DEVELOPMENT AND VALIDATION OF SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF SALBUTAMOL SULPHATE, AMBROXOL HYDROCHLORIDE AND CETIRIZINE HYDROCHLORIDE IN COMBINED PHARMACEUTICAL TABLET FORMULATION: A NOVEL TECHNIQUE FOR IN-VITRO DISSOLUTION STUDIES

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ABSTRACT

Objective: To develop a simple, rapid, accurate, precise and economical spectroscopic method for the simultaneous determination of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine hydrochloride in combined pharmaceutical tablet formulation and validate as per ICH guidelines.

Methods: In this method, the 6.8 pH phosphate buffer was selected for the developing spectral characteristics of the three drugs. The overlay spectra of Salbutamol Sulphate, Ambroxol hydrochloride and Cetirizine hydrochloride were resolved by making the use of simultaneous equation method based on measurement of absorbance at three wavelengths.

Results: The λ\textsubscript{max} of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine hydrochloride were found to be 276 nm, 244 nm and 230 nm. The method obeyed Beer Lambert's law in the concentration range of 10-100 µg/ml for SAL, 2-18 µg/ml for AMB HCl and 2-20 µg/ml for CET HCl. The high values of correlation coefficient (R) indicated good linearity of calibration plot for the drugs. Result of percentage recovery study confirms the accuracy of proposed method. Percentage RSD for precision and accuracy of the method was found to be less than 2%. LOD values for SAL, AMB, and CET were found to be 0.523 µg/ml, 0.450 µg/ml and 0.457 µg/ml, respectively. LOQ values for SAL, AMB, and CET were found to be 1.372 µg/ml, 1.424 µg/ml and 1.386 µg/ml, respectively.

Conclusion: A rapid, economical, accurate, precise and reproducible simultaneous equation spectroscopic method was developed and validated. The proposed method can be employed for routine analysis of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride in combined pharmaceutical tablet formulation.

Keywords: Salbutamol Sulphate, Ambroxol Hydrochloride, Cetirizine Hydrochloride, Simultaneous equation, Absorbivity, Absorbance, Absorption Maxima, ICH guidelines, Validation.

INTRODUCTION

Salbutamol Sulphate (SAL) (fig. 1) official in Indian Pharmacopoeia and British Pharmacopoeia, is chemically known as (RS)-1-(4-hydroxy-3-hydroxy-methylphenyl)-2-(tert-butylamino) ethanol sulphate. It is a white or almost white, crystalline powder. It is freely soluble in water, slightly soluble in ethanol (95 %) and in ether; very slightly soluble in dichloromethane. The drug is official in Indian Pharmacopoeia and British Pharmacopoeia [1, 2]. Salbutamol sulphate is a short-acting ß2-adrenergic receptor agonist used for the relief of bronchospasm in conditions such as asthma and COPD (Chronic obstructive pulmonary disease) [3].

Fig. 1: Chemical Structure of Salbutamol Sulphate

Ambroxol hydrochloride [AMB HCl] (fig. 2) official in Indian Pharmacopoeia and British Pharmacopoeia, is chemically Trans-4-[(2-amino-3, 5-dibromobenzyl) amino]-cyclohexanol hydrochloride. It is a white or yellowish crystalline powder. It is sparingly soluble in water; soluble in methanol; practically insoluble in methylene chloride [4, 5]. Ambroxol hydrochloride is a potent mucoytic & mucokinetic, capable of inducing bronchial secretion. It depolymerises mucopolysaccharides directly as well as by liberating lysosomal enzymes network of fibres in tenacious sputum is broken. It is particularly useful in if mucus plugs are present. Ambroxol hydrochloride (AMB) is semi-synthetic derivative of vasicine obtained from Indian shrub Adhatoda vasica. It is a metabolic product of bromhexine. Used in a variety of respiratory disorders including chronic bronchitis, also used in the treatment of cough [6].

