DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHODS FOR SIMULTANEOUS ESTIMATION OF SALBUTAMOL SULPHATE AND DOXOPHYLLINE IN COMBINED SOLID DOSAGE FORM

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ABSTRACT

Objective: Salbutamol sulphate (SBS) and doxophylline (DOX) was used for the treatment of asthma and bronchitis. In the present study, two simple, accurate, precise, reproducible and economical UV-spectroscopic methods (A and B) for simultaneous estimation of SBS and DOX in tablet dosage form have been developed.

Methods: In the present study the simultaneous estimation of SBS and DOX was carried out by two methods. Method A employs solving of simultaneous equations based on the measurement of absorbance at two wavelengths, 272 nm and 276 nm which are the λmax values of SBS and DOX respectively in phosphate buffer (pH 7.4). Method B is based on the principle of Q-Analysis where in, absorbance was measured at 225 nm (iso-absorptive point, λi) and 276 nm (λmax of DOX, λλ) in phosphate buffer (pH 7.4).

Results: Both SBS and DOX shows linearity at all the selected wavelengths and obeys beae’s law in the concentration range of between 0.2-1.6µg/ml and 0.1-3.5µg/ml at 276 nm; 0.2-1.6 µg/ml and 0.1-4.5 µg/ml at 272 nm and 0.2-2.0µg/ml and 0.2-3.5µg/ml at iso-absorptive point 225 nm. Recovery studies for SBS and DOX were performed and the percentage recovery for both the drugs was obtained in the range of 97.45-98.63% (Method A) and 97.49-98.87 % (Method B) confirming the accuracy of the proposed method.

Conclusion: Both the methods showed good reproducibility and recovery with % RSD less than 2. Statistical validation of the data shows that the proposed methods can be successfully applied for the routine analysis of drugs in commercial tablets. Hence, it could be used in the analysis of laboratory samples and marketed formulations containing these two drugs in combined dosage form without the interference of common excipients.

Keywords: Simultaneous equation method, Q-absorbance ratio method, Salbutamol, Doxophylline

INTRODUCTION

SBS is chemically (RS)-2-(hydroxymethyl)-4-{1-hydroxy-2-[(2-methyl-2-propenyl)-amino]ethyl} phenol sulfate (2:1) (fig. 1). It is β2-adrenergic receptor agonist used for the relief of bronchial asthma and chronic obstructive pulmonary disease. Selective β2-adrenergic receptor stimulant that causes the relaxation of the smooth muscles through the increase of the intracellular cyclic adenosine monophosphate (cAMP) by stimulating the β2 adrenergic receptors. DOX inhibits phosphodiesterase (PDE IV) activities with consequent increase of cyclic AMP that determines relaxation of smooth muscle. DOX appears to have decreased affinity toward adenosine A1 and A2 receptors which may account for the better safety profile of the drug. DOX does not interfere with calcium influx into the cells or antagonize calcium channel blockers. Unlike aminophylline it has low secretagogue activity and suitable for asthmatic patients with peptic ulcer disease. DOX is used in the treatment of bronchial asthma, chronic obstructive pulmonary disease (COPD) and chronic bronchitis [5-6].

SBS is official in European Pharmacopoeia [7], which describes a microtitrimetric, conductometric, HPLC, UV-spectrophotometry and immunoaffinity-chromatography. Some analytical methods for quantitative determination of doxophylline in pharmaceutical formulations are described in literature are some of reported methods used for analysis. UV-spectrophotometry estimation of doxophylline in biological samples, plasma and serum [13-19].

Extensive literature survey has revealed that no UV spectroscopic method is reported for simultaneous determination of SBS and DOX in combine dosage form. The UV spectrophotometric analysis is often preferred in quality control testing and ordinary laboratories due to its broader availability, suitability and ease of use. Therefore, in the present work successful attempt has been made to estimate both the drugs simultaneously by two simple UV spectrophotometric methods i.e. simultaneous equation method and Q-absorbance ratio method in combine dosage form. The proposed methods were optimized and validated as per ICH guidelines [20-26].

Fig. 1: Structure of SBS [27]
Absorbance measurements were made on double beam UV-Visible spectrophotometer, model 1800, Shimadzu, Japan, with software UV Probe 2.10 and 1 cm matched quartz cells.

