Original article

Use of bromate-bromide mixture as a reagent for the determination of dothiepin hydrochloride in pharmaceuticals

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Abstract:

The present study describes one titrimetric and two spectrophotometric methods for the determination of dothiepin hydrochloride (DOTH) in bulk drug and in tablets. The methods employed bromate-bromide mixture in acid medium as an oxidimetric as well as brominating reagent and meta-cresol purple (MCP) as auxiliary reagents. The described methods are based on the oxidation and bromination of DOTH by the bromine generated *in situ* by the action of the acid on the bromate-bromide mixture. In titrimetry, DOTH is treated with a known excess amount of bromate-bromide mixture in acid medium followed by the determination of unreacted bromine iodometrically. Spectrophotometry involves the addition of a measured excess of bromate-bromide reagent in acid medium to DOTH followed by determination of the residual bromine by reacting with a fixed amount of MCP and measuring the absorbance at 540 nm (method A) or 460 nm (method B). Titrimetry allows the determination over the range of 3.0-10.0 mg DOTH whereas in spectrophotometry, Beer's law is obeyed in the concentration ranges of 0.5-7.0 and 1.0-16.0 μg mL^{-1} DOTH for method A and method B, respectively. The important analytical and sensitivity parameters are also reported for both spectrophotometric methods. The proposed methods were applied successfully to the determination of DOTH in raw material and commercial tablets. Statistical comparison of the results was performed using Student's t-test and F-ratio 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; Bromate-bromide; Meta-cresol purple; Titrimetry; Spectrophotometry; Pharmaceuticals

Introduction

Dothiepin hydrochloride (DOTH), (dosulepin hydrochloride; (E)-3-dibenzo[*b,e*]thiepin-11(6*H*)-ylidene-*N, N*-dimethyl-1-propan-1-amine hydrochloride) [1], is a tricyclic antidepressant drug used widely to treat the depression and the anxiety frequently associated with depressive illness [2]. DOTH, whether in its raw material form or capsule form, is assayed in the British Pharmacopoeia [3] by a non-aqueous titration method. The literature survey reveals that several techniques have been reported for the determination of DOTH in pharmaceuticals. Among these methods are flow injection potentiometry [2], high-performance liquid chromatography (HPLC) [4-8], capillary electrophoresis [9], voltammetry [10], ion-selective electrode potentiometry [11], conductometry [12,13], spectrofluorimetry [14,15] and visible spectrophotometry [14-20].

To the best of our knowledge, there are only two reports [12,13] on the titrimetric determination of DOTH in which the end point was located conductometrically. The first report [12] is based on the titration of DOTH with potassium salts of trioxalato-ferrate (III), trioxalato-chromate (III), trioxalato-aluminate (III) and trioxalato-cobaltate (III) which allowed the determination in the ranges of 6.63-29.87, 3.31-49.78, 9.95-49.78 and 3.31-33.19 mg DOTH, respectively. The second report [13] used phosphotungstic and phosphomolybdic acids as titrants and permitted the determination of the drug in the range of 9.95-49.78 mg DOTH. Also, seven reports [14-20] on the use of visible spectrophotometry were found in the literature for the determination of DOTH in pharmaceuticals. Abdellatef *et al.* [14] have reported a method based on the condensation reaction of DOTH 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 acetate buffer has been reported by Walash *et al.* [15]. Taha [16] has reported two methods for the determination 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 [17-20] based on the formation of ion-pair complexes for the determination of DOTH by reacting of the drug with bromophenol blue [17,18], bromothymol blue [17], bromocresol purple and bromophenol red [17], thymol blue [18], methyl orange and orange G [19], chromotrope 2R and rose Bengal [20] in buffer medium followed by extraction in to organic solvents were also found in the literature. Taha *et al.* [18] 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. An assay method based on ternary complex formation between cobalt thiocyanate and DOTH was reported by Hassan [19]. However, the reported methods suffered from one or the other disadvantage such as poor sensitivity, use of organic solvents, rigid pH control, heating or extraction step, less stability of the measured species, complicated experimental setup and meticulous control of experimental variables as can be seen from Table 1.