Fig. 2: Chemical Structure of Ambroxol Hydrochloride

Cetirizine hydrochloride [CET HCl] (fig. 3) official in Indian Pharmacopoeia and British Pharmacopoeia, is chemically [2-[4-(4-chlorophenyl) phenylmethyl]-1-piperaziny]ethoxy]acetic acid and the active metabolite of the pipazine H+ - receptor antagonist hydroxizine. It is white or almost white powder; freely soluble in water, practically insoluble in acetone and in methylene chloride [7, 8]. It is a non-sedative second generation anti-histamine drug used in the treatment of seasonal allergic rhinitis, perennial allergic rhinitis, chronic urticaria also used as adjuvant in seasonal asthma. Cetirizine inhibits the release of histamine and of cytotoxic mediators from platelets, as well as eosinophil chemotaxis during the secondary phase of allergic response [9].

Original Article

DEVELOPMENT AND VALIDATION OF SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF SALBUTAMOL SULPHATE, AMBROXOL HYDROCHLORIDE AND CETIRIZINE HYDROCHLORIDE IN COMBINED PHARMACEUTICAL TABLET FORMULATION: A NOVEL TECHNIQUE FOR IN-VITRO DISSOLUTION STUDIES

Fig. 1: Chemical Structure of Salbutamol Sulphate

Fig. 2: Chemical Structure of Ambroxol Hydrochloride

Fig. 3: Chemical Structure of Cetirizine Hydrochloride
The combination of these three drugs is not official in any pharmacopoeia; hence, no official method is available for the simultaneous estimation of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride in combined tablet dosage form. Literature survey revealed that there are several methods that have been reported for the estimation of these drugs individually or in combination with other drugs by using UV spectrophotometry, [22–33], chromatographic methods such as RP-HPLC [22–33], RP-HPLC [34], RP-HPLC [35–37] and LC-MS [38]. As per literature, no analytical method could be traced for the analysis of SAL, AMB, and CET in combined tablet dosage form. Therefore, simpler, compared to methods such as chromatography and estimation of SAL, AMB, and CET in combined tablet dosage form. Spectrophotometric methods of analysis are more economic and simpler, compared to methods such as chromatography and electrophoresis. Hence an attempt has been made to develop new simultaneous equation spectrophotometric method which is simple, rapid, reproducible, and economical method for simultaneous estimation of SAL, AMB, and CET in combined tablet dosage form. The presently developed method was validated as per International Conference on Harmonization guidelines (ICH) [39-40].

MATERIALS AND METHODS

Apparatus and Instrument
A double UV Visible Spectrophotometer (UV- 1800 Shimadzu, Japan) was used. Absorption and overline spectra of both test and standard solutions were recorded over the wavelength range of 200-400 nm using 1 cm quartz cell at fast scanned speed and fixed slit width of 1.0 nm. All weighing of ingredients were done on digital weighing balance (DV 215 CD Ohaus, USA) and bath sonicator (PCI analytical Pvt. Ltd) was also used in study. Glasswares used in each procedure were air oven.

Reagents and materials
Pure drug samples of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine hydrochloride were supplied as gift sample by Trojan Pharma Baddi, Himachal Pradesh, India. All other chemicals and reagents used were of analytical grade.

Selection of common solvent
Phosphate buffer of pH 6.8 was selected as common solvent for developing spectral characteristics of drug. The selection was made after assessing the solubility of the drugs in different solvents.

Preparation of standard stock solution
Standard stock solution of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine hydrochloride were prepared by dissolving 10 mg of Salbutamol Sulphate, 10 mg of Ambroxol Hydrochloride and 10 mg of Cetirizine Hydrochloride separately in 10 ml of pH 6.8 Phosphate buffer solution and sonicated for 15 minutes in bath sonicator and filtered through whatman filter paper in order to get dilution of 1 mg/1 ml i.e. 1000 µg/ml.

Determination of absorption (λ) maxima
By appropriate dilution of standard stock solutions of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride with pH 6.8 phosphate buffer solution, solution containing 10 µg/ml of Salbutamol Sulphate, 10 µg/ml of Ambroxol Hydrochloride and 10 µg/ml of Cetirizine Hydrochloride was scanned separately and then in mixture form in the range of 200-400 nm.

