Development of Chromatographic Technique for Simultaneous Estimation of Lovastatin and Diltiazem Hydrochloride

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Abstract

Rapid, precise, accurate, specific and sensitive reverse phase liquid chromatographic (RP-HPLC) method has been developed for the simultaneous estimation of Diltiazem hydrochloride and Lovastatin in their tablet formulation. The chromatographic method was standardized using a Kromasil C₁₈ column (300 mm x 4 mm i.d., 10 µm particle size) with UV detection at 237 nm and flow rate of 1 ml/min. The mobile phase consisting of methanol: water (90:10: v/v). The retention time for the Diltiazem hydrochloride and Lovastatin was found to be 4 min and 5.62 min respectively. Propranolol was selected as an internal standard. Good linearity was obtained for the calibration curve of Diltiazem hydrochloride and Lovastatin and was found to be r² = 0.9925 and r² = 0.9992 respectively. Tablet analysis and recovery results were found to be 99.95±0.79, 99.95±0.79, 99.68±0.34 and 99.65±0.46 for Lovastatin and Diltiazem hydrochloride respectively, where n = 9.

Keyword: Diltiazem hydrochloride; Lovastatin; Propranolol; RP-HPLC.

INTRODUCTION

Lovastatin (LOV) (Figure 1) is a HMG-CoA reductase inhibitor used in the treatment of hyperlipidemia. Chemically LOV is \([1S-{1\alpha(R*)},3\alpha,7\beta,8\beta(2S*,4S*)], 8a\)-2-methylbutanoic acid, 1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo2H-pyran-2-yl)ethyl]-1-aphthalenylester. Several analytical methods such as HPLC²⁴ and capillary isotachophoresis² are reported in the literature for estimation of the Lov alone in biological fluids and pharmaceutical dosage forms.

Diltiazem HCl (DTZ) (Figure 1) chemically known as (2S-cis)-3-(acetyloxy-5-[2-(dimethylamino) ethyl]-2, 3-dihydro-2-(4-methoxy-Phenyl)-1,5benzothia-zepin4 (5H)-one monohydrochloride. is a Calcium channel blocker⁶. The drug is mentioned in Martindale; The Extra Pharmacopoeia⁷. A survey of literature revealed different analytical methods including High Performance Liquid Chromatography⁸⁹. Few spectrophotometric methods have also been reported¹⁰¹² for estimation of DTZ from its pharmaceutical dosage forms.

There is no any approved formulation of combination of DTZ and LOV. The designing of bilayer floating tablets of DTZ and LOV to give immediate release of LOV and controlled release of DTZ has been carried out by Kulkarni et. al¹³.

There is no any reported method for simultaneous determination of LOV and DTZ in their combined dosage form. In the present work, an attempt has been made to develop and validate a simple, sensitive and reproducible liquid chromatographic method for the quality control and routine analysis of LOV and DTZ in tablet dosage form.

MATERIALS AND METHODS

Apparatus:

a) A Pharmaspec, UV-1700 recording spectrophotometer (Schimadzu, Japan) with spectral bandwidth (resolution) of 2 nm and...
Wavelength accuracy ± 0.3 nm (with automatic wavelength correction) was employed for all measurements using a matched pair of 10mm quartz cell.

b) The HPLC system was a PC based Jasco series comprising of a pump PU-2080 and a UV-2070 detector. Manual injections were carried out using a Rheodine injector with a fixed 20 µl external loop. The chromatographic separations were performed on a 10 µm Kr 100-C18 column (300 mm * 4. mm i.d.), operating at ambient temperature, using a mobile phase consisting of methanol: acetonitrile: (50:50 %v/v).

c) Shimadzu AY 120 analytical balance was used for weighing.

d) PCI Ultrasonicator was used for sonication.

**Materials:**

**Pure samples:**

Pure drug sample of DTZ was procured from Cipla Ltd., Mumbai and LOV was procured from Biocon Ltd., Bangalore.

