Use of alizarin red S as a chromogenic agent for the colorimetric determination of dothiepin hydrochloride in pharmaceutical formulations

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Abstract The present study describes two simple, rapid, selective and cost-effective spectrophotometric methods for the determination of dothiepin hydrochloride (DOTH), an antidepressant drug, in bulk drug and pharmaceutical formulations. The first method (method A) is based on the formation of yellow colored ion-pair complex between DOTH and alizarin red S (ARS) in acid medium which was extracted into dichloromethane and the absorbance was measured at 445 nm. The second method (method B) is based on the breaking of the yellow DOTH–ARS ion-pair complex in alkaline medium followed by the measurement of the violet color free dye at 570 nm. Under the optimized conditions, Beer's law is obeyed over the concentration ranges of 2.50–55.0 and 1.00–35.0 µg ml⁻¹ DOTH for method A and method B, respectively. The molar absorptivity, Sandell's sensitivity, detection and quantification limits are also calculated. The methods were validated for intra-day and inter-day accuracy and precision; selectivity and robustness and ruggedness. The proposed methods were applied successfully to the determination of DOTH in pure drug and commercial formulations. The accuracy and reliability of the proposed methods were further established by parallel determination by the official method and also by recovery studies via standard addition technique.

1. Introduction

Dothiepin hydrochloride (DOTH) (dosulepin hydrochloride; 3-dibenzo[b,e]thiepin-11(6H)-ylidene-N,N-dimethyl-1-propanamine hydrochloride) is a tricyclic antidepressant drug (Merck Index, 2006). It is used to treat patients who experience difficulty in sleeping and a loss of appetite (Chen et al., 2008). The drug is official in the British Pharmacopoeia (BP, 2003) which describes a non-aqueous titration method for its determination with potentiometric end point detection. Several analytical methods have been published for the determination of DOTH in pharmaceuticals and include high-performance
liquid chromatography (HPLC) (Sane et al., 1989; Slais and Subert, 1980; Li and Irwin, 1979; Pawlak et al., 1990; Pawlak and Clark, 1989), capillary electrophoresis (Clark et al., 1992), voltammetry (Bishop and Hussein, 1984), ion-selective electrode potentiometry (Hosny, 2007), flow injection potentiometry (Abdel-Ghani et al., 2006a), conductometry (Youssef, 2005; Abdel-Ghani et al., 2004b), spectrofluorimetry (Abdellatef et al., 2006; Walash et al., 2010) and visible spectrophotometry (Abdellatef et al., 2006; Walash et al., 2010; Taha, 2003; Sane et al., 1988; Taha et al., 2002; Hassan, 2008).

To the best of our knowledge, six reports on the use of visible spectrophotometry were found in the literature for the determination of DOTH in pharmaceuticals. Abdellatef et al. (2006) have reported one method based on the condensation of the drug with the mixed anhydrides of malonic and acetic acids at 60 °C. An assay method based on the formation of a binary complex with eosin in acetate buffer has been reported by Walash et al. (2010). Taha (2003) has reported two methods for the assay of DOTH based on either kinetic oxidation reaction of the drug with alkaline potassium permanganate or reaction of the drug with 4-chloro-7-nitrobenzofurazan (NBD-Cl) in the presence of sodium bicarbonate. Few reports (Sane et al., 1988; Taha et al., 2002; Hassan, 2008) based on the formation of ion-pair complexes for the determination of DOTH by reacting of the drug with bromophenol blue (Sane et al., 1988), bromothymol blue and bromoresol purple (Sane et al., 1988), bromophenol red (Sane et al., 1988), thymol blue (Taha et al., 2002), methyl orange and orange G (Hassan, 2008) in acid medium were also found in the literature. Taha et al. (2002) have reported two methods based on charge-transfer complex formation between DOTH and 2,3-dichloro-5,6-dicyano-p-benzoquinone or p-chloranilic acid. A method based on ternary complex formation between cobalt thiocyanate and DOTH was reported by Hassan (2008). However, many of the previously reported methods suffered from one or the other disadvantage such as pH control, less stability of the measured species, narrow linear dynamic range, heating step, use of expensive chemical and/or complicated experimental setup.

