Simultaneous determination of Salbutamol sulphate and doxophylline in tablets by reverse phase liquid chromatography

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ABSTRACT

A simple, isocratic reverse phase liquid chromatographic method has been developed and validated for simultaneous determination of Salbutamol Sulphate (SBS) and Doxophylline (DOX) present in tablet dosage forms. Chromatographic separation achieved isocratically on Luna C18 column (5 µm, 150mm x 4.60mm) and Acetonitrile/KH2PO4 buffer (40:60, v/v, pH 3.0 with OPA) as mobile phase, at a flow rate of 0.5 ml/min. Detection was carried out at 225 nm. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the ICH guidelines. The retention time for SBS and DOX was found to be 3.14±0.015 and 5.73±0.06 min respectively. Linearity for SBS and DOX was in the range of 4-20 µg/ml and 400-2000 µg/ml respectively. The mean recoveries obtained for SBS and DOX were 98.54 and 98.79% respectively and RSD was less than 2. The correlation coefficients for all components are close to 1. The relative standard deviations for three replicate measurements in three concentrations of samples in tablets are always less than 2%. Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of SBS and DOX in tablets.

Key Words: Salbutamol Sulphate, Doxophylline, HPLC, UV detection.

INTRODUCTION

Salbutamol sulphate (SBS) chemically, bis [(1RS)-2-[(1, 1-dimethylethyl) amino]-1-[4-hydroxy-3-(hydroxymethyl) phenyl] ethanol] sulphate (Figure 1A), is a β2-adrenergic receptor agonist used for the relief of broncho-spasms in conditions such as asthma and chronic obstructive pulmonary disease [1-3]. Selective β2-adrenoceptor stimulant that causes the relaxation of the smooth muscles through the increase of the intracellular cyclic adenosine monophosphate (cAMP) due to this, bronchial and uterine muscles get relaxed, the peripheral vessels are dilated and heart rate increases[4]. Activation of the β2 adreno-receptors opens ATPase channels and
drives potassium from the extra cellular to the intracellular space [5]. This both decreases extracellular hyperkalaemia and increases intracellular potassium, so decreasing the chance of arrhythmia [6]. Doxophylline (DOX) chemically, 7(1, 3 dioxolone-2-yl methyl) theophylline (Figure 1B) is a bronchodilator xanthine drug which has the therapeutic properties of theophylline with lower incidence of side-effects [7]. Doxophylline do not affect gastric acid secretion; either in vivo or in-vitro; unlike theophylline. The lack of side effects with doxophylline indicates that the drug can be used safely & effectively in the treatment of COLD [8]. Doxophylline inhibits Phosphodiesterase (PDE IV) activities with consequent increase of cyclic AMP that determines relaxation of smooth musculature. Doxophylline appears to have decreased affinities toward adenosine A1 & A2 receptors which may account for the better safety profile of the drug. Doxophylline does not interfere with calcium influx into the cells or antagonize calcium channel blockers [9]. Unlike aminophylline it has low secretagogue activity & suitable for asthmatic patients with peptic ulcer disease [10]. Doxophylline (DOX) is used in the treatment of bronchial asthma, chronic obstructive pulmonary disease (COPD) and chronic bronchitis.

Salbutamol sulphate (SBS) is official in European Pharmacopoeia [11], which describes a potentiometric titration in non-aqueous medium, British Pharmacopoeia [12] and Indian Pharmacopoeia [13]. Salbutamol sulphate alone or in combination with other drugs is reported to be estimated by HPLC in pharmaceutical dosage form [14-19], plasma [20], titrimetric and spectrophotometric [21], TLC [22], Microtitrimetric [23], Conductometric [24], UV & HPLC [25], UV-spectrophotometry [26-35] and Immunoaffinity-chromatography [36]. Some analytical methods for quantitative determination of doxophylline in pharmaceutical formulations are described in literature like UV-Spectrophotometry [37], in biological samples, plasma [38] and serum [39-40].

Extensive literature survey reveals that no RP-HPLC method is reported for simultaneous determination of SBS and DOX in tablet dosage form. Fixed dose combination containing doxophylline (400 mg) and salbutamol (4 mg) is available in tablet form in the market. Therefore, an attempt was made to develop a new, rapid and sensitive RP-HPLC method for the simultaneous determination of SBS and DOX in tablet dosage form. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines [41], which is mandatory also.

