Analytical Method Development and Validation of Doxofylline and Terbutaline Sulfate by RP-HPLC Method

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The objective of present work was to develop and validate a simple, accurate, precise HPLC method for the estimation of doxofylline and terbutaline sulfate. The chromatographic separation was achieved on a Hypersil BDSC 18 column(4.6x250 mm,5µm particle size). Different mobile phase systems in different proportions were tried. For HPLC method a mobile phase consisting of Methanol and Acetonitrile (80:20) produced symmetric peak shape with good resolution for both the drugs. Next, the drugs were chromatographed under different flow rates from which a flow rate of 1.0 ml/min was selected. The retention times of Doxofylline and Terbutaline sulfate were found to be 2.869 min and 3.942 min, respectively. The proposed method was found to have excellent linearity in the concentration range of 20-80mg/ml with correlation coefficient r²=0.999 and 0.999 for Doxofylline and Terbutaline sulfate respectively. The method was validated for linearity, precision, LOD, LOQ and robustness. The proposed method optimized and validated as per ICH guidelines.

Keywords: Doxofylline, Terbutaline, chromatographed.

1. INTRODUCTION

Doxofylline and Terbutaline sulfate are anti-asthmatic drugs. Doxofylline is chemically:7-(1,3-dioxolan-2-ylmethyl)-1,3-dimethylpurine-2,6-dione. It has a molecular weight of 266g/mol. Doxofylline is solid and it is soluble in methanol. Terbutaline sulfate is 3-
Benzenediol, 5-[(1,1-dimethylethyl)amino]-1-hydroxyethyl]-sulfate(2:1)(salt). (±)-a-[(tert-Butylamino)methyl]-3,5-dihydroxybenzyl alcohol sulfate (2:1)(salt). It has a molecular weight of 548g/mol. Terbutaline sulfate in low doses acts relatively, selectively at beta-adrenergic receptors to cause bronchodilation and relax the pregnant uterus. Terbutaline sulfate is white to gray, crystalline powder and it is soluble in methanol. 

Terbutaline sulfate is effective in the treatment of conditions characterized by bronchoconstriction such as asthma, chronic obstructive pulmonary disease, and severe allergic reactions. It is also used in the treatment of bronchitis and emphysema. It is available in various forms such as oral tablets, inhalers, and nasal sprays.

**Chemical Structure**

![Doxofylline](image)

**Doxofylline**

![Terbutaline sulfate](image)

**Terbutaline sulfate**

**Preparation of standard solution**

Stock solutions were prepared by dissolving 10mg of Doxofylline and 10mg of Terbutaline sulfate in mobile phase separately. Aliquots of standard solution of Doxofylline and Terbutaline sulfate were transferred into 10ml volumetric flasks and solutions were made up to the volume to yield concentrations of Doxofylline and Terbutaline sulfate.

**Preparation of sample solution**

For analysis of commercial formulation, 10 tablets of DOXOLL-TL of Doxofylline 400 mg and Terbutaline sulfate 5mg were weighed the average weight was calculated and powdered. A quantity equivalent to 400mg of Doxofylline and 5mg of Terbutaline sulfate was weighed and transferred to a 100ml volumetric flask which contain mobile phase and then shake it for 10mins and sonicate it for 20mins. The solution was allowed to stand at a room temperature for 20-30mins and filter it through a whatmann filter paper. Then suitable aliquots of formulation solution were prepared...
Validation of analytical method

ACCURACY: Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. Recovery studies of the drug were carried out for determining accuracy parameter. Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. It was done by mixing known quantity of standard drugs with the analyzed sample formulation and the contents were reanalyzed by the proposed method. This was carried out in 50% 100% and 150% levels.  

PRECISION: The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The Precision expressed as standard deviation or relative standard deviation.

a. System precision: Standard solution prepared as per test method and injected five times.

b. Method precision: Prepare five sample preparations individually using the single as per test method and injected each solution. 

LINEARITY: The linearity of analytical method is the ability to elicit test results that are directly proportional to the concentration of analyte in the sample within the given range. The linearity was performed by seven different concentrations, which were injected and calibration curve were plotted. The linearity of Doxofylline and Terbutaline sulfate was found to be in the range of 20-80µg/ml respectively. The chromatograms of the resulting solutions were recorded. The plot showing linearity and range study for Doxofylline and Terbutaline sulfate is shown in figure.

Fig 3: Plot of linearity and range study for Doxofylline

Fig 4: Plot of linearity and range study for Terbutaline sulfate

Ruggedness:

a) System to System variability: System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. A comparison of both the results obtained on two different HPLC systems, shows that the assay test method is rugged for System to system variables.

Robustness: The robustness of an analytical procedure are a measure of its capacity to remain unaffected by small, but deliberate changes in the method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase and flow rate. % RSD of assay was calculated.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD of an analytical procedure is the lowest concentration of an analyte in a sample which can be detected but not necessarily quantitated as an exact value where as LOQ is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.
4. CONCLUSION

The proposed RP-HPLC method for the estimation of the Doxofylline and Terbutaline sulfate in the pharmaceutical dosage form were simple, reliable and selective providing satisfactory accuracy and precision with lower limits of detection and quantification. The recoveries achieved was good by RP-HPLC method. The methods can be recommended for routine and quality control analysis of these drugs in the pharmaceutical dosage forms. In this proposed method symmetrical peaks with good resolution were obtained.

Table 2: summary of analysis of doxofylline and terbutaline sulfate by RP-HPLC method

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Labeled amount, mg/tablet</th>
<th>Estimated Amount, mg/tablet</th>
<th>% Label claim</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOXO</td>
<td>400</td>
<td>397.56</td>
<td>99.39</td>
<td>1.53</td>
</tr>
<tr>
<td>TER</td>
<td>5</td>
<td>4.79</td>
<td>95.80</td>
<td>1.13</td>
</tr>
</tbody>
</table>

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6. REFERENCES


