DEVELOPMENT AND VALIDATION UV-VISIBLE SPECTROPHOTOMETRIC METHOD OF ESTIMATION OF TERBUTALIN SULPHATE IN BULK DRUG BY USING NINHYDRIN.

Shital Godse¹, Gajanan A. Vaishnav², Sushama Vaishnav-Hota², Angadi S.S.², Patil S. J.²

¹Smt. Kashibai Navale College of Pharmacy, Kondhwa - Pune 411048, Maharashtra State, INDIA.
²Department of Pharmaceutical Analysis, Yash Institute of Pharmacy, South City, Waluj Road, Aurangabad 431 134, Maharashtra State, INDIA.

ABSTRACT
An accurate, precise and simple spectrophotometric method for the determination of Terbutaline sulphate based on the formation of a yellow color product with Ninhydrin in the presence of sodium bicarbonate with an λ_{max} at 346 nm. The Reaction heating temperature is 80°C in 30 min. The calibration curve is linear over the range of 10-60 µg/ml and the regression equation y=0.0263x+0.2 with a regression coefficient (r) of 0.98 (n=10). The limit of Detection (LOD) and limit of Quantification (LOQ) calculated as per ICH guidelines are 18.15µg/ml and 55µg/ml, respectively.

KEYWORDS: Terbutaline sulphate, UV-Spectrophotometer, Ninhydrin.

INTRODUCTION
Bronchial asthma is characterized by hyper responsiveness of trachobranchial smooth muscle to a verity of stimuli, resulting a narrowing of air tubes, often accompaning by increased secretion, muscle edema and muscle plugging, symptom include dysporea, wheezing, cough and may limitation of activity. Terbutaline sulphate it is used in Bronchial asthma¹ is a (RS)-2-(tert-butylamino)-1-(3,5 dihydroxyphenyl) ethanol sulphate, is a beta2 bronchodilator.² All ammonia, primary amine and secondary amine are detected by ninhydrin. Ammonia and primary amine produce purple color with ninhydrin and form purple color product. And
secondary amine produce yellow color product, imines such as pipecolic acid and prolin, arginine, asparagin the indol ring of tryptophan, the sulfhydrl group of cystin and guanine and cyanide this all are secondary amine are also react with ninhydrin and produce yellow color complex. \[3\] Reaction of secondary amine with ninhydrin used for detection of herbal constituent\[4,5\], detection of basic organic drug and there metabolites in urine.\[6\] And ninhydrin based forensic investigation.\[7\] It is officially in I.P.\[8\], B.P.\[9\], U.S.P\[10\], E.P.\[11\], at present, different methods to determine Terbutalin sulphate have been employed, liquid chromatography\[12\], HPTLC\[13\], Gas chromatography, Mass Spectrometry\[14\], Flourimetry.\[15\]

\[
\text{HO-CH}_{2}\text{-CH}_{2}-\text{N}^{+}\text{CH}_{3}\text{-CH}_{2}\text{-CH}_{3}\cdot \text{H}_{2}\text{SO}_{4} \]

**MATERIAL AND METHOD**

**Apparatus**

The spectral measurements were carried out by using a shimadzu UV-Visible spectrophotometer (model no.1800 japan) with matched quartz cell of 1cm optical path length was used to measure absorbance, weighing balance, vortex mixer etc.

**Reagent and chemical**

Terbutalin sulphate bulk drug (gift sample from shreya life science Ltd Aurangabad, Ninhydrin power purchased from DEEPA Chemical Industries, (Aurangabad, Maharashtra) all reagent and solvent A.R. grade from S.D. fine chemical, Mumbai, distilled water.

**Experimental Method**

**Preparation of standard stock solution**

1) Preparation of standard stock solution

stock solution (A): Accurately weight 100 mg Terbutalin was dissolved in Freshly prepared Distilled Water in 100 ml volumetric flask and volume was made up to the mark with the Distilled water i.e.1000µg/ml.

Stock solution (B): from these stock solution (A) pipette out 10ml of solution and dilute up to 100ml with Distilled Water to prepare (100µg/ml) was stock 2. from the stock 2 solution
pipette out 1 ml, 2 ml, 3 ml, 4 ml, 5 ml dilute each up to 10 ml respectively. to prepare 10, 20, 30, 40, 50….100 µg/ml solution.

