SIMULTANEOUS QUANTIFICATION OF SALBUTAMOL SULPHATE AND AMBROXOL HYDROCHLORIDE BY RP-HPLC AND HPTLC IN BULK DRUG AND DOSAGE FORM

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ABSTRACT

Present work describes a precise, accurate and reproducible Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) and High Performance Thin Layer Chromatographic (HPTLC) methods for simultaneous estimation of Salbutamol sulphate and Ambroxol hydrochloride. In the RP-HPLC method, the drugs were resolved using a mobile phase of acetonitrile: 50 mM disodium hydrogen phosphate buffer (containing 0.1% triethylamine, pH adjusted to 4.2 using ortho phosphoric acid) (28:72 v/v) on an Inertsil, ODS -3V C18 (250 X 4.6 mm), 5μm column in isocratic mode. The retention time of salbutamol sulphate and ambroxol hydrochloride were 2.59 and 6.15 min, respectively. In the HPTLC method, the chromatograms were developed using a mobile phase of methanol: ethyl acetate: toluene: ammonia (4:1.5:5.6:1.0 v/v) on precoated plate of silica gel 60 F254 and quantified by densitometric absorbance mode at 231 nm. The Rf values of salbutamol sulphate and ambroxol hydrochloride were, 0.38 and 0.59 respectively. Recovery values of 98.09 – 102%, percentage relative standard deviation of < 1.38 and correlation coefficient of 0.996–0.998 shows that the developed methods were accurate and precise. As per ICH guidelines the results of the analysis were validated in terms of specificity, limit of detection, limit of quantification, linearity, precision and accuracy and were found to be satisfactory. These methods can be employed for the routine analysis of tablets containing salbutamol sulphate and ambroxol hydrochloride.

Keywords: Salbutamol sulphate, Ambroxol hydrochloride, RP-HPLC, HPTLC, Validation

INTRODUCTION

Salbutamol sulphate (SAL), bis [(1RS)-2-[(1, 1-dimethylethyl) amino]-1-[4-hydroxy-3-(hydroxymethyl) phenyl] ethanol] sulphate, is beta-adrenoceptor agonist used for the relief of broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease 1, 2. Ambroxol hydrochloride (AMB), trans-4-((2-amino-3, 5-dibromobenzyl) amino) cyclohexanol hydrochloride. Ambroxol reduces bronchial hyper-reactivity and acts as a mucolytic and cough suppressant. Both SAL and AMB are official in Indian pharmacopoeia. Combination of SAL and AMB is used for the treatment of asthma and bronchitis 3, 4. Literature survey reveals that salbutamol in combination with other drugs has been estimated by UV spectrophotometric methods 5-9, HPTLC 10, RP-HPLC 11, TLC method12. The methods reported for simultaneous determination of ambroxol in combination with other drugs are UV spectrophotometric methods 13-16, RP-HPLC 17-21, HPTLC 22 and LC-MS/MS 23. No HPTLC method and only one HPLC method 24 have been found to be reported for the simultaneous estimation of salbutamol sulphate and ambroxol hydrochloride in combination. The aim of the work was to introduce a simple, accurate and reproducible isocratic RP-HPLC and HPTLC methods for simultaneous determination of SAL and AMB. The proposed methods were optimized and validated as per ICH guidelines 25.

MATERIALS AND METHODS

Chemicals and Reagents

SAL and AMB were obtained as gift samples from Gens Pharma International Pvt Ltd, Pune and Elder Pharmaceuticals Ltd., Mumbai respectively. Disodium hydrogen phosphate, HPLC grade Water and Acetonitrile were procured from Merck Ltd, Mumbai, India. Orthophosphoric acid was purchased from Research Lab, Fine Industries, Mumbai. Methanol, ethyl acetate, toluene, ammonia all were of analytical grade (Merck Ltd, Mumbai). The commercial formulation of SAL and AMB is available in ratio of 1:15 (Sal-Mucolite) tablet procured from local market.

Instrumentation

The HPLC system, Jasco PU-2080 Plus, with manual Rheodyne injector facility operates at 20 μl capacity per injection was used. The column used was Inertsil, C18 (250 X 4.6 mm), 5μm and the detector consisted of UV/VIS (Jasco UV 2075-Plus) operated at 231 nm. The data were acquired and processed using Borwin software version 1.5.
HPTLC was performed in Camag HPTLC (Camag, Muttenz, Switzerland) system, equipped with Linomat 5 sampler applicator, twin trough plate development chamber, and CAMAG TLC III scanner.