**Preparation of standard stock solution**

Standard stock solutions (20 µg/ml) of both SBS and DOX were prepared separately by dissolving accurately weighed (2.0 mg) quantity of pure SBS and DOX in 100 ml volumetric flask and diluting up to the mark with phosphate buffer (pH 7.4) to get working standard solution of each containing 20µg/ml of both SBS and DOX.

**Preparation of working standard solutions**

From the above stock solution desired concentrations were prepared by transferring specific volume to separate 10 ml volumetric flasks and volume was made up to 10 ml with phosphate buffer.

**Determination of isoabsorptive point and absorption maxima**

By appropriate dilution of standard solutions of SBS and DOX with phosphate buffer (pH 7.4), solutions containing 10 µg/ml of both drugs were scanned separately in the range of 200-400 nm against phosphate buffer (pH 7.4) as blank. The overlaying spectrum was also obtained to determine isoabsorptive point and wavelength of maximum absorbance \( \lambda_{\text{max}} \) of both the drugs.

**Methods**

**Simultaneous equation method (Method A)**

1µg/ml solutions of SBS and DOX were prepared separately in phosphate buffer (pH 7.4) and the solutions were scanned against blank in the entire UV range to determine the \( \lambda_{\text{max}} \) values. Clear peaks were observed at 272 nm for SBS and 276 nm for DOX. Hence these wavelengths were chosen as \( \lambda_{\text{max}} \) values for each drug respectively (fig. 1). Standard solutions of SBS and DOX in the concentration range 0.1-5µg/ml were prepared in the phosphate buffer (pH 7.4) and the absorbance of these solutions was measured at 272 nm and 276 nm. Calibration curves were plotted to verify the Beer’s law and the absorptivity values calculated at the respective wavelength for both the drugs. The absorptivity values were reported in table 1. [21-24]

The concentration of two drugs in mixture was calculated by using the following equations:

\[
Q = \frac{Q_1}{Q_2} = \frac{A_1}{A_2}
\]

Where, \( Q_1 \) and \( Q_2 \) are the concentrations of SBS and DOX measured in gm/100 ml in sample solutions, \( A_1 \) and \( A_2 \) are absorbance of mixture at selected wavelengths 272 nm and 276 nm respectively.

**Absorbance ratio method/Q-analysis (method B)**

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that for a substance, which obey Beer’s law at all wavelength, the ratio of absorbance at any two wavelengths in constant value independent of concentration or path length. E. g. two dilutions of the same substance give the same absorbance ratio \( A_1/A_2 \). In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in admixture by the absorbance ratio method, absorbance are measured at two wavelengths, one being the \( \lambda_{\text{max}} \) of one of the component (\( \lambda_1 \)) and the other being wavelength of equal absorptivity of two components (\( \lambda_2 \)) i.e. an isoabsorptive point.

A series of standard solutions of SBS and DOX in the concentration range of 0.1-5 µg/ml were prepared in phosphate buffer and the absorbance of these solutions was measured at 225 nm (isoabsorptive point) and 276 nm (\( \lambda_{\text{max}} \) of DOX) (fig. 1). Calibration curves were plotted to verify the Beer’s law and the absorptivity values calculated at the respective wavelength for both the drugs. The absorptivity values were reported in table 1. [21-24]

The concentration of two drugs in mixture was calculated by using the following equations:

\[
C_x = \frac{A_2 - a_1 x_1 - a_2 x_2}{a_2 y_1 - a_1 y_2}, \quad \text{and} \quad C_y = \frac{A_1 x_2 - A_2 x_1}{a_2 y_1 - a_1 y_2}
\]

Where, \( C_x \) and \( C_y \) are the concentrations of SBS and DOX.

**Validation of proposed method (Method A and B)**

The method was validated according to ICH guidelines for validation of analytical procedures in order to determine linearity, sensitivity, accuracy and precision for each analyte [20].

**Linearity**

Appropriate dilutions of working standard solutions for SBS and DOX were prepared in the concentration range of 0.1-5µg/ml and 0.1-3µg/ml, respectively and analyzed as per the developed methods A and B. Calibration curves were generated and the linearity was evaluated by the least square regression method. The results are reported in table 1, 5.