2) **Preparation of 0.4% Ninhydrin:** Accurately weight 400 mg of Ninhydrin and dissolved it in 100 ml of Distilled Water.

3) **Preparation of Sodium bicarbonate:** Accurately weight 25 gm of sodium bicarbonate and dissolved in 100 ml of distilled water.

**Preparation of calibration curve.**

**Scanning of Terbutalin sulphate by UV- Spectrophotometery in Ninhydrin (0.4%).**

Fresh aliquots from standard stock solution was pipette out and separately and add 1 ml of Ninhydrin and 1 ml of sodium bicarbonate and make final volume up to 10 ml with distilled water to got final concentration range in between 10-50 µg/ml. The solution of Terbutalin in bulk drug at highest dilution i.e. (40µg/ml) were scanned in the spectrum mode for 400 and 200 nm wavelength range \( \lambda_{\text{max}} \) at 346nm was found to be as fig.1.

![Figure 1 Spectra of Terbutalin Sulphate](image-url)

**METHOD VALIDATION**

**Linearity**

Calibration Curve was constructed by plotting absorbance against concentration for Terbutalin sulphate. Beer's law limits, molar absorptive, linear regression equations and
sandell sensitivity were calculated for drug (Table 1). The correlation coefficient was found to be 0.982 indicating excellent linearity over beer's law limit.

Table 1: Observation table for calibration curve of Terbutalin sulphate.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Concentration range(µg/ml)</th>
<th>Absorbance(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.36</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.66</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>1.01</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>1.39</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>1.61</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>1.92</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>2.01</td>
</tr>
<tr>
<td>8</td>
<td>80</td>
<td>2.14</td>
</tr>
<tr>
<td>9</td>
<td>90</td>
<td>2.48</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>2.90</td>
</tr>
</tbody>
</table>

Figure 2 Calibration curve of Terbutalin sulphate at 346nm.

Sensitivity

The detection limit (LOD) and limit of quantification (LOQ) were calculated using the following equation according to ICH.\(^{[16]}\) The results obtained are compiled in Table 2.

LOD=3.3σ/s
LOQ=10σ/s

Where, σ=the std. deviation of replicate blank response.
S=the slop of the calibrated curve.
**Precision**

Precision of the method were calculated in the term of intermediate precision (intra-day and inter-day),\(^4\) two concentration of Terbutalin sulphate were analyzed in three replicates during the same day (intra-day precision). and two days (inter-day precision). The RSD value of intra-day and inter-day studies showed good precision. 6 dilution of 40 µg/ml concentration of Terbutalin sulphate were prepared and assay by proposed method An acceptance criteria of % RSD less than 2.5% was used.\(^{17}\) The result obtained are compiled in Table 3.

**Accuracy**

Accuracy of an analytical method is the closeness between the reference value and the found value.\(^{18}\) Accuracy was evaluated as %RE between the measured concentration and actual concentration for Terbutalin sulphate. The results obtained are compiled in Table 3.

**Recovery Study**

The accuracy and validity of the proposed method were further ascertained by performing recovery studies. 50µg/ml solution was spiked with 100µg/ml at three concentration level (80, 100 and 120%). 6 dilution of 45, 50 and 55 µg/ml concentration of Terbutalin sulphate were prepared and assay by proposed method An acceptance criteria of % Recovery between 95-105% was used.\(^{17}\) The result obtained are compiled in Table no 3.

**RESULT AND DISCUSSION**

Now a day’s Ninhydrin is used to detect ammonia, primary and secondary amine. All primary amine and ammonia produce purple color with ninhydrin and secondary amine produce yellow color complex with Ninhydrin.\(^{19}\) The present author found that ninhydrin form yellow color product with Terbutalin sulphate in the present of saturated solution of NaHCO\(_3\) and without employing any salt. All absorbance measured were \(\lambda_{\text{max}}\) at 346 nm. Reaction between ninhydrin and Terbutalin sulphate did not give any color product in the absence of NaHCO\(_3\). four parameter Volume of NaHCO\(_3\), concentration of Ninhydrin and heating time were optimized. Different concentration of NaOH, Acetate buffer (Ph 5.5) were used to study the effect of Ph on the reaction between ninhydrin and Terbutalin sulphate. Since no color was formed the reaction is specific in bicarbonate medium. When more than 1 ml volume of NaHCO\(_3\) and heating time increased violet color produce but it is unstable and turn to stable yellow color. All measurement was made at 346 nm against reagent blank.
Optimization of the Reaction conditions\textsuperscript{[20, 21]}