Calibration plots for salbutamol sulphate, ambroxol hydrochloride and cetirizine hydrochloride
The calibration plots were constructed for Salbutamol Sulphate, Ambroxol Hydrochloride and for Cetirizine hydrochloride using pH 6.8 phosphate buffer solutions at their respective absorption maxima in order to find the linearity range of drugs at their respective absorption maxima.

Development of simultaneous equation
The absorbances were measured at all the selected wavelengths and absorbivities for three drugs were determined at all three wavelengths. The concentrations of drugs in sample solution were determined by using following formula:
\[
A_\lambda = a_x C_x + a_y C_y + a_z C_z \quad \text{(1)}
\]
\[
A_{244} = a_x C_x + a_y C_y + a_z C_z \quad \text{(2)}
\]
\[
A_{230} = a_x C_x + a_y C_y + a_z C_z \quad \text{(3)}
\]
Where, \(C_x, C_y\), and \(C_z\) are the concentration of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride, \(A_x, A_y\), and \(A_z\) are absorbance at 276 nm, 244 nm and 230 nm respectively, \(a_x\), \(a_y\), and \(a_z\) are absorbivities of Salbutamol Sulphate at 276 nm, 244 nm and 230 nm respectively; \(a_x, a_y,\) and \(a_z\) are absorbivities of Ambroxol Hydrochloride at 276 nm, 244 nm and 230 nm respectively; \(a_x, a_y,\) and \(a_z\) are absorbivities of Cetirizine Hydrochloride at 276 nm, 244 nm and 230 nm respectively.

Validation of proposed method
The optimized UV spectrophotometric method was completely validated according to the procedure described in ICH guidelines. The performance parameters evaluated for the method were linearity, precision, accuracy, limits of detection and quantitation, and assay of drug.

Linearity
The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride.

Precision
Repeatability
The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions \(n = 6\) for Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride (10 µg/ml for each drug) without changing the parameter of the proposed spectrophotometry method.

Intermediate precision
Precision of method was determined in terms of intraday and interday variations (%RSD). Intraday precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Interday precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days.

Limit of detection and Limit of quantitation
The limit of detection (LOD) and limit of quantitation (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (4) and (5), respectively.
\[
\text{LOD} = 3.3 \delta ... ... (4)
\]
\[
\text{LOQ} = 10 \delta ....... (5)
\]
Where, \(\delta\): standard deviation of y-intercept and \(S\): slope of calibration curve.

Recovery (Accuracy) Studies
In order to check the accuracy, reproducibility and precision of the proposed method, recovery study was carried out by taking

Fig. 3: Chemical Structure of Cetirizine Hydrochloride
standard mixture solution of SAL, AMB and CET and absorbances were determined at 276 nm, 244 nm and 230 nm respectively.

Assay (Analysis) of Drug

Ten tablets (200 mg) were powdered in a mortar pestle and the blend equivalent to 2 mg of Salbutamol Sulphate, 7.5 mg of Ambroxol Hydrochloride and 5 mg of Cetirizine Hydrochloride were weighed and dissolved in 100 ml of pH 6.8 phosphate buffer solutions.

The solution was sonicated for 15 minutes, filtered through whatman filter paper, suitably diluted with pH 6.8 phosphate buffer and the drug content was analyzed form simultaneous equation method by using double beam UV spectrophotometer at 276 nm, 244 nm and 230 nm respectively. Each sample was analyzed in triplicate, the result of which was given in table 8.

RESULTS AND DISCUSSION

Selection of Absorption maxims

Wavelengths of absorption maxims were determined for three drugs. SAL showed absorption maxima at 276 nm, AMB HCl showed absorption maxima at 244 nm, CET HCl showed maximum absorbance at 230 nm with reference to British Pharmacopoeia and Indian Pharmacopoeia.

