SPECTROPHOTOMETRIC DETERMINATION OF DOTHIPEIN HYDROCHLORIDE IN PHARMACEUTICALS THROUGH ION-PAIR COMPLEXATION REACTION

Two simple, sensitive and extraction-free spectrophotometric methods are described for the determination of dothiepin hydrochloride (DOTH) in both pure form and in pharmaceutical tablets. The methods are based on ion-pair complex formation between dothiepin base (DOT) and two acidic dyes, namely, bromophenol blue (BPB) or bromocresol green (BCG) with absorption maximum at 425 nm for BPB method or 430 nm for BCG method. Beer’s law is obeyed over the concentration ranges of 1.0-15.0 and 1.0-17.5 µg mL⁻¹ DOT for BPB and BCG methods, respectively. The molar absorptivity values and Sandell’s sensitivity values are reported for both methods. The limits of detection (LOD) and quantification (LOQ) were calculated to be 0.18 and 0.53 µg mL⁻¹ for BPB method, and 0.17 and 0.50 µg mL⁻¹ for BCG method, respectively. The stoichiometry of the complex in either case was found to be 1:1 and the conditional stability constant (Kf) of the complexes has also been calculated. The proposed methods were applied successfully to the determination of DOTH in pure form and in its tablet form with good accuracy and precision. Statistical comparison of the results was performed using Student’s t-test and variance ratio F-test at 95% confidence level and there was no significant difference between the official and proposed methods with regard to accuracy and precision. Further, the validity of the proposed methods was confirmed by recovery studies via standard addition technique.

Keywords: dothiepin hydrochloride; ion-pair complex; bromophenol blue; bromocresol green; pharmaceutical analysis.

Dothiepin hydrochloride (DOTH) (dosulepin hydrochloride; 3-dibenzo[b,e]thiepin-11(6 H)-ylidene-N,N-dimethyl-1-propanamine hydrochloride) [1] is a tricyclic antidepressant resembling amitriptyline in structure and is prescribed to treat major depressive disorders, particularly in the elderly or where there is underlying heart disease [2]. The drug is official in the British Pharmacopoeia [3] which describes a non-aqueous titration method with potentiometric end point detection for its determination. Several techniques have been reported for the determination of DOTH in pharmaceuticals and include high-performance liquid chromatography (HPLC) [4-8], capillary electrophoresis [9], voltammetry [10], ion-selective electrode potentiometry [11], flow injection potentiometry [12], conductometry [13,14] or spectrofluorimetry [15,16].

To the best of our knowledge, seven reports [15-21] on the use of visible spectrophotometry were found in the literature for the determination of DOTH in pharmaceuticals. Abdellatef et al. [15] have reported a method based on the condensation of the drug with the mixed anhydrides of malonic and acetic acids at 60 °C. An assay procedure based on the formation of a binary complex with eosin in acetic buffer has been reported by Walash et al. [16]. Taha [17] has reported two methods for the determination of DOTH based on either kinetic oxidation reaction of the drug with alkaline potassium permanganate or re-action of the drug with 4-chloro-7-nitrobenzofurazan (NBD-Cl) in the presence of sodium bicarbonate.
Sane et al. [18] have developed four methods based on the formation of ion-pair complexes by reacting of the drug with four acidic dyes, namely, bromophenol blue, bromothymol blue, bromocresol purple and bromophenol red. Another four methods, two methods based on the formation of ion-pair complexes between DOTH and bromophenol blue as well as thymol blue; and two methods based on charge-transfer complex formation reaction between DOTH and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone or p-chloranilic acid, have been reported by Taha et al. [19]. Three assay methods were reported by Hassan [20]. Two methods were based on the formation of ion-pair complexes between the drug and two dyes, namely, methyl orange and orange G dyes; the third method is based on ternary complex formation between cobalt thiocyanate and DOTH. Abdulrahman and Basavaiah [21] have developed two methods, the first method based on the formation of ion-pair complex between DOTH and alizarin red S (ARS) in acid medium, whereas the second method is based on the breaking of the DOTH-ARS ion-pair complex in alkaline medium. However, the reported methods suffered from one or more disadvantage such as poor sensitivity, rigid pH control, heating or extraction step, complicated experimental setup and meticulous control of experimental variables as can be seen from Table 1.