Extractive spectrophotometric procedures have received considerable attention for quantitative determination of many organic compounds of pharmaceutical interest (Mostafa et al., 2002; Ashour et al., 2006; Harikrishna et al., 2008; Milano and Cardoso, 2005; Rajendraprasad et al., 2010). Also, alizarin red S (ARS) has been widely used as an ion-pairing reagent for quantitative analysis of many drugs in pharmaceutical formulations (Basavaiah et al., 1999; Farhadi and Maleki, 2002; Amin, 2002; Farhadia et al., 2003; Kamel et al., 2008). Therefore, the aim of this study was directed to develop two accurate, selective, precise and inexpensive procedures for the determination of DOTH in pharmaceuticals based on ion-pair complex formation using alizarin red S as a reagent.

2. Experimental

2.1. Instrument

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

2.2. Materials

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 Dothipen 50 from Micro Labs Ltd., Distt. solan, Himachal Pradesh, India.

2.3. Reagents and chemicals

All the reagents and solvents used were of analytical reagent grade and distilled water was used throughout the study.

1. Alizarin red S (S. d. Fine Chem., Mumbai, India): 0.05% (w/v) solution in water.
2. Hydrochloric acid (Merck, Mumbai, India, sp. gr. 1.18): 0.1 M in water.
3. Potassium hydroxide (Merck, Mumbai, India): 1.0% (w/v) in methanol.
4. Standard stock solution: A stock standard solution of 100 mg ml⁻¹ of DOTH was prepared by dissolving accurately weighed 25 mg of pure drug in water and diluting to the mark with the same solvent in a 250 ml calibrated flask.

2.4. Recommended procedure

2.4.1. Method A (based on the measurement of ion-pair complex)

Different aliquots (0.25, 0.50–5.50 ml) of a standard DOTH (100 μg ml⁻¹) solution were accurately transferred into a series of 125 ml separating funnels and the total volume was adjusted to 6.0 ml by adding adequate quantity of water. To each funnel 5 ml of 0.1 M HCl was added, followed by 4 ml of 0.05% ARS solution. The content was mixed well and after 10 min, the formed ion-pair complex was extracted with 10 ml of dichloromethane after shaking for 1 min. The two phases were allowed to separate and the dichloromethane layer was dried over anhydrous sodium sulphate and the absorbance of the yellow DOTH–ARS ion-pair complex was measured at 445 nm against a reagent blank.

2.4.2. Method B (based on the measurement of the free form of ARS from the broken ion-pair)

Into a series of 125 ml separating funnels, 5.0 ml aliquots of a standard solution (100 μg ml⁻¹ DOTH) were transferred separately and the total volume in each separating funnel was adjusted to 6.0 ml by adding 1 ml of water. To each funnel were added 5 ml of 0.1 M HCl and 4 ml of 0.05% ARS solution. The content was mixed thoroughly and after 10 min, the ion-pair complex was extracted with 10 ml of dichloromethane by shaking for 1 min. The two layers were allowed to separate and the organic layer of all separating funnels was passed over anhydrous sodium sulphate and then collected in a 50 ml dry standard flask. Varying aliquots (0.1–3.5 ml) of this organic layer, the ion-pair complex of DOTH–ARS (50 μg ml⁻¹ in DOTH), were transferred into a series of 5 ml standard flasks and the total volume was adjusted to 3.5 ml by adding dichloromethane.
To each flask, 1.5 ml of 1.0% alcoholic KOH was added, the content was mixed well and the absorbance of the violet colored species was measured at 570 nm against the reagent blank.

2.4.3. Procedure for commercial tablets

Ten tablets each containing 75 mg or 50 mg of DOTH were weighed and finely powdered. An amount of the powder equivalent to 10.0 mg of DOTH was accurately weighed and transferred to a 100 ml volumetric flask, 60 ml of water was added and the content was shaken thoroughly for about 20 min. The volume was diluted to the mark with water, mixed well and filtered using Whatman no. 42 filter paper. First 10 ml portion of the filtrate was rejected and a suitable aliquot of the filtrate (containing 100 μg ml⁻¹ DOTH) was used for the assay by the recommended procedure of method A. The ion-pair complex DOTH–ARS (50 μg ml⁻¹ in DOTH, prepared in method A) of the tablets was used for assay by applying the procedure described in method B.

