoside (Fig 4).
2. Method development
Methanol, THF (15%) and Acetate buffer (pH adjusted up to 6) in the ratio of 51:09:40 v/v were employed as a mobile phase. The present RP – HPLC- PDA method for the quantification of DRT and ETR in bulk and pharmaceutical combined dosage forms, revealed as simple, accurate and precise method with significant shorter retention time of 5.46 and 6.24min respectively.

3. Method validation
The Proposed method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, and robustness. System suitability was established by injecting standard solution and results were given in (Table 1). The chromatograms were checked for the appearance of any extra peaks. No chromatographic interference from the tablet excipients was found. Peak purity was verified by confirming homogeneous spectral data for DRT and ETR.

Table 1. System suitability parameters and results of precision study

<table>
<thead>
<tr>
<th>Compound</th>
<th>System Suitability</th>
<th>Precision of the Method (n=9)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Parameter</td>
<td>Value</td>
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<tr>
<td></td>
<td></td>
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<td>Drotaverine</td>
<td>Therotical plates</td>
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<td>Peak Tailing</td>
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<td></td>
<td>Peak Tailing</td>
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<td></td>
<td>% R.S.D.</td>
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Specificity
The specificity of the HPLC method was illustrated, where complete separation of DRT and ETR was noticed in presence of tablet placebo. In addition there was no any interference at the
retention time of DRT and ETR in the chromatogram of tablet solution. In peak purity analysis with photo diode array detector, purity angle was always less than purity threshold for all the analytes. This shows that the peak of analytes was pure and excipients in the formulation did not interfere the analytes.

**Linearity and range**
For the construction of calibration curves, six calibration standard solutions were prepared over the concentration range. Linearity was determined for DRT in the range of 1.6-80 μg ml\(^{-1}\) and for ETR 1.8-90 μg ml\(^{-1}\). The correlation coefficient (’r’) values were >0.999 (n = 6). Typically, the regression equations for the calibration curve was found to be \(y = 4.9495x - 5.4337\) for DRT, \(y = 7.1375x - 2.6187\) for ETR.

**Limit of detection (LOD) and Limit of quantitation (LOQ)**
The LOD and LOQ values were found to be 0.11 and 0.33 μg/ml and 0.13 and 0.4 μg/ml for DRT and ETR, respectively.

**Method accuracy**
To ensure the reliability and accuracy of the method, the recovery studies were carried out by adding a known quantity of drug with pre analyzed sample and contents were reanalyzed by the proposed method. Accuracy was evaluated by injecting five times at three different concentrations equivalent to 50,100, and 150% of the active ingredient, by adding a known amount of DRT and ETR standard to a sample of known concentration and calculating the recovery of DRT and ETR with RSD (%), and % recovery for each concentration. The mean % recoveries were in between 100.10-100.67% and were given in (Table 2).

![Table 2: Results of Tablet analysis and accuracy studies](image)

**Precision and ruggedness of the method**
The intraday and inter-day variations of the method were determined using nine replicate injections of three concentrations and analyzed on the same day and three different days over a period of two weeks. The result revealed the precision with %RSD (0.47% and 0.29% for DRT) and (0.36% and 0.45% for ETR), respectively for intraday and inter day. Ruggedness of the method (intermediate precision) was estimated by preparing six dilutions of the DRT and ETR as per the proposed method and each dilution injected in duplicate using different column and analyst on different days.

**Solution stability**
In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed in several conditions like at fridge, table top and in auto sampler over a
period of 12 h at room temperature. The result showed that for solutions, the retention time and peak area of DRT and ETR remained almost unchanged (% R.S.D. less than 2.0) and no significant degradation within the indicated period, which was sufficient to complete the whole analytical process.

System suitability
To know reproducibility of the method system suitability test was employed to establish the parameters such as tailing factor, theoretical plates, limit of detection and limit of quantification of the drugs.

Method Robustness
Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, and System suitability parameters were found to be within acceptable limits. Results were shown in (Table3) indicating that the test method was robust for all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variation</th>
<th>System Suitability</th>
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</table>

CONCLUSION

The mean percentage dissolution of DRT and ETR in 0.1 N HCL from tablet dosage form was found within the limit.

Analytical RP-HPLC method was developed and validated for quantitative determination of DRT and ETR from two tablet formulations. The manuscript describes, first time this type of method was reported for this combination. All the parameters for the two titled drugs met the
criteria of ICH guidelines for method validation. The method is very simple, specific, reliable, rapid and economic. As the peaks are well separated and there is no interference by excipients peaks with total runtime of 8 min, which makes it especially suitable for routine quality control analysis work and Dissolution studies were also carried out to know the percentage release from the drug combination and we found that there is a release of 93.83 & 96.89% for DRT & ETR. The results of Dissolution studies were shown in (Fig 5).

![Dissolution profile of DRT and ETR.](image)

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