Table 1. Comparison of the existing visible spectrophotometric methods and the proposed methods (NBD-Cl: 4-chloro-7-nitrobenzofurazan, DDQ: 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, NR: Not reported)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Reagents used</th>
<th>Methodology</th>
<th>$\lambda_{\text{max}}$ nm</th>
<th>Linear range, $\mu$g mL$^{-1}$</th>
<th>$\varepsilon$ / L mol$^{-1}$ cm$^{-1}$</th>
<th>LOD $\mu$g mL$^{-1}$</th>
<th>Reaction time, min</th>
<th>Remarks</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Anhydrides of malonic and acetic acids</td>
<td>Condensation product measured</td>
<td>329</td>
<td>0.5-2.5, 9.84 x 10$^4$</td>
<td>10.17</td>
<td>40</td>
<td>Heating is required and measurements done at shorter wavelengths</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Eosin</td>
<td>Ion-pair complex of DOTH with eosin measured</td>
<td>540</td>
<td>1.0-10.0, NR</td>
<td>0.18</td>
<td>-</td>
<td>Use of buffer of pH 3.7, pH dependent</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Alkaline KMnO$_4$</td>
<td>Manganate species measured</td>
<td>610</td>
<td>4-24, NR</td>
<td>1.00</td>
<td>25</td>
<td>Less selectivity</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>
| 4.      | a) Bromophenol blue  
b) Bromothymol blue  
c) Bromocresol purple  
d) Bromophenol red | Ion-pair complex measured | 420 | 2-16, NR | 2-12, NR | 4-24, NR | Extraction is required, use of buffer of different pHs, pH dependent | 18 |
| 5.      | DDQ  
P-chloranilic acid | Radical anion measured | 460 | 10-100, 3.60 x 10$^3$ | NR | 25 | Less sensitive | 19 |
| 6.      | Bromophenol blue  
Thymol blue | Ion-pair complex measured | 415 | 2.0-18.0, 2.40 x 10$^4$ | 3 | Extraction is required, use of buffer of pH 3.6, pH dependent | 20 |
| a) Methyl orange  
b) Orange G  
c) Cobalt thiocyanate | Ion-pair complex measured | 413 | 5-40, 9.80 x 10$^3$ | 0.06 | - | Extraction is required, use of buffer of pH 2.7 | 20 |
| 7.      | a) Alizarin red S (ARS)  
b) ARS-KOH | Ion-pair complex measured | 485 | 2.5-55.0, 5.15 x 10$^3$ | 0.52 | 10 | Extraction is required, less sensitive | 21 |
| 8.      | a) Bromophenol blue (BPB)  
b) Bromocresol green (BCG) | Ion-pair complex measured | 425 | 1.0-15.0, 1.98 x 10$^4$ | 0.18 | 5 | Simple, fast, sensitive, extraction-free and no pH-adjustment, no heating step, only one reagent is required | 21 |

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The visible spectrophotometric methods employing ion-pair extraction procedures and found in the literature are based on the formation of an ion-pair complex between DOTH and anionic dyes at a specific pH. In this case, the ion-pair was extracted into an organic solvent, which is immiscible with water, and the concentration of the resulting ion pair in the organic phase is determined spectrophotometrically. The ion-pair extraction technique has some difficulties and inaccuracies due to incomplete extraction or the formation of emulsions between the hydrocarbon solvent and the basic compound-containing solution. In response to the problems resulting from extraction of the ion-pair complex, few articles were published for the analysis of pharmaceutical compounds through ion pair formation without extraction [22-24].

The aim of the present study was to develop two extraction-free spectrophotometric methods for the determination of DOTH in pharmaceutical formulations based on the formation of ion-pair complexes. The formed ion-pair complexes between DOT and two acidic dyes, namely, bromophenol blue (BPB) and bromocresol green (BCG) require no extraction step and are measured directly in chloroform. The proposed methods are superior to the reported methods with respect to speed, simplicity, sensitivity and cost effectiveness.