The scientific references found in the CAS and SCI database, relating to green analytical chemistry or environmental-friendly analytical methods have been growing significantly in recent years and the recent development of new analytical methods with good characteristics such as selectivity and sensitivity are not sufficient; modern analytical methods need to be green [21]. Hence, the aim of this study was to develop three methods for the determination of DOTH based on oxidation and bromination reactions of DOTH by an eco-friendly agent (i.e. bromine-generated *in situ* by the action of the acid on bromate-bromide mixture). The methods employ titrimetric and spectrophotometric techniques, and use of bromate-bromide mixture and meta-cresol purple (MCP) as reagents. The proposed methods offer the advantage of simplicity, speed, accuracy and precision besides being free from interference from common tablet excipients.

Materials and Methods

Instrument

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

Table 1 Comparison of the existing visible spectrophotometric methods and the proposed methods

Sl. No	Reagent/s used	Methodology	λ_{max} (nm)	Linear Range, $\mu g\ mL^{-1}$ and (ε, L mol^{-1} cm^{-1})	LOD, $\mu g\ mL^{-1}$	Remarks	Ref.
1.	Anhydrides of malonic and acetic acids	Condensation product measured	329	0.5-2.5 ($\varepsilon = 9.84 \times 10^4$)	10.17	Heating is required and measurements done at shorter wavelengths.	14
2.	Eosin	Ion-pair complex of DOTH with eosin measured	540	1.0-10.0 (NR)	0.18	Use of buffer of pH 3.7, pH dependent.	15
3.	a) Alkaline $KMnO_4$	Manganate species measured	610	4-24 (NR)	1.00	Rate measurement requires judicious control of several variables. Less sensitive and time consuming.	16
	b) NBD-Cl	Charge-transfer complex measured	470	50-250 (NR)	12.50		
4.	a) Bromophenol blue	Ion-pair complex measured	420	2-16 (NR)	NR	Extraction is required, use of organic solvents, pH dependent.	17
	b) Bromothymol blue	Ion-pair complex measured	420	2-16 (NR)	NR		
	c) Bromocresol purple	Ion-pair complex measured	420	2-12 (NR)	NR		
	d) Bromophenol red	Ion-pair complex measured	420	4-24 (NR)	NR		
5.	a) DDQ	Radical anion measured	460	10-100 ($\varepsilon = 3.60 \times 10^3$)	NR	Less sensitive, use of organic solvents.	18
	b) P-chloranilic acid	Radical anion measured	525	20-160 ($\varepsilon = 2.50 \times 10^3$)	NR	Extraction is required, use of organic solvents, pH dependent.	
	c) Bromophenol blue	Ion-pair complex measured	415	2.0-18.0 ($\varepsilon = 2.40 \times 10^4$)	NR		
	d) Thymol blue	Ion-pair complex measured	413	5-40 ($\varepsilon = 9.80 \times 10^3$)	NR		
6.	a) Methyl orange	Ion-pair complex measured	423	0.2-12.0 ($\varepsilon = 3.04 \times 10^4$)	0.06	Extraction is required, use of organic solvents and pH dependent. Less sensitive and use of organic solvents.	19
	b) Orange G	Ion-pair complex measured	498	0.5-10.0 ($\varepsilon = 2.32 \times 10^4$)	0.15		
	c) Cobalt thiocyanate	Ternary complex of DOTH with cobalt thiocyanate measured	625	3.2-80.0 ($\varepsilon = 3.60 \times 10^3$)	0.98		
7.	a) Chromotrope 2R	Ion-pair complex measured	525	8-40 ($\varepsilon = 7.80 \times 10^3$)	0.27	Extraction is required, use of organic solvents and pH dependent. Use of surfactant and pH dependent.	20
	b) Rose bengal (with extraction)	Ion-pair complex measured	555	16-44 ($\varepsilon = 4.60 \times 10^3$)	0.26		
	c) Rose bengal (extraction-free)	Ion-pair complex measured	575	2-20 ($\varepsilon = 1.8 \times 10^4$)	0.17		
8.	Bromate-bromide mixture-meta cresol purple	Color of the dye in acid medium measured	540	0.5-7.0 ($\varepsilon = 3.65 \times 10^4$)	0.27	Sensitive, no heating or extraction step, no use of organic solvents, use of eco-friendly chemicals.	This Work
		Bromo-derivative of the dye measured	460	1.0-16.0 ($\varepsilon = 1.65 \times 10^4$)	0.75		