2.4.4. Procedure for the selectivity study

The selectivity was evaluated by both placebo blank analysis and recovery studies. A placebo blank, the commonly employed excipients added to the formulations, consisting of 50 mg starch, 30 mg lactose, 30 mg acacia, 30 mg calcium gluconate, 50 mg talc, 50 mg magnesium stearate and 30 mg sodium alginate was prepared as described under the Section 2.4.3 and then subjected to analysis. A synthetic mixture was prepared by adding 10 mg of pure DOTH to the above mentioned placebo blank and the mixture was homogenized. Following the same procedure for tablets, the synthetic mixture solution was prepared and a suitable quantity was subjected for analysis by both the methods.

3. Results and discussion

3.1. Absorption spectra

The reaction of DOTH with ARS in an acidic medium to form a yellow ion-pair complex was investigated and this complex was extracted into dichloromethane. The absorption spectra (Fig. 1) of the formed ion-pair complex was recorded at 370–540 nm against the reagent blank solution and exhibited a maximum absorption at 445 nm (method A). In method B, this DOTH–ARS ion-pair complex was treated with alcoholic KOH to yield a maximum absorbance as can be seen in Fig. 5. The results showed that 1.5 ml of 0.05% ARS solution was selected as the difference between the absorbance of the blank and the measured species is a maximum (Fig. 4). Similarly, the effect of alcoholic KOH concentration required to break the ion-pair complex and formation of the dianionic form of the dye in method B was studied by measuring the absorbance of solutions containing a fixed concentration of 50 μg ml⁻¹ DOTH and different amounts of the ARS at 445 nm. The results showed that 4.0 ml of 0.05% ARS solution was selected as the difference between the absorbance of the blank and the measured species is a maximum (Fig. 4).

3.2. Reaction mechanism

The anionic dyes such as ARS form ion-pair complex with the nitrogenous drug in acid medium. Protonation of DOTH can be carried out easily, as the nitrogen atom present in the drug is bonded to two electron donating methyl groups. So, when the DOTH treated with ARS dye in acid medium, a yellow ion-pair complex extractable into dichloromethane is formed and the possible mechanism for the formation of ion-pair complex (method A) is given in Fig. 2. Canamares et al. (2006) have shown that alizarin can exist in three different forms (neutral absorbing at 433 nm, monoionized absorbing at 526 nm and dionized absorbing at 567 nm) based on different pHs 3.4, 10.0 and 12.8, respectively. Nemodruk and Karalova (1969) have stated that alizarin red S has a yellow color in acid solutions (pH < 5) which becomes a violet on increasing the pH to about 11. They explain that this phenomenon is due to the dissociation of the alizarin red S molecule at the two phenol group and forming dianionic form of the dye (ARS⁻²). So, when the DOTH–ARS ion-pair complex, formed in method A, is treated with alcoholic KOH, the ion-pair complex will break and the yellow color will change to violet color due to the formation of dianionic form of the dye (ARS⁻²). The possible mechanism for method B is illustrated in Fig. 3.

3.3. Optimization of reaction variables

A number of preliminary experiments established optimum conditions necessary to achieve complete reaction formation, quantitative extraction of the ion-pair complex, complete breaking of the ion-pair complex and highest sensitivity. Optimum condition was fixed by varying one parameter at a time while keeping other parameters constant and observing its effect on the absorbance at the respected wavelength.