EXPERIMENTAL

Instrument

A Systronics model 106 digital spectrophotometer (Ahmedabad, Gujarat, India) equipped with 1 cm matched quartz cells was used for all absorbance measurements.

Materials and reagents

Pharmaceutical grade dothiepin hydrochloride (DOTH), certified to be 99.60% pure, was received from Abbott India Ltd., Mumbai, India. The following pharmaceutical preparations were purchased from commercial sources in the local market and subjected to analysis: Prothiaden 75 from Abbott India Ltd., Mumbai, India, and Dothip 50 from Micro Labs Ltd., Distt. solan, Himachal Pradesh, India.

All reagents and solvents used were of analytical grade and distilled water was used throughout the study; bromophenol blue (BPB) (LOBA Chemie, Mumbai, India): 0.025% (w/v) solution in chloroform (Merck, Mumbai, India); bromocresol green (BCG) (Merck, Mumbai, India): 0.025% (w/v) solution in chloroform.

Standard dothiepin base solution

An accurately weighed amount of 0.0281 g of pure dothiepin hydrochloride was dissolved in 20 mL of water; the solution was rendered alkaline with 0.1 M sodium hydroxide and 30 mL of water was then added. This solution was quantitatively transferred into a 125 mL separating funnel and the dothiepin base was extracted with 4×20 mL of chloroform. The mixed chloroform extracts and washings were passed through anhydrous sodium sulphate, transferred into a 100 mL calibrated flask and the solution was diluted to the mark with chloroform. The resulting solution (250 μL in dothiepin base) was diluted appropriately with chloroform to get a working concentration of 25 μg mL⁻¹ DOT for use in both the methods. This extraction procedure was described by Taha et al. [19] to prepare the dothiepin base.

Assay procedures

BPB method

Different aliquots (0.2–3.0 mL) of a standard DOT base (25 μg mL⁻¹) solution were transferred into a series of 5 mL calibrated flasks using a micro burette and the total volume was brought to 3.0 mL by adding chloroform. To each flask, 1.0 mL of 0.025% (w/v) BPB solution was added and the mixture was diluted to the volume with chloroform and mixed well. The absorbance of each solution was measured at 425 nm against the reagent blank after 5 min.

BCG Method

Aliquots (0.2-3.5 mL) of a standard DOT base (25 μg mL⁻¹) solution were transferred into a series of 5 mL calibrated flasks, as described above. To each flask was then added 1.0 mL of 0.025% (w/v) BCG solution and diluted to the volume with chloroform and mixed well. The absorbance of each solution was measured at 430 nm against the reagent blank after 5 min.

Assay procedure for tablets

Ten tablets were weighed accurately and ground into fine powder. An accurately weighed amount of the powder equivalent to 25 mg of DOT base was transferred into a 50 mL calibrated flask and dissolved in 30 mL of water. The solution was shaken thoroughly for about 15-20 min, diluted to the mark with water and filtered using a Whatman No. 42 filter paper. The filtrate was rendered alkaline with 0.1 N sodium hydroxide using a pH-meter, transferred into a 125 mL separating funnel and the dothiepin base was extracted with 4×20 mL of chloroform. The remaining steps are followed as described above under the preparation of “Standard dothiepin base solution”.

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**Assay procedure for the effect of interference**

Effect of interference was evaluated by both placebo blank and synthetic mixture analyses. A placebo blank, the commonly employed excipients added to the formulations, consisting of 50 mg starch, 30 mg lactose, 30 mg acacia, 20 mg calcium gluconate, 50 mg talc, 20 mg magnesium stearate and 20 mg sodium alginate was prepared as described above under “Assay procedure for tablets” and then subjected to analysis using 1.5 and 2.0 mL of placebo blank solution by BPB and BCG methods, respectively. A synthetic mixture was prepared by adding 11.3 mg of pure DOT to 60 mg of the above mentioned placebo blank and the mixture was homogenized. Following the same procedure for tablets, the synthetic mixture solution (100 μg mL⁻¹ DOT) was prepared and subjected to analysis using 1.5 and 2.0 mL of synthetic mixture solution (25 μg mL⁻¹ DOT) by BPB and BCG methods, respectively.