**Chemical and reagents:**

For spectrophotometric work double distilled water, methanol and acetonitrile (Loba chemie Pvt. Ltd. Mumbai, India) were of pure analytical grade. For HPLC work double distilled water, acetonitrile and methanol (Spectrochem Pvt. Ltd. Mumbai, India) were of HPLC grade.

**Preparation of standard and sample solutions:**

**Standard stock solutions:**

Standard stock solutions containing DTZ and LOV were prepared by transferring 25mg of DTZ and LOV separately into two 25 ml volumetric flasks. It was then dissolved in 10 ml of mobile phase and ultrasonicated for 10 minutes. The final volume of the solution was made up to 25ml with mobile phase to get stock solutions containing 1000 µg. ml⁻¹ of DTZ and LOV.

The propranolol HCl (PRO) (Figure 1) was selected as an internal standard. Standard stock solution containing PRO

![Structures of diltiazem (DTZ), lovastatin (LOV) and propranolol (PRO)](image)
was prepared by dissolving 12.5 mg of PRO in 15 ml of mobile phase in a 25 ml volumetric flask. It was then ultrasonicated for 10 minutes and then final volume of solution was made up to 25 ml with mobile phase to get final concentration as 500 µg ml⁻¹ of PRO.

*Working solutions:*

For HPLC method, 1 ml of the Standard stock solutions containing LOV and DTZ were transferred in two separate 10 ml volumetric flasks and diluted to the mark with mobile phase to get a final concentration of 100 µg ml⁻¹ of LOV and DTZ.

*Linearity:*

Dilutions containing 110-180 µg ml⁻¹ of DTZ and 40-110 µg ml⁻¹ of LOV were prepared by proper dilutions of primary stock solution with mobile phase to obtain working standards. To each 10 ml volumetric flask 1.0 ml of PRO was added as an internal standard and the final volume was made up with the mobile phase. A 20 µl of sample solution was injected into the chromatographic system using fixed volume loop injector and chromatograms were allowed to run for 15 min acquisition. The flow rate was maintained at 1 ml/min at ambient temperature and the eluents were monitored at 237 nm. The separation was done on a C18 column using developed mobile phase which contains methanol: water (90:10: v/v). The corresponding slope, intercept and coefficient of correlation values are shown in Table 1.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>DTZ</th>
<th>LOV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Slope</td>
<td>0.0163</td>
<td>0.1215</td>
</tr>
<tr>
<td>2.</td>
<td>Intercept value</td>
<td>0.0114</td>
<td>0.0325</td>
</tr>
<tr>
<td>3.</td>
<td>Correlation coefficient</td>
<td>0.9994</td>
<td>0.9999</td>
</tr>
<tr>
<td>4.</td>
<td>Limit of Detection (µg ml⁻¹)</td>
<td>3.25</td>
<td>3.70</td>
</tr>
<tr>
<td>5.</td>
<td>Limit of Quantitation (µg ml⁻¹)</td>
<td>9.8</td>
<td>11.2</td>
</tr>
</tbody>
</table>

*Assay of laboratory prepared mixtures:*

*Laboratory prepared mixtures:*

Dilutions containing 110-180 µg ml⁻¹ of DTZ and 40-110 µg ml⁻¹ of LOV were prepared by proper dilutions of primary stock solution with mobile phase to obtain working standards. To each 10 ml volumetric flask 1.0 ml of PRO was added as an internal standard and the final volume was made up with the mobile phase. Chromatograms of different laboratory-prepared mixtures containing different concentrations of the two drugs were recorded. The chromatographic conditions were applied for each laboratory-prepared mixture and the concentrations of LOV and DTZ were calculated by substituting in the regression equations.