From the overlain spectra of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride (fig. 4); three wavelengths 276 nm, 244 nm and 230 nm, \( \lambda_{\text{max}} \) of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine hydrochloride were selected for further spectroscopic studies. Therefore, for simultaneous equation method, wavelengths selected for analysis were 276 nm for SAL, 244 nm for AMB HCl and 230 nm for CET HCl.

Linearity of Calibration curves

From the calibration plot of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride at their respective absorption maxima, the linearity was observed in the concentration range of 10–100 µg/ml for Salbutamol Sulphate, 2-18 µg/ml for Ambroxol Hydrochloride and 2-20 µg/ml for Cetirizine Hydrochloride at all three wavelengths, which were validated by least square method. Coefficient of correlation (R) was found to be 0.999 for Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride as given in table 4. The high value of correlation coefficient (R) also indicates good linearity of calibration curve for the drugs as shown in fig. 5, fig. 6 and fig. 7.

### Table 1: Absorbance and Absorptivity of Salbutamol Sulphate at 276 nm, 244 nm and 230 nm respectively

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>*Absorbance at 276 nm ± S. D</th>
<th>*Absorbance at 244 nm ± S. D</th>
<th>*Absorbance at 230 nm ± S. D</th>
<th>Absorptivity at 276 nm</th>
<th>Absorptivity at 244 nm</th>
<th>Absorptivity at 230 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.071 ± 0.002</td>
<td>0.026 ± 0.003</td>
<td>0.250 ± 0.003</td>
<td>0.0071</td>
<td>0.0026</td>
<td>0.025</td>
</tr>
<tr>
<td>20</td>
<td>0.138 ± 0.003</td>
<td>0.043 ± 0.004</td>
<td>0.485 ± 0.002</td>
<td>0.0069</td>
<td>0.0022</td>
<td>0.024</td>
</tr>
<tr>
<td>30</td>
<td>0.211 ± 0.002</td>
<td>0.064 ± 0.002</td>
<td>0.713 ± 0.001</td>
<td>0.0070</td>
<td>0.0021</td>
<td>0.024</td>
</tr>
<tr>
<td>40</td>
<td>0.264 ± 0.001</td>
<td>0.067 ± 0.001</td>
<td>0.930 ± 0.002</td>
<td>0.0066</td>
<td>0.0017</td>
<td>0.023</td>
</tr>
<tr>
<td>50</td>
<td>0.327 ± 0.002</td>
<td>0.081 ± 0.002</td>
<td>1.151 ± 0.003</td>
<td>0.0065</td>
<td>0.0016</td>
<td>0.023</td>
</tr>
<tr>
<td>60</td>
<td>0.385 ± 0.003</td>
<td>0.084 ± 0.003</td>
<td>1.362 ± 0.002</td>
<td>0.0064</td>
<td>0.0014</td>
<td>0.023</td>
</tr>
<tr>
<td>70</td>
<td>0.459 ± 0.001</td>
<td>0.108 ± 0.002</td>
<td>1.599 ± 0.002</td>
<td>0.0066</td>
<td>0.0015</td>
<td>0.023</td>
</tr>
<tr>
<td>80</td>
<td>0.512 ± 0.001</td>
<td>0.109 ± 0.002</td>
<td>1.794 ± 0.004</td>
<td>0.0064</td>
<td>0.0014</td>
<td>0.022</td>
</tr>
<tr>
<td>90</td>
<td>0.571 ± 0.001</td>
<td>0.122 ± 0.003</td>
<td>1.969 ± 0.002</td>
<td>0.0063</td>
<td>0.0014</td>
<td>0.022</td>
</tr>
<tr>
<td>100</td>
<td>0.628 ± 0.002</td>
<td>0.146 ± 0.002</td>
<td>2.125 ± 0.003</td>
<td>0.0063</td>
<td>0.0015</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Mean

- \( \bar{a}_1 = 0.0066 \)
- \( \bar{a}_2 = 0.0017 \)
- \( \bar{a}_3 = 0.023 \)

*Each value is the average of three determinations*
Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride in tablet dosage form: The optical characteristics such as Lambert’s law limit, Regression equation, Slope, Intercept, correlation coefficient, were calculated and are summarized in table 4.