**RESULTS AND DISCUSSION**

BPB and BCG are dyes of sulphonphthalein type and the color of such dyes is due to the opening of lactoid ring and subsequent formation of quinoid group [25]. Dothiepin base forms ion-pair complexes with acidic dyes such as BPB and BCG since it contains a basic nitrogen atom, bonded to two electron donating methyl groups, which can be protonated easily. After protonation of the drug, the protonated DOT forms ion-pair complexes with these anionic dyes. The possible reaction pathway is proposed and illustrated in Scheme 1.

**Absorption spectra**

The reaction of BPB or BCG with DOT results in the formation of intense yellow colored products. The absorption spectra of the yellow colored ion-pair complexes were recorded at 370-500 nm against the corresponding blank solutions as shown in Figure 1. The resulting yellow colored ion-pair complexes showed maximum absorbance at 425 and 430 nm for BPB method and BCG method, respectively. The measurements were thus made at these wavelengths for bulk and tablet samples.

**Optimization of reaction variables**

Optimum reaction variables for quantitative determination of the formed ion-pair complexes were established via various preliminary experiments such as choice of organic solvent, concentration of the dye and reaction time.

Since hydrochloride salt of dothiepin is not soluble in many organic solvents such as chloroform and dichloromethane, the dothiepin hydrochloride was neutralized and then the base was extracted into chloroform. The procedures followed for the preparation of “Standard dothiepin base solution” recommend the use of chloroform as extracting solvent for the drug under study. So, in order to select the suitable

![Scheme 1. The possible reaction pathway for the formation of DOT and BPB/BCG ion-pair complexes.](image-url)
solvent for preparation of BPB and BCG solutions, both dyes were prepared separately in different solvents such as 1,4-dioxane, chloroform, dichloromethane and 1,2-dichloroethane, and the same solvent was used as diluting solvent. Then, the reaction of DOT with BPB or BCG was carried out in the solvents mentioned above and the absorbance of each solution was measured at the selected wavelengths against the corresponding blank. The results showed that 1,2-dichloroethane could not be used for preparation of dyes solutions in both methods, as the difference between the absorbance of the sample and blank was negligible. Also, the absorbance of the reagents blanks was somewhat high while using 1,4-dioxane as a solvent to prepare the dyes solutions, but chloroform and dichloromethane were found suitable to be used for preparation of BPB and BCG solutions. Since, chloroform was the recommended solvent in the extraction procedure [19] for the preparation of the drug solution (DOT base), the same was preferred as a solvent to prepare BPB and BCG solutions.

The effect of the dye-concentration on the intensity of the color developed at the selected wavelengths was studied by measuring the absorbances of solutions containing different amounts of the reagents BPB and BCG and fixed concentrations of 10 and 12.5 µg mL\(^{-1}\) DOT for BPB and BCG methods, respectively. The results showed that maximum color intensity of the complex was achieved with 1.0 mL of both BPB and BCG solutions and any excess dyes did not affect the absorbance of the complex (Figure 2).

The addition of the dye solution resulted in an immediate full color development at room temperature and the formed ion-pair complexes were stable for at least 1.0 h in both methods. The reaction was found complete and quantitative when the reaction mixture was allowed to stand for 5 min and any delay in the absorbance measurements of the formed ion-pair complexes up to 1.0 h had no pronounced effect on the measured absorbance.

Composition of the ion-pair complexes

Job’s method of continuous variations of equimolar solutions [26] was employed to establish the composition of the ion-pair complex formed between DOT and BPB/BCG. In this method, solutions of 8.46×10\(^{-5}\) mol L\(^{-1}\) standard DOT and 8.46×10\(^{-5}\) mol L\(^{-1}\) dye (BPB/BCG) were mixed in varying volume ratios in such a way that the total volume of each mixture was kept the same at 5.0 mL. The absorbance of each solution was plotted against the mole fraction of DOT (Figure 3). The plot reached a maximum value at a mole fraction of 0.5 which indicated the formation of 1:1 (DOT:dye) ion-pair complexes and confirm the presence of one basic nitrogen containing group. The conditional stability constants (K\(_F\)) of the formed ion-pair complexes were calculated [27] from the data of continuous variations method and found to be 2.44×10\(^7\) and 2.02×10\(^7\) for DOT-BPB and DOT-BCG complexes, respectively.