*Assay of formulation:*

Formulations containing a DTZ 120 mg and LOV 50 mg (Kulkarni et al.¹³) were analyzed using this method. Twenty tablets of each formulation were accurately weighed and average weight was calculated. These tablets were then ground to a fine powder. An accurately weighed tablet powder equivalent to 120 mg of DTZ and 50 mg LOV of the formulation was transferred to a
separate 50 ml volumetric flask containing 25 ml of mobile phase. Prepared solution was sonicated for 10 minutes for proper solubilisation of the drug and final volume was made up to 50 ml with mobile phase. This solution was then filtered through Whatman’s filter paper No.41. Three aliquots in suitable concentration were prepared in triplicate to get nine solutions. Finally each solution was filtered through hydrophilic PVDF 40.4 µm size syringe filter. Filtered aliquots are analyzed using proposed method. The concentration of DTZ and LOV present in the sample solution was calculated by using the formula, \( Y = A + B \times \text{Conc.} \), where, \( A = \) slope, \( B = \) intercept and \( Y = \) response. The results of Analysis of formulation developed are shown in Table 2. The statistical data obtained for the determination of DTZ and LOV in tablet formulations by the proposed method is shown in Table 3. The Figure 2 shows chromatographic separation of diltiazem, lovastatin and propranolol.

**Limit of Detection (LOD) and Limit of Quantitation (LOQ):**

The LOD and LOQ were separately determined based on the calibration curves data. The standard deviation of the y-intercepts and slope of the regression lines were used in calculating these values using formulae give below.

\[
\text{LOD} = 3.3 \times \sigma / S \\
\text{LOQ} = 10 \times \sigma / S
\]

Where, \( \sigma = \) standard deviation of the response
\( S = \) slope of the calibration curve
The results are reported in Table 1.

**Precision Study:**

Precision study was performed for three consecutive days at three times to find out intra-day and inter-day variations. The results of precision studies are reported in Table 2.

**Recovery Study:**

Recovery studies were performed by standard addition method at three levels i.e., 80%, 100% and 120%. Known amounts of standard DTZ and LOV were added to pre-analyzed samples and they were subjected to proposed HPLC. Results of recovery studies are shown in Table 2.

**Table 2. Result of Formulation Analysis, Precision and Recovery Study**

<table>
<thead>
<tr>
<th>Precision</th>
<th>% Lable Claim Estimated (Mean(^a) ± % RSD)</th>
<th>Recovery Estimated (Mean(^a) ± % RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DTZ</td>
<td>LOV</td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning</td>
<td>101.04 ± 0.21</td>
<td>98.33 ± 0.24</td>
</tr>
<tr>
<td>Afternoon</td>
<td>98.51 ± 0.52</td>
<td>99.40 ± 0.35</td>
</tr>
<tr>
<td>Evening</td>
<td>99.46 ± 0.82</td>
<td>99.63 ± 0.55</td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning</td>
<td>100.19 ± 0.13</td>
<td>100.59 ± 0.21</td>
</tr>
<tr>
<td>Afternoon</td>
<td>99.21 ± 0.45</td>
<td>98.78 ± 0.32</td>
</tr>
<tr>
<td>Evening</td>
<td>101.04 ± 0.21</td>
<td>98.33 ± 0.24</td>
</tr>
</tbody>
</table>

\( a: \) Number of readings (n=6), RSD: Relative Standard Deviation
Development of Chromatographic Technique for Simultaneous Estimation of Lovastatin and Diltiazem HCL

Table 3. System Suitability Parameters

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>DTZ</th>
<th>LOV</th>
<th>PRO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Theoretical Plates</td>
<td>2171.96</td>
<td>4520.67</td>
<td>383.68</td>
</tr>
<tr>
<td>2.</td>
<td>Asymmetry</td>
<td>1.71</td>
<td>1.14</td>
<td>1.78</td>
</tr>
<tr>
<td>3.</td>
<td>Resolution</td>
<td>3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Retention Time in minutes (Mean ± % RSD)</td>
<td>4.0±0.49</td>
<td>5.6±0.56</td>
<td>8.8±0.25</td>
</tr>
<tr>
<td>5.</td>
<td>Selectivity</td>
<td>1.78</td>
<td>1.37</td>
<td>1.54</td>
</tr>
<tr>
<td>6.</td>
<td>Calibration Curve (μg ml⁻¹)</td>
<td>110-180</td>
<td>40-110</td>
<td>50</td>
</tr>
</tbody>
</table>