3.3.1. Effect of reagents concentration

The effect of the ARS concentration in method A was studied by measuring the absorbance of solutions containing a fixed concentration of 40 μg ml⁻¹ DOTH and different amounts of the ARS at 445 nm. The results showed that 4.0 ml of 0.05% ARS solution was selected as the difference between the absorbance of the blank and the measured species is a maximum (Fig. 4). Similarly, the effect of alcoholic KOH concentration required to break the ion-pair complex and formation of the dianionic form of the dye in method B was studied by measuring the absorbance of solutions containing a fixed concentration of ion-pair complex (20.0 μg ml⁻¹; in DOTH) and different volumes of 1.0% alcoholic KOH at 570 nm. The results showed that 1.5 ml of 1.0% (w/v) alcoholic KOH was sufficient to yield a maximum absorbance as can be seen in Fig. 5.

3.3.2. Effect of pH on the ion-pair complex formation

The effect of pH of the aqueous phase was studied by extracting the colored complex in the presence of either hydrochloric acid or acidic buffers of pH 3.0–4.0. It was noticed that the maximum color intensity was observed using hydrochloric acid. Also, the formed ion-pair complex is pH independent since no remarkable changes were observed while using different concentrations of HCl such as 0.1, 0.3 and 0.5 M. Further, 5.0 ml of 0.1 M HCl gave reproducible results and it was fixed throughout the study.

3.3.3. Selecting of the extracting solvent

Three organic solvents viz., chloroform, dichloromethane and 1,2-dichloroethane were examined for quantitative extraction of the formed ion-pair complex. From the results shown in Table 1, dichloromethane was selected as extracting solvent due to its efficiency, the greater stability of the extracted ion-pair complex (> 24 h), its high sensitivity, maximum absorbance of the measured species and shortest time to reach the equilibrium between both phases.
3.3.4. Effect of time, sequence of addition and stability

The effect of contact time between DOTH and ARS in the presence of 0.1 M HCl (method A) was studied in the time range of 0–25 min before extraction and it was found that 10 min is the minimum time to achieve maximum absorbance at 445 nm. Shaking times of 0.5–3 min was studied and the results showed that 1.0 min is sufficient to produce a constant absorbance. Hence a shaking time of 1.0 min was used throughout. In method B, the effect of the time required to break the ion-pair complex was studied after the addition of alcoholic KOH to the ion-pair complex and it was noticed that the breaking of the complex was instantaneous. There was no appreciable change in the absorbance of the measured species if the order of addition of the reactants was varied. The absorbance of the yellow ion-pair complex remained stable for more than 24 h at room temperature (method A) and the absorbance of the violet color of the dianionic form of ARS (method B) was found to remain stable for about 1 hr.

3.3.5. Effect of number of extractions and volume of organic solvent

The results showed that one extraction was adequate to achieve a quantitative recovery of the complex and 10.0 ml of the organic solvent i.e. dichloromethane was sufficient for efficient extraction of the colored species.
3.4. Composition of the ion-pair complex

The composition of the ion-pair complex formed in method A between DOTH and ARS was established by applying Job’s method of continuous variations. In this method, \(6.03 \times 10^{-4}\) M solutions of DOTH and ARS were used and mixed in varying volume ratios in such a way that the total volume of the drug and ARS was kept at 5 ml in the total volume of 15 ml of aqueous layer. The absorbance of extracted ion-pair in each instance was measured and plotted against the mole fraction of the drug (Fig. 6). The plot reached a maximum value at a mole fraction of 0.5 which indicated that a 1:1 (DOTH: ARS) ion-pair complex is formed through the electrostatic attraction between positive protonated DOTH and ARS anion. The conditional stability constant \(K_f\) of the ion-pair complex was calculated (Amin et al., 2007) from the data of continuous variations method and found to be \(4.65 \times 10^5\).

3.5. Method validation

The proposed methods were validated in agreement with the current ICH guidelines (ICH, 1996).