Method validation

Analytical parameters

Under optimum experimental conditions for DOTH determination, a linear relation was found between the absorbance and concentration of DOT in the ranges of 1.0-15.0 and 1.0-17.5 µg mL\(^{-1}\) for BPB and
BCG methods, respectively. Beer’s law is obeyed and the equations of the lines being: 

\[ Y = -0.0033 + 0.0684X \]  
for BPB method and 

\[ Y = 0.0050 + 0.0542X \]  
for BCG method, where \( Y \) is the absorbance and \( X \) is concentration in \( \mu g \ mL^{-1} \). The correlation coefficients (\( r \)) of the calibration plots are calculated to be 0.9997 and 0.9988 for BPB and BCG methods, respectively, confirming a linear increase in the absorbance with increasing the concentration of DOT. The molar absorptivity values are calculated to be \( 1.98 \times 10^4 \) and \( 1.62 \times 10^4 \) L mol\(^{-1}\) cm\(^{-1}\) with Sandell’s sensitivity values of 0.0149 and 0.0182 \( \mu g \ cm^{-2} \) for BPB and BCG methods, respectively.

The limits of detection (\( LOD \)) and quantification (\( LOQ \)) for the proposed methods were calculated using the following equations [28]:

\[
LOD = \frac{3.3\sigma}{S}, \quad LOQ = \frac{10\sigma}{S}
\]

where \( \sigma \) is the standard deviation of \( n \) replicate determinations under the same conditions as for the sample analysis in the absence of the analyte and \( S \) is the sensitivity, namely the slope of the calibration graph.

The calculated \( LOD \) are 0.18 and 0.17 \( \mu g \ mL^{-1} \) with \( LOQ \) 0.53 and 0.50 \( \mu g \ mL^{-1} \) for BPB and BCG methods, respectively.

**Precision and accuracy**

The precision of the proposed methods was calculated in terms of intermediate precision (intra-day and inter-day) [29]. Three different concentration levels of DOT (within the working limits) were analyzed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The percentage relative standard deviation (\( RSD \)) values were \( \leq 2.04\% \) (intra-day) and \( \leq 2.17\% \) (inter-day) indicating high precision of the proposed methods (Table 2). Also, the accuracy of the proposed methods was evaluated as percentage relative error (\( RE \), %) between the measured concentrations and taken concentrations for DOT (Bias, %), and from the results shown in Table 2, it is clear that the accuracy is satisfactory (\( RE \leq 2.60\% \)).

**Effect of interference**

The effect of interference of the proposed methods for the analysis of DOTH was evaluated by analysis of placebo blank solution as shown under “Assay procedure for tablets” and the resulting absorbance readings in both methods were same as reagent blank, inferring no interference from the placebo. Non interference from placebo was further confirmed by carrying out recovery study from synthetic mixture with percent recoveries of 99.14\pm1.76 and 98.72\pm1.98 for BPB and BCG methods, respectively. These results confirm the non interference in the proposed methods in the presence of the commonly employed excipients added to the formulations.

**Robustness and ruggedness**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the proposed methods was evaluated by making small incremental changes in

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**Table 2. Evaluation of intra-day and inter-day precision and accuracy**

<table>
<thead>
<tr>
<th>Method</th>
<th>DOT taken, ( \mu g \ mL^{-1} )</th>
<th>Intra-day (n = 7)</th>
<th>Inter-day (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DOT found, ( \mu g \ mL^{-1} )</td>
<td>Precision(^{b})</td>
<td>Accuracy(^{c})</td>
</tr>
<tr>
<td>BPB</td>
<td>5.0</td>
<td>4.95</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>7.62</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.93</td>
<td>2.04</td>
</tr>
<tr>
<td>BCG</td>
<td>5.0</td>
<td>4.91</td>
<td>1.94</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>7.58</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>10.19</td>
<td>1.15</td>
</tr>
</tbody>
</table>