**RP-HPLC method**

**Chromatographic Conditions**

Optimizations of chromatographic condition were carried out using mobile phase, acetonitrile: 50 mM disodium hydrogen phosphate buffer, containing 0.1% triethylamine (28.72 v/v), pH 4.2 was adjusted using orthophosphoric acid. Prior to deliver into the system, mobile phase was filtered through 0.45 μ membrane filter and sonicate for 10 min. The samples were introduced by injector with a 20 μl sample loop. The analysis was carried out under isocratic conditions using flow rate 1 ml/min at 10°C and chromatograms were recorded at 231 nm.

**Preparation of standard stock solution**

Weighed accurately 2 mg of SAL and 30 mg of AMB, transferred to a 10 ml volumetric flask, it was dissolved in 5 ml of mobile phase and sonicate for 10 min. Finally the volume was made up to mark with mobile phase.

**Preparation of working standard solution**

From the standard stock solution, 0.1ml solution was pipetted out in 10 ml volumetric flask and volume was made up to the mark with mobile phase.

**Analysis of tablet formulation**

Twenty tablets (sal-mucolite) were weighed, triturated, mixed thoroughly and average weight of tablet was calculated. Accurately weighed quantity of tablet powder equivalent to 2 mg of SAL was transferred to 10 ml volumetric flask, it was dissolved in 5 ml of mobile phase and sonicate for 10 min. The resultant solution was filtered through 0.45μm membrane filter, diluted to volume with methanol (Table 1).

**RESULTS AND DISCUSSION**

**Table 1: Analysis data of Salbutamol Sulphate and Ambroxol Hydrochloride**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Label claim</th>
<th>RP-HPLC</th>
<th>HPTLC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% estimated ± S.D. % RSD</td>
<td>% estimated ± S.D. % RSD</td>
</tr>
<tr>
<td>SAL</td>
<td>2 mg</td>
<td>99.52 ± 1.7414</td>
<td>1.7497</td>
</tr>
<tr>
<td></td>
<td>30 mg</td>
<td>98.64 ± 1.2545</td>
<td>1.2718</td>
</tr>
<tr>
<td>AMB</td>
<td></td>
<td>98.97 ± 1.9086</td>
<td>1.9284</td>
</tr>
</tbody>
</table>

SD -Standard deviation, RSD- Relative standard deviation

**Validation**

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

The signal-to-noise ratio (S/N) method was adopted for the determination of limit of detection and limit of quantification. The limit of detection was estimated as three times the S/N ratio and the limit of quantification was estimated as ten times the S/N ratio.

**Specificity**

Specificity is the ability of a method to discriminate between the analyte of interest and other components that may present in the sample. The specificity of the method was evaluated to ensure separation of SAL and AMB and was demonstrated by assaying samples of SAL and AMB tablet.

**Linearity**

For RP-HPLC method, different standard solutions were prepared by diluting standard stock solution with mobile phase in concentration 0.5, 1.0, 1.5, 2.0, 2.5, 3 μg/ml for SAL and 7.5, 15, 22.5, 30, 37.5, 45 μg/ml for AMB. The standard chromatograms were taken under standard chromatographic conditions.

For HPTLC the standard solutions were prepared by diluting standard stock solution with methanol in concentration range of 100, 120, 140, 160, 180, and 200 ng/spot for SAL and 1500, 1800, 2100, 2400, 2700 and 3000 ng/spot for AMB. The peak area was plotted against corresponding concentrations to obtain the calibration graph.

**Precision**

Precision of analytical methods were expressed in relative standard deviation (RSD) of a series of measurements. The intra-day and inter-day precisions of the proposed methods were determined by estimating the corresponding responses (i.e. three concentrations / three replicates each) of the sample solution on the same day and on three different days respectively.

**Recovery**

To check the accuracy of the proposed method, recovery studies were carried out by standard addition method. A known amount of standard SAL and AMB corresponding to 80, 100 and 120% of the label claim was added to preanalysed sample of tablet. The recovery studies were carried out in triplicate at each level.

**RESULTS AND DISCUSSION**

In RP-HPLC method, chromatographic conditions were optimized to obtain an adequate separation of eluted compounds. Mobile phase and flow rate selection was based on peak parameters such as tailing, theoretical plates, capacity factor etc. The system with acetonitrile: 50 mM disodium hydrogen phosphate buffer (containing 0.1% triethylamine, pH 4.2 adjusted by using orthophosphoric acid) (28:72 v/v) with 1 ml/min flow rate is quite robust. A typical chromatogram for SAL and AMB is shown in Fig 2.

The optimum wavelength for detection was 231 nm at which better detector response for drugs was obtained. The average retention times for SAL and AMB was found to be 2.59 and 6.15 min with
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linear range of 0.5-3 µg/ml \( (r^2 = 0.998) \) and 7.5-45 µg/ml \( (r^2 = 0.998) \) respectively (Table 2). The method was found to be precise after quantification of six replicates of SAL and AMB with RSD less than 2.0% (Table 3). The recovery values were found to be 98.48-100.82% (Table 4).