Development of simultaneous equation

The absorptivity values were found approximately same for all the concentrations hence all three drugs obeyed Beer Lambert’s law in an indicated concentration range as given in table 1, 2 and 3.

Substituting the values of \( a_x \), \( a_{x_2} \), \( a_{x_3} \), \( a_y \), \( a_{y_2} \), \( a_{y_3} \), \( a_z \), \( a_{z_2} \), \( a_{z_3} \) from table 1, table 2 and table 3 in eqn. 1, 2 and 3, the following equation was designed for the simultaneous estimation of Ambroxol Hydrochloride and Cetirizine Hydrochloride in tablet dosage form:

- At 276 nm \( A_1 = 0.0066 C_s + 0.0030 C_a + 0.0050 C_c \) .... (6)
- At 244 nm \( A_2 = 0.0017 C_s + 0.025 C_a + 0.0063 C_c \) .... (7)
- At 230 nm \( A_3 = 0.023 C_s + 0.019 C_a + 0.0338 C_c \) .... (8)

Where \( C_s \), \( C_a \) and \( C_c \) are the concentration of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride respectively, \( A_1 \), \( A_2 \) and \( A_3 \) are absorbance values at 276 nm, 244 nm and 230 nm respectively, 0.0066, 0.0017 and 0.023 are absorbivities of Salbutamol Sulphate at 276 nm, 244 nm and 230 nm respectively; 0.0030, 0.025 and 0.019 are absorbivities of Ambroxol Hydrochloride at 276 nm, 244 nm and 230 nm respectively; 0.0050, 0.0063 and 0.0338 are absorbivities of Cetirizine Hydrochloride at 276 nm, 244 nm and 230 nm respectively. By solving equation (6), (7) and (8) simultaneously, the concentration of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride in combined tablet dosage form can be calculated.

Validation of Proposed Method

The validation parameters were studied at all the wavelengths for the proposed method. The optical characteristics such as \( \lambda_{\text{max}} \), Beer’s Lambert’s law limit, Regression equation, Slope, Intercept, correlation coefficient, were calculated and are summarized in table 4.

Linearity

The linearity was observed in the concentration range of 10–100 \( \mu g/mL \) for Salbutamol Sulphate, 2-18 \( \mu g/mL \) for Ambroxol Hydrochloride and 2-20 \( \mu g/mL \) for Cetirizine Hydrochloride at all three wavelengths, which were validated by least square method. Coefficient of correlation (R) was found to be 0.999 for Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride. Regression parameters are mentioned in table 4.

LOD and LOQ

LOD and LOQ of the drug were calculated as per ICH guideline. LOD values for SAL, AMB, and CET were found to be 0.523 \( \mu g/mL \), 0.450 \( \mu g/mL \), and 0.457 \( \mu g/mL \) respectively. LOQ values for SAL, AMB, and CET were found to be 1.372 \( \mu g/mL \), 1.424 \( \mu g/mL \), and 1.386 \( \mu g/mL \) respectively (Table 4). These data show that the proposed method is precise and sensitive for the determination of SAL, AMB, and CET.

Repeatability

Instrumental precision study was carried out by repeatability study. The % RSD values for SAL, AMB, and CET were found to be 1.6609, 1.9596, and 1.8547 respectively given in table 5. Low relative standard deviation (<2) indicates that the proposed method is repeatable.

Intermediate precision (Reproducibility)

Precision studies were carried out to study the intra-day and inter-day variations of the responses. The intra-day and inter-day precisions were determined, results of which are given in table 6.

Recovery (Accuracy) studies

Known amounts of Salbutamol Sulphate (20, 40, and 60 \( \mu g/mL \)), Ambroxol Hydrochloride (4, 8, and 12 \( \mu g/mL \)) and Cetirizine Hydrochloride (6, 10 and 14 \( \mu g/mL \)) were added to a pre-quantified sample solution, and the amount of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride were estimated by proposed method.