\(^{a}\)Mean value of \( n \) determinations; \(^{b}\)relative standard deviation (\%); \(^{c}\)Bias (\%) = \(|\text{found} - \text{taken}|/\text{taken}|\times 100
two experimental variables, namely, volume of the dye (0.8, 1.0 and 1.2 mL) and the reaction time (4, 5 and 6 min). The analysis was performed at the altered experimental conditions by taking three different concentrations of DOT base and the effect of the changes on the absorbance reading of the resulted complexes in both methods was studied and found to remain unaffected as shown by the RSD values in the range of 0.82 to 1.56%, confirming the robustness of the proposed methods. Ruggedness of the proposed methods was expressed as RSD of the same procedure applied by three analysts and also by a single analyst performing analysis on three different cuvettes. The results presented in Table 3 showed that no statistical differences between different analysts and instruments suggesting that the proposed methods were rugged.

**Application to pharmaceutical formulations**

The proposed methods were applied to the determination of DOTH in two representative tablets prothiaden 75 and dothip 50. The results compiled in Table 4 were compared with those obtained by the official method [3] by means of Student’s t-test for accuracy and F-test for precision at 95% confidence level. The official method [3] described a non-aqueous titration of the drug with aceto perchloric acid and determining the end-point potentiometrically. As can be seen from Table 4, the calculated t and F values at 95% confidence level did not exceed the tabulated values of 2.78 and 6.39, respectively, indicating no significant differences between the proposed methods and the official method with respect to accuracy and precision.

**Recovery study**

The accuracy and validity of the proposed methods were ascertained by performing the recovery experiment *via* the standard addition procedure. Pre-analyzed tablet powder was spiked with pure drug at three different concentration levels (50, 100 and 150% of the quantity present in the tablet powder) and the total was measured using the proposed methods. The determination with each level was repeated three times and the results of this study presented in Table 5 indicated that the excipients commonly present in the formulations did not interfere in the assay.

**CONCLUSIONS**

The present study describes for the first time the use of extraction-free ion-pair complexation reaction for the spectrophotometric determination of DOTH in bulk drug as well as in pharmaceutical samples. Among the proposed methods, the BPB method is more sensitive than BCG method as can be seen from the molar absorptivity values and Sandell’s sensitivity values of both methods. The proposed methods require only dyes as reagents which are cheaper and readily available; no pH-adjustment is required and the procedures do not involve any critical reaction

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**Table 3. Robustness and ruggedness of the proposed methods (in both methods, the volume of the dye was 0.8, 1.0 and 1.2 mL and the reaction time was 4, 5 and 6 min)**

<table>
<thead>
<tr>
<th>Method</th>
<th>DOT taken, µg mL⁻¹</th>
<th>Robustness (RSD, %)</th>
<th>Ruggenedness (RSD, %)</th>
<th>Inter-analysts (n = 3)</th>
<th>Inter-instruments (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPB</td>
<td>5.00</td>
<td>1.56</td>
<td>0.82</td>
<td>0.74</td>
<td>2.76</td>
</tr>
<tr>
<td></td>
<td>7.50</td>
<td>0.96</td>
<td>0.88</td>
<td>0.93</td>
<td>2.29</td>
</tr>
<tr>
<td></td>
<td>10.00</td>
<td>1.04</td>
<td>1.13</td>
<td>0.66</td>
<td>1.86</td>
</tr>
<tr>
<td>BCG</td>
<td>5.00</td>
<td>0.97</td>
<td>1.24</td>
<td>0.92</td>
<td>2.71</td>
</tr>
<tr>
<td></td>
<td>7.50</td>
<td>1.36</td>
<td>1.18</td>
<td>1.21</td>
<td>2.91</td>
</tr>
<tr>
<td></td>
<td>10.00</td>
<td>1.22</td>
<td>1.39</td>
<td>1.08</td>
<td>2.24</td>
</tr>
</tbody>
</table>

