Development of HPLC method for simultaneous estimation of ambroxol, guaifenesin and salbutamol in single dose form

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A simple, accurate and precise High Performance Liquid Chromatographic (HPLC) method has been developed for simultaneous determination of ambroxol, guaifenesin and salbutamol in single dosage form. The method has been validated as per the guidelines of ICH and FDA. The finalized Reverse Phase–HPLC method is revealed with significant shorter retention time of 15 min with simple isocratic program. The separation is achieved on C- 8, 5 µm; 250 mm × 4.6 mm column with flow rate 1.0 mL per minute in isocratic mode using disodium hydrogen-ortho-phosphate buffer (pH 4.5) and methanol as mobile phase. Column oven temperature is maintained at 25°C and observations are recorded at 220 nm. The method is simple, accurate, reproducible and short and can be used for simultaneous analysis of ambroxol, guaifenesin and salbutamol in several single dose form formulation available in the market.

Keywords: Ambroxol, guaifenesin, salbutamol, HPLC method, simultaneous determination

Ambroxol hydrochloride is an expectorant and a mucolytic agent used in the treatment of bronchial asthma and chronic bronchitis. It has also been reported to have a cough suppressing effect and anti-inflammatory action. Recently, the inhibition of nitric oxide dependent activation of soluble guanylate cyclase was suggested as one of the molecular mechanism of the therapeutic action of ambroxol hydrochloride, also used in pulmonary alveolar proteinosis in pulmonary distress and infant respiratory distress syndrome. Guaifenesin, the glyceryl ether of guaiacol (a constituent of guaiac resin from the wood of Guajacum officinale Linné), is another drug thought to act as an expectorant by increasing the volume and reducing the viscosity of secretions in the trachea and bronchi. It is a component of numerous cough and cold preparations available worldwide. Interestingly, another established role for guaifenesin is as an anesthetic agent in veterinary medicine. Salbutamol is yet another drug typically used to treat bronchospasm and chronic obstructive pulmonary disease. With increasing research and development activities in the pharma sector, multi-salt drugs are being introduced in the market. Several drugs with the combination of ambroxol, guaifenesin and salbutamol have been launched in the market in recent years and Ambrex (Grandix Pharma), Simbro (Silicon Pharma), Bromo plus (Angel Labs), Xalphen (Alpic Remedies) Asthacure AM (Swiss Medicare), Axalin (Aronex Life science) and Tustross (Kaytross Healthcare) are a few of them. While the availability of multi-salt drug is an advantage to the consumer, it is a challenge for the testing and validating agencies as it needs a suitable method for simultaneous estimation of such salts as available in the drug. Quality control laboratory plays a vital role in determining the purity and safety of the pharmaceutical preparations hence it requires the development of highly specific and sensitive analytical methods to analyze the drug molecule and its dosage form. An extensive survey of recent literature by Mohamed A. Korany et al., followed by this group’s own literature search indicates that no simple, short and accurate HPLC method is available till date for simultaneous determination of ambroxol, guaifenesin, and salbutamol in single dose form. Thus, the aim of this study is to develop a simple high performance liquid chromatographic method for determination of ambroxol, guaifenesin, and salbutamol in single dosage form. The developed method is sort, simple, accurate and precise.

Materials and Methods

Reagents and Materials

Pure standards of all active ingredients (with minimum of 98.5% purity) were used in the current research work. HPLC grade methanol (Merck India Ltd), HPLC grade water, disodium hydrogen-orthophosphate anhydrous (Na₂HPO₄) (S D Fine Chemicals), electronic analytical balance (Mettler Toledo) and micro pipette (10-500 µL) were employed in the study. All glassware employed in the study were cleaned with hot water followed by acetic anhydride then acetone and dried in hot air oven.
whenever required. Working temperature was maintained between 18-25°C.

HPLC instrument and chromatographic conditions
The HPLC system (Agilent 1200 series) was loaded with EZ Chrome software with PDA detector. Isocratic elution of mobile phase with flow rate of 1.0 mL per min was performed on C-8 analytical column (Princeton sphere C-8, 5 µm; 250 mm × 4.6 mm). The run time was set for 15 min and column temperature maintained at 25°C. The volume of injection was 20 µL. Prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase. The eluent was measured at 220 nm.

Preparation of buffer (0.01 M)
1.4196 g of disodium hydrogen-ortho-phosphate anhydrous (Na₂HPO₄) was weight and dissolved in 1000 mL water. The desired pH was achieved with ortho-phosphoric acid solution having concentration 5% v/v.

Preparation of standard stock solution
10 mg each of ambroxol, guaifenesin and salbutamol were weighed in 10 mL volumetric flasks separately and dissolved in mobile phase (phosphate buffer pH 4.5 and methanol in the ratio of 40:60. After the immediate dissolution, the volume was made upto the mark with mobile phase). These standard stock solutions thus prepared contain 1000 µg mL⁻¹ each of ambroxol, guaifenesin and salbutamol.