NBD-Cl: 4-chloro-7-nitrobenzofurazan, DDQ: 2,3-dicloro-5,6-dicyano-1,4-benzoquinone, NR: Not reported.

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

Reagents and chemicals

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

A stock standard solution of bromate-bromide mixture equivalent to 5 mM $KBrO_3$ and 10-fold molar excess of KBr was prepared by dissolving accurately weighed 0.209 g of potassium bromate (S. D. Fine-Chem. Ltd., Mumbai, India) and 1.488 g of potassium bromide (Merck, Mumbai, India) in water and diluting to volume in a 250 mL calibrated flask, and directly used in the titrimetric method. Another stock standard solution of $KBrO_3$-KBr equivalent to 500 μg mL^{-1} $KBrO_3$ was prepared in a 100 mL calibrated flask and diluted with water to get bromate-bromide solutions containing 45 and 135 μg mL^{-1} in $KBrO_3$ for use in spectrophotometric method A and method B, respectively.

Solutions of 3 M hydrochloric acid (Merck, Mumbai, India; sp. gr. 1.18), 5% (w/v) potassium iodide (Merck, Mumbai, India), 0.03 M sodium thiosulphate (Sisco-chem Industries, Mumbai, India, assay 98%) and 1% (w/v) starch indicator GR (LOBA Chemie Pvt. Ltd., Mumbai, India) were prepared in water. A solution of 800 μg mL^{-1} meta-cresol purple (MCP) (LOBA Chemie Pvt. Ltd., Mumbai, India) was prepared in a minimum amount of 0.1 N sodium hydroxide and made up to the required volume with water. The solution of 800 μg mL^{-1} MCP was used for method B and diluted appropriately with water to get 100 μg mL^{-1} MCP for use in method A.

Standard dothiepin hydrochloride solution

A stock standard solution equivalent to 1.0 mg mL^{-1} of DOTH was prepared by dissolving accurately weighed 100 mg of pure drug in water and diluted to the mark in a 100 mL calibrated flask with the same solvent. The solution (1 mg mL^{-1} DOTH) was used in titrimetric work and diluted appropriately with water to get the working concentrations of 20 and 40 μg mL^{-1} DOTH for use in spectrophotometric method A and method B, respectively.

Recommended methods

Titrimetry

Different volumes (3-10 mL) of standard DOTH (1 mg mL^{-1}) solution were measured accurately, transferred into a 100 mL Erlenmeyer flask and the total volume was made to 10 mL with water. The solution was acidified by adding 2 mL of 3 M HCl followed by the addition of 10 mL of bromate-bromide mixture (5 mM in $KBrO_3$) using a pipette. The content was mixed well and the flask was kept aside for 10 min with occasional swirling. Then, 5 mL of 5% (w/v) potassium iodide was added to the flask and the liberated iodine was titrated with 0.03 M sodium thiosulphate to a starch end point. A blank titration was performed under the same conditions taking 10 mL of water. The drug content in the measured aliquot was calculated from the following equation:

$$\text{Amount(mg)} = \frac{(B\text{-}S) \times M_W \times R}{n} \qquad (1)$$

where B is volume of the titrant in the absence of the drug, S is volume of the titrant in the presence of the drug, M_W is relative molecular mass of the drug, R is molarity of bromate in the bromate-bromide mixture and n is the reaction stoichiometry (number of moles of bromate reacting with each mole of DOTH = 1).