![Figure1. Chemical structures of (A) Salbutamol (B) Doxophylline](image)

**MATERIALS AND METHODS**

**Instrumentation**

Liquid chromatographic system from Shimadzu (LC-20AT) comprising of manual injector, double reciprocating plunger pump LC-20ATVp for constant flow and constant pressure delivery

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Reagents and chemicals
Analytically pure sample of SBS and DOX was kindly supplied by Aristo Pharmaceuticals Limited Bhopal, India. Acetonitrile, Potassium dihydrogen phosphate and concentrated orthophosphoric acid was of HPLC grade supplied by Merck Ltd., India. The pharmaceutical dosage form used in this study was Doxoril Plus 4 (Mecleods Pharmaceutical Ltd Mumbai) tablets containing 400 mg doxophylline and 4 mg salbutamol were purchased from the local drug market. Triple distilled water was generated in house.

Chromatographic condition
The isocratic mobile phase consisted of Acetonitrile/KH$_2$PO$_4$ buffer (pH 3.0 with OPA) in the ratio of (40:60 v/v), flowing through the column at a constant flow rate of 0.5 ml/ min. A Luna C$_{18}$ column (5 µm, 150mm x 4.60mm) was used as the stationary phase. Although the SBS and DOX have different λ$_{max}$ viz 225, 277 and 221, 274 nm respectively, but considering the chromatographic parameter, sensitivity and selectivity of method for two drugs 225 nm was selected as the detection wavelength for UV-PDA detector.

Standard preparation
Standard stock solution
Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 100 ml of diluent which was a mixture of ACN and KH$_2$PO$_4$ buffer in the ratio of 40:60 (pH 3.0) to get concentration of 1000 µg/ml.

Working standard solution
Working standard solutions were prepared by taking dilutions ranging from 4-20, 400-2000 µg/ml for SBS and DOX respectively.

Sample preparation
Twenty tablets of Doxoril Plus 4 were weighed and ground to a fine powder. An accurately weighed powder sample equivalent to 400 mg of DOX and 4 mg SBS were transferred to 100 ml of volumetric flask. Drug was extracted with three 20ml quantities of mixture of diluent. The flask was sonicated for about 10 min to solubilize the drug and the volume was made up to mark and filtered through whatmann filter paper No. 42, finally different concentrations of tablet sample were prepared by serial dilution technique.

RESULTS AND DISCUSSION
Chromatography
The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting of acetonitrile/KH$_2$PO$_4$ buffer (40:60, v/v, pH 3.0 with OPA) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5/min were studied. A flow rate of 0.5 ml/min gave an optimal signal to noise ratio with a reasonable separation time. Using a reversed-phase C18 column, the retention times for SBS and DOX was found to be 3.14±0.015and 5.73±0.06 min respectively. Total time of analysis was less than 6 min. The maximum absorption of SBS and DOX together as detected at 225 nm and this wavelength was chosen for the analysis. The concentration of SBS is low, hence the AUC is not noticeable in comparison to DOX, and therefore its peak is not clearly visible on the same scale in
chromatogram (Figure 2). By minimizing the scale, the peak corresponds to SBS is clearly visible (Figure 3).

**Figure 2. Representative chromatogram of Salbutamol sulphate and Doxophylline**

**Figure 3. Representative chromatogram of Salbutamol sulphate and Doxophylline (by minimizing the scale)**

**System suitability**
System suitability parameters such as number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in Table 1. The number of theoretical plates for SBS and DOX were 1802.67 and 7197.67 respectively.

**Table1. System suitability parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Salbutamol</th>
<th>Doxophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time*</td>
<td>3.14±0.015</td>
<td>5.73±0.06</td>
</tr>
<tr>
<td>No. of theoretical plate*</td>
<td>1802.67±26.469</td>
<td>7197.67±64.45</td>
</tr>
<tr>
<td>Tailing factor*</td>
<td>1.44±0.020</td>
<td>1.11±0.02</td>
</tr>
<tr>
<td>HETP*</td>
<td>0.14±0.003</td>
<td>0.03±0.001</td>
</tr>
<tr>
<td>Linearity range</td>
<td>4-20 µg/ml</td>
<td>400-2000 µg/ml</td>
</tr>
</tbody>
</table>

* Each value is the Mean ± S.D of six determinations
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Linearity
SBS and DOX showed a linearity of response between 4-20 and 400-2000 µg/ml respectively. The linearity was represented by a linear regression equation as follows.

\[ Y \,(\text{SBS})= 380847 \text{conc.} + 296419 \quad (r^2=0.9997) \]
\[ Y \,(\text{DOX})= 357757 \text{conc.} + 277353 \quad (r^2=0.9999) \]

Accuracy
Accuracy of the method was calculated by recovery studies at three levels by standard addition method (Table 2). The mean percentage recoveries obtained for salbutamol and doxophylline were 98.54 and 98.79%, respectively.