Assay of Drugs

Based on the above results obtained, it can be concluded that the proposed spectroscopic method for simultaneous estimation of
Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride is rapid, economical, accurate, precise and reproducible. The utility of the developed method has been demonstrated by analysis of combined dose tablet formulation. Hence, the proposed method can be employed for quantitative estimation of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride in combined tablet dosage form. Simultaneous equation method can be used to carry out in-vitro dissolution study in combined tablet formulation of these drugs.

Table 4: Regression analysis data and summary of validation parameters for the proposed method

<table>
<thead>
<tr>
<th>Optical Characteristics</th>
<th>Salbutamol Sulphate</th>
<th>Ambroxol Hydrochloride</th>
<th>Cetirizine Hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ&lt;sub&gt;max&lt;/sub&gt; (nm)</td>
<td>276</td>
<td>244</td>
<td>230</td>
</tr>
<tr>
<td>Linearity range (µg/ml)</td>
<td>10-100</td>
<td>2-18</td>
<td>2-20</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = 0.006x + 0.016</td>
<td>y = 0.025x - 0.002</td>
<td>y = 0.032x + 0.011</td>
</tr>
<tr>
<td>(y = mx + c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.006</td>
<td>0.025</td>
<td>0.032</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.016</td>
<td>0.002</td>
<td>0.011</td>
</tr>
<tr>
<td>Correlation coefficient (R)</td>
<td>0.999</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.523</td>
<td>0.450</td>
<td>0.457</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>1.372</td>
<td>1.424</td>
<td>1.386</td>
</tr>
<tr>
<td>Precision (% RSD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability (n=6)</td>
<td>1.6609</td>
<td>1.9596</td>
<td>1.8547</td>
</tr>
<tr>
<td>Intra-day (n=3)</td>
<td>1.2877</td>
<td>1.6166</td>
<td>1.7930</td>
</tr>
<tr>
<td>Inter-day (n=3)</td>
<td>1.4785</td>
<td>1.6238</td>
<td>1.4048</td>
</tr>
</tbody>
</table>

As per the ICH guidelines, the method validation parameters checked were linearity, LOD and LOQ, repeatability, intermediate precision, accuracy (recovery) studies and assay of drug.

Table 5: Repeatability Data for Proposed Method

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance of SAL at 276 nm</th>
<th>Absorbance of AMB at 244 nm</th>
<th>Absorbance of CET at 230 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 ppm</td>
<td>0.069</td>
<td>0.043</td>
<td>0.076</td>
</tr>
<tr>
<td>2</td>
<td>10 ppm</td>
<td>0.070</td>
<td>0.041</td>
<td>0.074</td>
</tr>
<tr>
<td>3</td>
<td>10 ppm</td>
<td>0.072</td>
<td>0.041</td>
<td>0.073</td>
</tr>
<tr>
<td>4</td>
<td>10 ppm</td>
<td>0.070</td>
<td>0.042</td>
<td>0.072</td>
</tr>
<tr>
<td>5</td>
<td>10 ppm</td>
<td>0.071</td>
<td>0.041</td>
<td>0.074</td>
</tr>
<tr>
<td>6</td>
<td>10 ppm</td>
<td>0.069</td>
<td>0.042</td>
<td>0.073</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.070</td>
<td>0.042</td>
<td>0.074</td>
</tr>
<tr>
<td>SD</td>
<td>0.001169</td>
<td>0.00081</td>
<td>0.00137</td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td>1.6609</td>
<td>1.9596</td>
<td>1.8547</td>
<td></td>
</tr>
</tbody>
</table>