**Accuracy (Recovery studies)**

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels according to ICH guidelines. A series of solutions of SBS and DOX at 60%, 100%, and 120% of the standard preparation in the ratio of the formulation were prepared and checked for accuracy by determining the absorbance values at \( \lambda_{\text{max}} \) of 272 nm and 276 nm (Method A) and 225 nm and 276 nm (method B) respectively. To a fixed concentration of the formulation, varying concentrations of pure drug solutions were added and percentage recoveries calculated. The result of the analysis is given in table 2, 3.
**Precision**

Precision is the degree of repeatability of analytical method under normal operational conditions. The precision of the assay was determined by repeatability (intraday) and intermediate (interday) and reported as %RSD for a statistically significant number of replicate measurement. The intermediate precision was studied by comparing the assays on three different days and the results documented as standard deviation and %RSD.

Precision studies were performed in triplicate at three different concentration levels covering the entire linearity range for SBS and DOX. The result of the analysis is given in table 4.

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

The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ of the proposed method were determined by using calibration curve: (table 5)

\[
\text{LOD} = \frac{3.3 \sigma}{s}, \\
\text{LOQ} = \frac{10 \sigma}{s}
\]

Where, \(\sigma\) is mean standard deviation of y-intercepts of regression lines, \(s\) is slope of the standard curve.

**Assay of tablets formulation**

For estimation of drugs in the commercial formulations, twenty tablets containing 400 mg DOX and 4 mg of SBS were weighed and average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drugs, quantity of powder equivalent to 1 mg of SBS and 100 mg of DOX was transferred to 100 ml volumetric flask and dissolved in sufficient quantity of phosphate buffer. It was sonicated for 30 min and volume was made up to obtain a stock solution 10μg/ml of SBS and 1000μg/ml of DOX. This solution was then filtered through whatmann filter paper #42. Further dilutions were made from this stock solution to get required concentration. In method A, the concentration of SBS and DOX was determined by measuring absorbance of sample solutions at 272 nm (\(\lambda_{\text{max}}\) of SBS) and 276 nm (\(\lambda_{\text{max}}\) of DOX) using simultaneous equation. In method B, the concentration of both SBS and DOX was determined by measuring absorbance of sample solutions at 276 nm (\(\lambda_{\text{max}}\) of DOX; \(\lambda_1\)) and 225 nm (isosbestic point of both drugs; \(\lambda_2\)). The results of analysis and statistical validation for the marketed tablet formulation are reported in table 2-4. The results of recovery studies conducted by the addition of different amount of pure drugs at different levels to a tablet solution were found to be satisfactory.

**RESULTS AND DISCUSSION**

The simultaneous equation method is generally used to estimate two absorbing substances (SBS and DOX) each of which absorbs at the wavelength of the other drug. By constructing and placing values in simultaneous equations 3 and 4 the concentration of two drugs was determined. The absorption ratio method generally used to estimate two absorbing substances (SBS and DOX) each of which absorbs at the wavelength of the other by constructing and placing values in absorption ratio equation 5 and 6 to determine the concentration of SBS and DOX.

SBS and DOX exhibited maximum absorption at 272 nm and 276 nm (Method A), so using these wavelengths simultaneous equation method for analysis of SBS and DOX in combine form was developed. For Q-absorption method (Method B) of simultaneous analysis of SBS and DOX in combine form, 225 nm (iso-absorptive point) and 276 nm (\(\lambda_{\text{max}}\) of DOX) was used. Beer’s law were found to be obeyed in the concentration range between 0.2-1.6μg/ml and 0.1 to 3.5μg/ml at 276 nm; 0.2-1.6 μg/ml and 0.1-4.5 μg/ml at 272 nm and 0.2 to 2.0μg/ml and 0.2 to 3.5μg/ml at iso-absorptive point 225 nm for SBS and DOX respectively (Method A and B). Calibration curves were prepared for both the drugs at 276 nm, 272 nm and 225 nm (fig. 4-6, table 1, 5). The overlain UV-absorption spectra of SBS (272 nm) and DOX (276 nm) showed isosbestic point (225 nm) in ethanol is shown in fig. 3. All calibration curve obtained was linear with correlation coefficient \(r^2\) greater than 0.998. Hence the relationship between the concentrations and absorbances of SBS and DOX showed linearity (table 5).