1. Effect of heating time

The effect of heating time on the absorption intensity was studied. Different heating times in a boiling water bath (at 100°C) from 5 min until 1 hr. were tried. It was found that heating for about 30 min (at 100°C) gave maximum absorption intensity (fig 1).

![Figure 3. Effect of heating time on the reaction of Ninhydrin with 40µg/ml Terbutalin.](image)

2 Effect of pH

The effect of pH on the reaction was studied over the pH 9. It was found that Sodium bicarbonate of pH 9 was the optimum for Terbutalin. Different volume of buffer ranging from 0.5 ml to 3 ml was tried.

![Figure 4. Effect of pH on the reaction of Ninhydrin with 40µg/ml Terbutalin.](image)
3 Effect of Ninhydrin concentration
Different volumes of Ninhydrin ranging from 1 ml to 5 ml were tried. Maximum color intensity was increasing with the volume of Ninhydrin.

![Figure 5. Effect of volume of ninhydrin on the reaction with 40µg/ml Terbutalin.](image)

4 Effect of solvent
Water, acetone and acetonitril were tried. Water gave the best results for Terbutalin sulphate.

5. Stoichiometric Relationship
The stoichiometry of the reaction product formed between the cited drug and ninhydrin was investigated by applying the continuous variation method. The molar ratio of ninhydrin to drug (2:1) (reagent: drug).

![Figure 6. Continuous variation plot for Terbutalin sulphate.](image)
Table no 2. Regression parameter and sensitivity value.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Linearity range(µg/ml)</td>
<td>10-100</td>
</tr>
<tr>
<td>2</td>
<td>λmax(nm)</td>
<td>346</td>
</tr>
<tr>
<td>3</td>
<td>Ɛ(L/mol/cm)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Sandell sensitivity(µg/ml)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Slope(b)</td>
<td>0.0263</td>
</tr>
<tr>
<td>6</td>
<td>Intercept(a)</td>
<td>0.2</td>
</tr>
<tr>
<td>7</td>
<td>LOD µg/ml</td>
<td>18.15µg/ml</td>
</tr>
<tr>
<td>8</td>
<td>LOQ µg/ml</td>
<td>55µg/ml</td>
</tr>
<tr>
<td>9</td>
<td>Regression equation</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Correlation coefficient (r2)</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Table no 3. The % Recovery data of Terbutalin sulphate using ninhydrin.

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Level</th>
<th>Concentration taken</th>
<th>Concentration found</th>
<th>% RSD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80%</td>
<td>45µg/ml</td>
<td>44.01µg/ml</td>
<td>0.56</td>
<td>99.78%</td>
</tr>
<tr>
<td>2</td>
<td>100%</td>
<td>50µg/ml</td>
<td>49.84µg/ml</td>
<td>1.16</td>
<td>99.68%</td>
</tr>
<tr>
<td>3</td>
<td>120%</td>
<td>55µg/ml</td>
<td>54.18µg/ml</td>
<td>2.04</td>
<td>98.49%</td>
</tr>
</tbody>
</table>

5. CONCLUSION

This paper presents a spectrophotometric evaluation of Terbutalin sulphate with ninhydrin in the presence of NaHCO3 produce a yellow color product with absorbs maximally at 346nm. The proposed spectrophotometric method developed for the determination of Terbutalin sulphate uses readily available and inexpensive chemical compared to many reported method. The method is selective, reported and inexpensive.

5. ACKNOWLEDGMENT

Author thanks to wockhardt pharmaceutical Ltd. (Aurangabad, India) for providing pure Terbutalin sulphate.

REFERENCE

19. Value @ amrita virtual amrita laboratories universalizing education, Amrita vishva vidhyapeetham, (1956).