SD: Standard deviation, RSD: Relative Standard deviation

Table 6: Intra-day and inter-day precision data of SAL, AMB, and CET

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount Labeled [200 mg Tablet]</th>
<th>Percentage obtained (n=3)</th>
<th>SD intraday</th>
<th>Interday</th>
<th>% RSD intraday</th>
<th>Interday</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAL</td>
<td>2 mg</td>
<td>98.24</td>
<td>99.43</td>
<td>1.2656</td>
<td>1.4472</td>
<td>1.2877</td>
</tr>
<tr>
<td></td>
<td>2 mg</td>
<td>99.57</td>
<td>96.56</td>
<td>1.4787</td>
<td>1.6158</td>
<td>1.6166</td>
</tr>
<tr>
<td></td>
<td>2 mg</td>
<td>97.04</td>
<td>97.67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>98.28</td>
<td>97.89</td>
<td></td>
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<tr>
<td>AMB</td>
<td>7.5 mg</td>
<td>99.46</td>
<td>98.29</td>
<td>1.5800</td>
<td>1.6158</td>
<td>1.6166</td>
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<tr>
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<td>7.5 mg</td>
<td>96.36</td>
<td>98.89</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>Mean</td>
<td>97.38</td>
<td>101.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CET</td>
<td>5 mg</td>
<td>99.67</td>
<td>96.78</td>
<td>1.7526</td>
<td>1.3757</td>
<td>1.7930</td>
</tr>
<tr>
<td></td>
<td>5 mg</td>
<td>96.24</td>
<td>97.54</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Mean</td>
<td>97.75</td>
<td>97.92</td>
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</tbody>
</table>

SD: Standard deviation, RSD: Relative Standard deviation

Table 7: Recovery studies

<table>
<thead>
<tr>
<th>Salbutamol Sulphate (µg/ml)</th>
<th>Ambroxol Hydrochloride (µg/ml)</th>
<th>Cetirizine Hydrochloride (µg/ml)</th>
<th>Salbutamol Sulphate % recovery ± S. D (n=3)</th>
<th>Ambroxol Hydrochloride % recovery ± S. D (n=3)</th>
<th>Cetirizine Hydrochloride % recovery ± S. D (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>4</td>
<td>6</td>
<td>97.44±2.01</td>
<td>96.67±1.53</td>
<td>98.63±0.76</td>
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<tr>
<td>40</td>
<td>8</td>
<td>10</td>
<td>100.33±1.12</td>
<td>95.43±0.41</td>
<td>99.79±0.25</td>
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<tr>
<td>60</td>
<td>12</td>
<td>14</td>
<td>96.80±1.45</td>
<td>98.80±1.15</td>
<td>101.5±1.29</td>
</tr>
</tbody>
</table>

SD: Standard deviation
Table 8: Assay of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Salbutamol Sulphate</th>
<th>Ambroxol Hydrochloride</th>
<th>Cetirizine Hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount Present (mg)</td>
<td>2</td>
<td>7.5</td>
<td>5</td>
</tr>
<tr>
<td>(200 mg Tablet)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>% Drug Content ± SD</td>
<td>97.37 ± 1.885</td>
<td>94.51 ± 0.828</td>
<td>99.75 ± 1.324</td>
</tr>
<tr>
<td>% RSD</td>
<td>1.9364</td>
<td>0.8679</td>
<td>1.3278</td>
</tr>
</tbody>
</table>

SD: Standard deviation, RSD: Relative Standard deviation

CONCLUSION

The developed spectroscopic method i.e. simultaneous equation method is found to be simple, sensitive, accurate and precise and can be used for routine analysis of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride in combined tablet dosage form. The developed method was validated as per ICH guidelines. The results demonstrated that simultaneous equation method by spectrophotometer could be a useful technique for estimation of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride in combined tablet dosage form and cost of analysis is less as compared to RP-HPLC, HPTLC, RP-UPLC and LC-MS method. Hence simultaneous equation method can be conveniently used for routine quality control analysis of SAL, AMB HCl and CET HCl in its combined pharmaceutical tablet formulations.

ACKNOWLEDGEMENT

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CONFLICT OF INTERESTS

The authors declare that they do not have any financial and personal relationships with other people or any other organizations that could inappropriately influence this research work.

ABBREVIATIONS

SAL: Salbutamol Sulphate
CET HCl: Cetirizine Hydrochloride
AMB HCl: Ambroxol Hydrochloride
RSD: Relative Standard Deviation
SD: Standard Deviation

REFERENCES