3.5.1. Analytical parameters

Under optimum experimental conditions for DOTH determination, the standard calibration curves were constructed by

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### Table 1

<table>
<thead>
<tr>
<th>Solvent</th>
<th>(A_{\text{blank}})</th>
<th>(A_{\text{ion-pair}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>0.028</td>
<td>0.308</td>
</tr>
<tr>
<td>1,2-Dichloroethane</td>
<td>0.040</td>
<td>0.248</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>0.029</td>
<td>0.355</td>
</tr>
</tbody>
</table>

\(A_{\text{ion-pair}}\) Concentration of DOTH is \(25 \mu g ml^{-1}\).
plotting the absorbance versus concentration. The linear regression equations were obtained by the method of least squares and Beer’s law was obeyed over the concentration ranges stated in Table 2. Many parameters such as intercept \((a)\), slope \((b)\), molar absorptivity, Sandell’s sensitivity, correlation coefficient \((r)\), standard deviation of intercept \((S_a)\), standard deviation of slope \((S_b)\), limits of detection and quantification for both methods are summarized in Table 2. The linearity of calibration graphs was proved by the high values of the correlation coefficient \((r)\) and the small values of the \(Y\)-intercepts of the regression equations.

### 3.5.2. Precision and accuracy

The precision of the proposed methods was calculated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of DOTH were analyzed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The results of this study were summarized in Table 3 and the percentage relative standard deviation (%RSD) values were \(\leq 2.17\%\) (intra-day) and \(\leq 2.63\%\) (inter-day) indicating high precision of the methods. Also, the accuracy of the proposed methods was evaluated as percentage relative error (RE \%) and from the results shown in Table 3, it is clear that the accuracy is satisfactory (RE \(\leq 2.90\%\)).

### 3.5.3. Selectivity

The selectivity of the proposed methods for the analysis of DOTH was tested by placebo blank and synthetic mixture analyses as shown under the Section 2.4.4. From the placebo blank analysis, the results confirmed that the change in the absorbance with respect to the reagent blank was caused only by the analyte. Non interference from placebo was further confirmed by carrying out recovery study from synthetic mixture and the percent recoveries of DOTH were 99.34 \(\pm\) 2.06 and 98.84 \(\pm\) 1.94 for method A and method B, respectively. The results of this study emphasize the selectivity of the proposed methods in the presence of the commonly used excipients added to the formulations.

### 3.5.4. Robustness and ruggedness

The evaluation of the method robustness was done by interchanging some parameters namely, volume of HCl and the contact time for method A or volume of alcoholic KOH for method B and performing the analysis under the optimized experimental conditions. The effect of these changes on the absorbance reading of the colored systems in both methods were studied and found to be negligible confirming the robustness of the proposed methods. Method ruggedness 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 4 showed that no statistical differences between different analysts and instruments suggesting that the proposed methods were rugged.

### 3.5.5. Applications to analysis of pharmaceutical formulations

The proposed methods were successfully applied to the determination of DOTH in two representative tablets Prothiaden 75 and Dothip 50. The results obtained are compiled in Table 5 and were compared with those obtained by the official method (British Pharmacopoeia, 2003) by means of Student’s \(t\)-test for

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#### Table 3 Evaluation of intra-day and inter-day precision and accuracy.

<table>
<thead>
<tr>
<th>Methods</th>
<th>DOOTH(^a) 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>DOOTH found, (\mu g) ml(^{-1})</td>
<td>% RE(^b)</td>
<td>% RSD(^c)</td>
</tr>
<tr>
<td>Method A</td>
<td>20.0</td>
<td>19.61</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>29.46</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>39.50</td>
<td>1.25</td>
</tr>
<tr>
<td>Method B</td>
<td>10.0</td>
<td>10.12</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>15.34</td>
<td>2.27</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>20.29</td>
<td>1.45</td>
</tr>
</tbody>
</table>

\(^a\) Mean value of \(n\) determinations.

\(^b\) Relative error (%).

\(^c\) Relative standard deviation (%).

#### Table 4 Results of robustness and ruggedness study.

<table>
<thead>
<tr>
<th>Method</th>
<th>DOOTH taken, (\mu g) ml(^{-1})</th>
<th>Robustness</th>
<th>Ruggedness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parameters interchanged</td>
<td>Volume of HCl/alcoholic KOH(^a)</td>
<td>Reaction time(^b)</td>
</tr>
<tr>
<td>A</td>
<td>30.0</td>
<td>1.84</td>
<td>1.12</td>
</tr>
<tr>
<td>B</td>
<td>20.0</td>
<td>1.03</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^a\) In method A, the volumes of 0.1 M HCl were 4.8, 5.0 and 5.2 ml, and in method B the volumes of alcoholic KOH added were 1.3, 1.5 and 1.7 ml.