**Table 4. Comparison of assay results of the official and proposed methods (tabulated t-value at the 95% confidence level is 2.78; tabulated F-value at the 95% confidence level is 6.39)**

<table>
<thead>
<tr>
<th>Tablet brand name</th>
<th>Found (nominal amount±SD, %, mean value of five determinations)</th>
<th>Official method</th>
<th>Proposed methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BPG method</td>
<td>BCG method</td>
</tr>
<tr>
<td>Prothiaden 75</td>
<td>99.54±0.97</td>
<td>101.6±1.46</td>
<td>98.83±1.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 2.63</td>
<td>t = 0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 2.26</td>
<td>F = 2.96</td>
</tr>
<tr>
<td>Dothip 50</td>
<td>99.26±0.67</td>
<td>100.8±1.19</td>
<td>99.08±1.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 2.52</td>
<td>t = 0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 3.15</td>
<td>F = 3.88</td>
</tr>
</tbody>
</table>
conditions or tedious sample preparation. Moreover, both methods are simple, fast, accurate, extraction-free, adequately sensitive, can be performed at room temperature and free from interferences by common additives and excipients. Hence, the proposed methods can be readily adopted by pharmaceutical quality control laboratory for routine analysis.

Acknowledgments

Authors are thankful to Abbott India Ltd., Mumbai, India, for gifting the pure sample of dothiepin hydrochloride. The first author wishes to express his thanks to Al-Bayda’ University, Republic of Yemen for awarding research fellowship and to the authorities of the University of Mysore for permission and facilities to carry out the research work.

REFERENCES


Table 5. Results of recovery study by standard-addition method

<table>
<thead>
<tr>
<th>Formulation studied</th>
<th>BPB method</th>
<th>BCG method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DOT in tablet µg mL⁻¹</td>
<td>Pure DOT added µg mL⁻¹</td>
</tr>
<tr>
<td>Prothiaden 75</td>
<td>5.08</td>
<td>2.50</td>
</tr>
<tr>
<td>Dothip 50</td>
<td>5.08</td>
<td>5.00</td>
</tr>
</tbody>
</table>

*Mean value of three determinations
Preložene su dve osetljive spektrofotometrijske procedure za određivanje dotiepin hidrohlorida (DOTH) u čistoj formi i u farmaceutskim tabletama, bez primene ekstrakcije. Metode se zasnivaju na kompleksometrijskim reakcijama uz transfer naelektrisanja između dotiepin baze (DOT) i dve kisele boje, bromofenol plavo (BPB) ili bromokrezol zeleno (BCG). Obojeni proizvodi imaju apsorpcione maksimume na 425 nm za metodu BPB i na 430 nm za metodu BCG. Saglasnost sa Beer-ovim zakonom je postignuta u opsegu koncentracija DOT-a od 1,0-15,0 μg mL⁻¹ za metodu BPB i 1,0-17,5 μg mL⁻¹ za metodu BCG. Izračunate su vrednosti molarne apsorptivnosti i Sandel-ovi indeksi za obe metode. Vrednosti granica detekcije (LOD) i granica kvantifikacije (LOQ) za metodu BPB iznose 0,18 i 0,53 μg mL⁻¹, a za metodu BCG iznose 0,17 i 0,50 μg mL⁻¹. Nađeno je da je stehiometrijski sastav kompleksa 1:1. Takođe, izračunate su uslovne konstante stabilnosti kompleksa (Kᵢ). Predložene metode su uspešno primene za određivanje DOTH-a u čistoj formi i u tabletama. Rezultati su statistički obrađeni korišćenjem Studentovog testa i F testa za zadati nivo poverenja od 95%. Upoređujući tačnost i preciznost nije nađena značajna razlika između referentnih i predloženih metoda. Validacija predloženih metoda potvrđena je dobrom „recovery“ vrednostima tehnikom standardnog dodatka.

Ključne reči: dotiepin hidrohlorid; kompleksometrijske reakcije uz transfer naelektrisanja; bromofenol plavo; bromokrezol zeleno; farmaceutske analize.