In HPTLC method, chromatographic separation of the drugs was performed on aluminium plates precoated with silica gel 60 F254, with methanol:ethyl acetate:toluene:ammonia (4:1.5:5.6:1.0 v/v) as mobile phase. Chromatographic evaluation of the separated bands was performed at 231 nm. SAL and AMB were resolved satisfactorily with \( R_f \) values 0.38 and 0.59 respectively (Fig.3).

The method showed good linear response in concentration range of 100-200 ng /spot \( (r^2 = 0.998) \) and 1500-3000 ng /spot \( (r^2 = 0.996) \) for SAL and AMB respectively (Table 2).

The method was found to be precise after quantification of six replicates of SAL and AMB and RSD was found to be less than 2.0% (Table 3). The recovery values were 98.09-102% with S.D. of <1.28 (Table 4).

![RP-HPLC Chromatogram of Salbutamol Sulphate and Ambroxol Hydrochloride](image1)

![HPTLC Chromatogram of Salbutamol Sulphate and Ambroxol Hydrochloride](image2)

Table 2: Linear Regression data of Salbutamol Sulphate and Ambroxol Hydrochloride

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RP-HPLC</th>
<th>HPTLC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAL</td>
<td>AMB</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>0.15 (µg/ml)</td>
<td>0.5 (µg/ml)</td>
</tr>
<tr>
<td>Limit of quantitation</td>
<td>0.45 (µg/ml)</td>
<td>1.5 (µg/ml)</td>
</tr>
<tr>
<td>Retention time (min) and Rf</td>
<td>2.59</td>
<td>6.15</td>
</tr>
<tr>
<td>Correlation coefficient ( (r^2) )</td>
<td>0.998</td>
<td>0.998</td>
</tr>
<tr>
<td>Calibration range</td>
<td>0.5-3.0 (µg/ml)</td>
<td>7.5-45 (µg/ml)</td>
</tr>
<tr>
<td>Regression equation</td>
<td>( y = 23445x + 3595 )</td>
<td>( y = 17219x + 45935 )</td>
</tr>
</tbody>
</table>
CONCLUSION

The proposed RP-HPLC and HPTLC methods for the quantification of SAL and AMB in tablet were simple, precise, accurate, rapid and selective. The methods were found to be linear in wide range of concentration. The developed methods were free from interference due to the excipients present in tablet and can be used for routine simultaneous quantitative estimation of SAL and AMB in tablet.

ACKNOWLEDGMENT

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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Precision (n = 3)</th>
<th>RP-HPLC</th>
<th>HPTLC</th>
</tr>
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<tbody>
<tr>
<td>SAL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-day*</td>
<td>100.12 ± 1.7993</td>
<td>100.29 ± 0.3775</td>
<td>102.35 ± 0.5978</td>
</tr>
<tr>
<td>AMB</td>
<td>100.18 ± 1.8458</td>
<td>99.57 ± 1.3984</td>
<td>98.19 ± 1.4623</td>
</tr>
<tr>
<td>Inter-day*</td>
<td>99.69 ± 1.7644</td>
<td>100.61 ± 1.1645</td>
<td>100.36 ± 1.0915</td>
</tr>
<tr>
<td>AMB</td>
<td>100.12 ± 1.1319</td>
<td>100.32 ± 0.4031</td>
<td>102.82 ± 0.8645</td>
</tr>
<tr>
<td>AMB</td>
<td>99.97 ± 1.0126</td>
<td>99.31 ± 0.6797</td>
<td>101.07 ± 1.3613</td>
</tr>
<tr>
<td>AMB</td>
<td>98.87 ± 1.1870</td>
<td>99.62 ± 0.7307</td>
<td>100.66 ± 0.9714</td>
</tr>
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</table>

* Mean of three replicates

<table>
<thead>
<tr>
<th>Level of standard</th>
<th>Addition (%)</th>
<th>RP-HPLC</th>
<th>HPTLC</th>
</tr>
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<tbody>
<tr>
<td>SAL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>98.70 ± 0.0293</td>
<td>101.1 ± 1.1343</td>
<td>1.1521</td>
</tr>
<tr>
<td>100</td>
<td>100.82 ± 1.128</td>
<td>98.54 ± 1.2668</td>
<td>1.2895</td>
</tr>
<tr>
<td>120</td>
<td>98.99 ± 0.8259</td>
<td>98.89 ± 0.7375</td>
<td>0.7451</td>
</tr>
</tbody>
</table>

| AMB               | 98.48 ± 0.7924 | 100.10 ± 0.5142 | 0.5133 |
|                  | 99.17 ± 1.3777 | 102 ± 0.6740    | 0.6647 |
|                  | 98.75 ± 0.9383 | 99.28 ± 0.5958  | 0.5875 |