Table 2. Result of recovery studies with statically evaluation

<table>
<thead>
<tr>
<th>Serial. No.</th>
<th>Conc. of drug in preanalyzed samples (µg/ml)</th>
<th>Std. drug sol. Added (µg/ml)</th>
<th>Recovered amount* (µg/ml)</th>
<th>% Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBS</td>
<td>DOX</td>
<td>SBS</td>
<td>DOX</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>400</td>
<td>8</td>
<td>400</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>800</td>
<td>8</td>
<td>400</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>1200</td>
<td>8</td>
<td>400</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean of Nine determinations (3 replicates at 3 concentration level)

Repeatability
Five dilutions in three replicates were analyzed in same day for repeatability and results were found within acceptable limits (RSD < 2) as shown in Table 3.

Intermediate Precision
Five dilutions in three replicates were analyzed on two different days and by two analysts for day to day and analyst to analyst variation and results were found within acceptable limits (RSD < 2) as shown in Table 3.

Table 3. Result of precision

<table>
<thead>
<tr>
<th>Validation Parameter</th>
<th>Percentage Mean ± S.D*, (n=15)</th>
<th>Percentage RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBM</td>
<td>DOX</td>
</tr>
<tr>
<td>Repeatability</td>
<td>97.620±0.255</td>
<td>98.950±1.013</td>
</tr>
<tr>
<td>Intermediate precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day to Day</td>
<td>96.553±0.520</td>
<td>98.507±1.531</td>
</tr>
<tr>
<td>Analyst to Analyst</td>
<td>98.645±0.120</td>
<td>98.68±1.22</td>
</tr>
</tbody>
</table>

*Mean of fifteen determinations (3 replicates at 5 concentration level)

Robustness
As per ICH norms, small, but deliberate variations, by altering the pH or concentration of the mobile phase were made to check the method’s capacity to remain unaffected. The change was made in the ratio of mobile phase, instead of Acetonitrile/KH₂PO₄ buffer (40:60, v/v, pH 3.0 with OPA), Acetonitrile/KH₂PO₄ buffer (45:55, v/v, pH 3.0 with OPA) was used as a mobile phase. Results of analysis were summarized in Table 4.

Stability of sample solution
The sample solution injected after 12 hrs do not show any appreciable change. Results are shown in Table 5.
Table 4. Results of robustness

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Validation Parameter</th>
<th>% Mean*</th>
<th>S.D.</th>
<th>% R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBS</td>
<td>DOX</td>
<td>SBS</td>
<td>DOX</td>
</tr>
<tr>
<td>1</td>
<td>Robustness</td>
<td>99.70</td>
<td>100.2</td>
<td>0.21</td>
</tr>
</tbody>
</table>

*Mean of six determinations

Table 5 Stability data of SBS and DOX

<table>
<thead>
<tr>
<th>Hours</th>
<th>SBS 4 µg/ml</th>
<th>DOX 8µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>389873 ±0.21</td>
<td>2708862±0.63</td>
</tr>
<tr>
<td>6</td>
<td>388058 ±0.32</td>
<td>27089342±0.75</td>
</tr>
<tr>
<td>12</td>
<td>388271 ±0.25</td>
<td>27885432±0.83</td>
</tr>
</tbody>
</table>

Tablet analysis

Content of SBS and DOX found in the tablets by the proposed method are shown in Table 6. The low values of R.S.D. indicate that the method is precise and accurate.

Table 6 Result of marketed tablet analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Doxoril Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBM</td>
<td>DOX</td>
</tr>
<tr>
<td>Mean % estimated</td>
<td>100.31</td>
</tr>
<tr>
<td>Standard deviation(S.D.)</td>
<td>1.46</td>
</tr>
<tr>
<td>% Coefficient of variation</td>
<td>1.45</td>
</tr>
<tr>
<td>*Standard error (SE)</td>
<td>0.34</td>
</tr>
</tbody>
</table>

*Mean of nine determinations (3 replicates at 3 concentration level)

CONCLUSION

RP-HPLC method was developed and validated for simultaneous estimation of salbutamol sulphate and doxophylline in tablet dosage form. The developed method is suitable for the identification and quantification of binary combination of salbutamol sulphate and doxophylline. A high percentage of recovery shows that the method can be successfully used on a routine basis. Proposed method is simple, fast, accurate, precise and sensitive and could be applied for quality and stability monitoring of salbutamol sulphate and doxophylline combination.

REFERENCES