a: Number of readings (n=3), RSD: Relative Standard Deviation

Figure 2. Chromatographic separation of 150 μg ml⁻¹ of Diltiazem HCl (DTZ), 100 μg ml⁻¹ of Lovastatin (LOV) and 50 μg ml⁻¹ of Propranolol HCl (PRO) on C18 Column using methanol: water (90:10: v/v).

RESULTS AND DISCUSSION

The development of an analytical method for simultaneous estimation of drugs without previous chemical separation in multi-component pharmaceutical formulations has received considerable attention in recent years because of their importance in quality control of drugs and drug products.

In the present work an attempt has been made for of LOV and DTZ from the tablet formulations. Literature survey revealed that none of the method is reported for
simultaneous determination of LOV and DTZ from their tablet dosage form. Therefore the aim of this work was to develop simple analytical methods for the simultaneous determination of LOV and DTZ. This was achieved by development of RP-HPLC method. The methods were validated according to International Conference on Harmonization Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte. Eight point calibration curves were generated with appropriate volumes of working standard solutions for HPLC method. RP-HPLC method was developed for estimation of LOV and DTZ, which can be conveniently employed for routine quality control in pharmaceutical dosage forms. The chromatographic conditions were optimized in order to provide a good performance of the assay. In order to affect the simultaneous elution of the two components under isocratic conditions, different chromatographic conditions (organic modifier, flow rate, ionic strength, and pH) were investigated. RP-HPLC system consisting of Kr 100 C-18 (300 mm x 4. mm i.d.) provided good resolution for separation of DTZ and LOV. The mobile phase for the two drugs was selected based on its polarity. Mobile phase containing methanol alone or acetonitrile alone was found to elute the two compounds unresolved. Various ratios of Methanol: water was found to produce the chromatograms with very close retention times. Best resolution was achieved at the mobile phase composition of methanol: water in the ratio of 90:10 v/v, where the peaks of DTZ, LOV and PRO resolved. Flow rate of 1.0 ml/min. resulted in greater retention times and 1.2 ml/min. resulted in very close retention times with poor resolution. A flow rate of 1 ml/min. resulted in elution of all drugs within 15 minutes.

The sampling wavelength was selected after scanning the drug solutions in the mobile phase having concentration of 10 μg ml⁻¹ in the UV range of 200 - 400 nm on a UV spectrophotometer. 237 nm was selected as suitable wavelength for estimation. PRO was found to be a suitable internal standard for this study under the selected chromatographic conditions. The system suitability parameters are shown in Table 1. The method was specific as none of the excipients interfered with the analytes of interest. Hence, the method was suitably employed for assaying the formulation containing DTZ and LOV. An eight point calibration curve was constructed with working standards and was found linear (r² ≥0.9999) for each of the analytes over their calibration ranges. The proposed method was applied to the determination of DTZ and LOV in their pharmaceutical preparation. The results indicate satisfactory accuracy and precision of the method. The % recovery ± S. D. (n = 9) of the added DTZ and LOV was 99.65±0.46 and 99.68±0.34, respectively. From above data we have concluded that the proposed method can be applicable for the in process quality control for determination of DTZ and LOV in pharmaceutical preparations.

**CONCLUSION**

The statistical data have proven that developed HPLC method for simultaneous estimation of DTZ and LOV was found to be more accurate, precise, sensitive and selective. Therefore the proposed method could be applied for routine analysis in quality control laboratories for both in bulk and multi-component formulations.

**Acknowledgements**

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References