![Fig. 3: UV overlay spectrum of SBS and DOX showing isoabsorptive point](image1.png)

![Fig. 4: Calibration curve of SBS and DOX at 276 nm](image2.png)
The validation parameters were studied on marketed formulation at all the wavelengths for the proposed methods. As per IP, tablets should contain not less than 95.0% and not more than 105.0% of active ingredients of the stated amount. The average % drug content was found to be of 97.98% and 96.89-98.16% for SBS and DOX by Method A and Method B respectively, which was found to be within the acceptance limit with %RSD values less than the limit of 2%. Accuracy was determined by calculating the recovery by standard addition method. Results revealed percentage recovery more than 97.50% with % RSD value within the accepted limit for both the components by both methods at all the three levels of recovery analysis. Hence both the proposed methods were found to be accurate for estimation of SBS and DOX in tablet formulation. Both the methods were subjected for study of repeatability, intraday and interday precision for both the drugs. % RSD values for repeatability, intraday and interday precision were calculated and found to be well below the specified limit of 2% (%RSD<2) indicating good precision in the specified range (table 4). The sensitivity of the proposed methods was determined in terms of limit of detection (LOD) and limit of quantitation (LOQ). LOD values for SBS and DOX were found to be 0.048 and 0.064 μg/ml at 276 nm; 0.048 and 0.013 at 272 nm and 0.015 and 0.057 μg/ml at 225 nm. LOQ values for SBS and DOX were found to be 0.148 and 0.195 μg/ml at 276 nm; 0.158 and 0.042 μg/ml at 272 nm and 0.047 and 0.173 μg/ml at 225 nm. (table 5)

### Table 1: Absorptivity values (A1%, 1 cm) of SBS and DOX for methods A and B i.e at all three (225 nm, 276 nm and 272 nm) wavelengths

<table>
<thead>
<tr>
<th>Concentration of the solution (μg/ml)</th>
<th>Absorptivity, A(1%, 1 cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBS mean absorptivity (n=3)</td>
</tr>
<tr>
<td></td>
<td>225 nm</td>
</tr>
<tr>
<td>0.4</td>
<td>6400</td>
</tr>
<tr>
<td>0.6</td>
<td>6200</td>
</tr>
<tr>
<td>0.8</td>
<td>6237</td>
</tr>
<tr>
<td>1</td>
<td>6400</td>
</tr>
<tr>
<td>1.2</td>
<td>6375</td>
</tr>
<tr>
<td>1.4</td>
<td>6321</td>
</tr>
<tr>
<td>1.6</td>
<td>6231</td>
</tr>
<tr>
<td>Mean</td>
<td>6309.311</td>
</tr>
</tbody>
</table>

Each value represents mean value (n=3)

### Table 2: Statistical parameters for marketed formulation: Doxoril plus 4 (SBS and DOX) by methods A and B

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Drug</th>
<th>Label claim (mg)</th>
<th>Amount found</th>
<th>% Recovery</th>
<th>% R. S. D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method A</td>
<td>SBS</td>
<td>4</td>
<td>3.92</td>
<td>98</td>
<td>1.012</td>
</tr>
<tr>
<td></td>
<td>DOX</td>
<td>400</td>
<td>302.65</td>
<td>98.162</td>
<td>1.315</td>
</tr>
<tr>
<td>Method B</td>
<td>SBS</td>
<td>4</td>
<td>3.88</td>
<td>97</td>
<td>0.915</td>
</tr>
<tr>
<td></td>
<td>DOX</td>
<td>400</td>
<td>378.56</td>
<td>96.89</td>
<td>1.541</td>
</tr>
</tbody>
</table>

Each value is given in mean+% RSD
The UV spectrophotometric simultaneous equation method and without interference of excipients successfully applied for the analysis of pharmaceutical formulations that the methods were linear, accurate and precise and can be validated. From the statistical data, it was found that both the methods were linear, accurate, precise, and reproducible, rapid, and sensitive. The method could be applied successfully and economically for the simultaneous estimation of SBS and DOX in bulk drug and within acceptance limit relative standard deviation (RSD) values were 0.67 and 0.91 for methods A and B, respectively.

CONCLUSION

Two new, simple, sensitive and economical UV spectrophotometric methods were developed for the simultaneous analysis of SBS and DOX in bulk and in pharmaceutical formulations. The developed methods were validated and from the statistical data, it was found that the methods were linear, accurate, precise, and reproducible, rapid, and sensitive. The method could be applied successfully and economically for the simultaneous estimation of SBS and DOX in laboratory samples for efficient data generation and for combination formulations of these two drugs in the future.

REFERENCES


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