Preparation of mix working standard solutions
From the standard stock solution (1000 µg/mL) of ambroxol, guaifenesin and salbutamol 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40 mL from each stock solution was transferred in different 10 mL volumetric flasks. Volume was adjusted upto 10 mL with mobile phase. Mix standard solutions thus prepared contain 10, 15, 20, 25, 30, 35, and 40 µg/mL each of ambroxol, guaifenesin and salbutamol.

Results and Discussions

Method Development
Several trials were performed in order to optimize the standard method. The finalized RP–HPLC method is revealed as simple, accurate and precise with significant shorter retention time of 15 min with simple isocratic program. The typical chromatogram of standard solution is shown in Figure 1. Standard solution was injected into the system for five replicate injections and system suitability parameters (tailing factor, resolution ≥ 2.0 and theoretical plates) are cross checked and found to be good. Relative standard deviation for area and retention time of all ingredients was cross checked and found to give satisfactory results and meeting the ICH and FDA specifications6–10 (%RSD ≤ 2.0%). Summary of system suitability results were tabulated in Table I.

Method Validation
Specificity
The specificity for the proposed method demonstrated that the placebo and diluents have no interference with all active ingredients. Furthermore, well shaped peaks indicate the specificity of the method.

Linearity
The concentrations of all active ingredients were prepared from the standard stock solution by taking suitable volume (mL) and diluted upto 10 mL to

![Figure 1 — Typical chromatogram of standard solution](image-url)
obtain the desired concentrations for linearity in the range of 10-40 µg per mL (standard concentration is 40 µg per mL) with seven different concentration levels. The prepared solutions were filtered through 0.45 µm membrane filter and each of the dilution was injected into the system. The calibration curve for each ingredient was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). It was found to be linear in the concentration range 10-40 µg per mL with good correlation in between concentration and mean peak area. All linearity chromatograms and co-relation coefficient of peak area was found to be linear. Results were tabulated in Table II.

Precision

Precision of the method was determined for all active ingredients. The intra-day and inter-day variations were determined using five replicate injections of sample preparation and analyzed on the same day and three different days over a period of three days. Sample assay was determined with six different preparations and assay found to be within the limits (between 98.0 to 102.0%) and %RSD found to be satisfactory (0.36 and 0.46%).

Accuracy

Accuracy was confirmed by carrying out recovery study as per ICH norms, where, to a pre analyzed sample solution, known concentration of standard solution was added equivalent to 80, 100 and 120% of label claimed. The percentage of drug recoveries found for formulation at 80, 100 and 120% were 101.09, 100.6, and 100.8 for ambroxol, 101.08, 100.70, 100.77 for guaifenesin and 100.19, 100.3, 100.31 for salbutamol. As all the statistical results were within the range of acceptance i.e. % RSD less than 2.0 and S.D. less than 1.0, hence the method was accurate for simultaneous quantitative estimation of components in single dosage form.

Ruggedness

Ruggedness of the method (intermediate precision) was estimated by preparing six dilutions of the test sample (for all ingredients) as per the proposed method and each dilution injected in duplicate using different column and analyst on different days.

Robustness

The proposed method was validated by changing chromatographic parameters and system suitability parameters were found to be within acceptable limits. Results are tabulated in Table III. The results indicate that the method was robust for all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions.

LOD and LOQ study

The LOD values for ambroxol, guaifenesin and salbutamol were 0.14, 0.16, and 0.41 µg mL⁻¹ respectively. LOQ values for ambroxol, guaifenesin

<table>
<thead>
<tr>
<th>System suitability parameter</th>
<th>Ambroxol</th>
<th>Guaifenesin</th>
<th>Salbutamol</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tailing factor</td>
<td>1.49</td>
<td>1.44</td>
<td>1.59</td>
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<tr>
<td>Peak asymmetry</td>
<td>1.96</td>
<td>1.73</td>
<td>1.84</td>
<td>≤ 2.0</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>4588</td>
<td>5330.0</td>
<td>4002.6</td>
<td>≥ 4000</td>
</tr>
<tr>
<td>% RSD of replicate injections</td>
<td>0.3260</td>
<td>0.3148</td>
<td>0.4612</td>
<td>≤ 2.0</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ambroxol</th>
<th>Guaifenesin</th>
<th>Salbutamol</th>
</tr>
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<tbody>
<tr>
<td>Linearity range (µg/mL)</td>
<td>10-40</td>
<td>10-40</td>
<td>10-40</td>
</tr>
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<td>Regression coefficient (r²)</td>
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<td>0.9988</td>
<td>0.9996</td>
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<tr>
<td>Slope</td>
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<tr>
<td>Intercept</td>
<td>7440</td>
<td>24950</td>
<td>10208</td>
</tr>
</tbody>
</table>

Table I — Summary of system suitability study of assay method by HPLC

Table II — Data for linearity and range of developed method
and salbutamol were 0.41, 0.47 and 0.92 µg mL⁻¹ respectively. Data for LOD and LOQ of developed method are as reported in Table IV.

References


10 USP 25–NF 20, Validation of Compendial Methods Section (1225), United States Pharmacopoeal Convention, Rockville, Maryland, USA, 2002, 2256.