\(^b\) In method A, the reaction times were 8, 10 and 12 min.
accuracy and \( F \)-tests for precision at 95% confidence level. The official method described a non-aqueous titration of the drug with acetous perchloric acid and determining the end-point potentiometrically. As can be seen from the Table 5, the calculated \( t \)- and \( F \)-values at 95% confidence level did not exceed the tabulated values of 2.78 and 6.39, respectively, indicating that there were no significant differences between the proposed methods and the official method. To ascertain the accuracy and validity of the proposed methods, recovery experiment was performed via the standard addition procedure. To a fixed and known amount of DOTH in tablets powder (pre-analyzed), pure drug was added at three levels (50%, 100% and 150% of the quantity present in the tablets powder) and the total was measured by the proposed methods. The determination with each level was repeated three times and the results of this study presented in Table 6 indicated that the various excipients present in the formulations did not interfere in the assay.

<table>
<thead>
<tr>
<th>Tablet brand name</th>
<th>Found (% of nominal amount ± SD)</th>
<th>Proposed methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Official method</td>
<td>Method A</td>
</tr>
<tr>
<td>Prothiaden 75</td>
<td>99.54 ± 0.97</td>
<td>97.86 ± 1.35</td>
</tr>
<tr>
<td></td>
<td>( t = 2.26 )</td>
<td>( t = 2.02 )</td>
</tr>
<tr>
<td></td>
<td>( F = 1.94 )</td>
<td>( F = 1.56 )</td>
</tr>
<tr>
<td>Dothip 50</td>
<td>99.26 ± 0.67</td>
<td>98.22 ± 1.28</td>
</tr>
<tr>
<td></td>
<td>( t = 1.61 )</td>
<td>( t = 0.42 )</td>
</tr>
<tr>
<td></td>
<td>( F = 3.65 )</td>
<td>( F = 2.60 )</td>
</tr>
</tbody>
</table>

Tabulated \( t \)-value at the 95% confidence level is 2.78.
Tabulated \( F \)-value at the 95% confidence level is 6.39.

\* Mean value of five determinations.

### Table 6 Results of recovery study using standard addition method.

<table>
<thead>
<tr>
<th>Formulation studied</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DOTH taken, ( \mu g ml^{-1} )</td>
<td>Pure DOTH added, ( \mu g ml^{-1} )</td>
</tr>
<tr>
<td>Prothiaden 75</td>
<td>19.57</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td>19.57</td>
<td>20.00</td>
</tr>
<tr>
<td></td>
<td>19.57</td>
<td>30.00</td>
</tr>
<tr>
<td>Dothip 50</td>
<td>19.64</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td>19.64</td>
<td>20.00</td>
</tr>
<tr>
<td></td>
<td>19.64</td>
<td>30.00</td>
</tr>
</tbody>
</table>

\* Mean value of three determinations.

4. Conclusion

The results of this study demonstrate that it is possible to use alizarin red S as an ion-pairing reagent for the spectrophotometric determination of DOTH in bulk drug as well as in pharmaceutical samples. The proposed methods are highly reliable owing to the stability of the ion-pair complex and the dianionic form of the ARS which are ultimately measured. The methods can be performed at room temperature and make use of cheaper and readily available reagent. Moreover, the procedures do not involve any critical reaction conditions and no pH-adjustment is required. As can be seen from the molar absorptivity values of both methods, the method B is more sensitive than method A. From the Student’s \( t \)-test and \( F \)-test values, it is clear that the results obtained by the proposed methods are in a good agreement with those obtained by the official method and indicate a high accuracy and precision. Thus, the methods are useful for the quality control and routine analysis of DOTH in pharmaceutical preparations since there is no interference was observed from the common excipients that might be found in commercial formulations.

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