Spectrophotometry (method A, measuring MCP in acid medium)

Aliquots (0.25-3.5 mL) of a standard DOTH (20 μg mL^{-1}) solution were accurately transferred into a series of 10 mL calibrated flasks and the total volume was adjusted to 3.5 mL by adding a suitable quantity of water. To each flask, 2 mL of 3 M HCl was added followed by 1.0 mL of bromate-bromide (45 μg mL^{-1} in $KBrO_3$). The flasks were stoppered, content mixed and

allowed to stand for 15 min with occasional shaking. Then, 1 mL of 100 μg mL^{-1} MCP solution was added to each flask, and after 5 min, the mixture was diluted to the mark with water and mixed well. The absorbance of each solution was measured at 540 nm against a reagent blank.

Spectrophotometry (method B, measuring the brominated product of MCP)

Different aliquots (0.25-4.0 mL) of a standard 40 μg mL^{-1} DOTH solution were transferred into a series of 10 mL calibrated flasks using a micro burette and the total volume was brought to 4 mL by adding water. To each flask 3 mL of 3 M HCl and 1.0 mL of bromate-bromide mixture solution (135 μg mL^{-1} in $KBrO_3$) were added. The content was mixed well and the flasks were kept aside for 15 min with intermittent shaking. Finally, 1.0 mL of 800 μg mL^{-1} MCP solution was added to each flask and the volume was adjusted to the mark with water after 5 min. The absorbance of each solution was measured at 460 nm against a reagent blank which is free from drug and bromate-bromide mixture.

Procedure for tablets

Twenty tablets each containing 75 mg or 50 mg of DOTH were weighed and finely powdered. An amount of the powder equivalent to 100 mg of DOTH was accurately weighed and transferred to a 100 mL calibrated 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. The first 10 mL portion of the filtrate was discarded and a suitable aliquot of the filtrate was assayed by titrimetric procedure. The same tablet extract (1 mg mL^{-1} DOTH) was appropriately diluted with water to get 20 and 40 μg mL^{-1} with respect to DOTH for the assay by the spectrophotometric methods A and B, respectively.

Procedure for the selectivity study

The selectivity of the proposed methods was evaluated by placebo blank and synthetic mixture analyses. A placebo blank of the composition: 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 under "Procedure for tablets" and then subjected to analysis by the procedures described above. A synthetic mixture was prepared by adding 100 mg of pure DOTH to 100 mg of the above mentioned placebo blank and the mixture was homogenized. Following the same procedure for tablets, the synthetic mixture solution (1 mg mL^{-1} DOTH) was prepared and a suitable quantity was subjected for analysis by the titrimetric procedure. The same synthetic mixture solution (1 mg mL^{-1} DOTH) was then diluted stepwise with water to get working concentrations of 20 and 40 μg mL^{-1} in DOTH for use in the spectrophotometric methods A and B, respectively.

Results and Discussion

Dothiepin hydrochloride is reported to undergo oxidation through the heterosulphur in the thiepin ring to form sulphone as a stable product [10]. Also, the addition of bromine to an alkene is one of the classical reactions of organic chemistry [22]. Based on these observations, the reaction between DOTH and bromine, generated *in situ* by the action of the acid on bromate-bromide mixture, uses oxidation as well as electrophilic addition reactions. The oxidation reaction will occur at the heterosulphur which will oxidize to sulphone using two moles of bromine whereas the addition reaction of one mole bromine will be added to the aliphatic carbon-carbon double bond present in the DOTH. The reaction between unreacted bromine and MCP produces a large decrease in the molar absorptivity at 540 nm and increase in the molar absorptivity at 460 nm due to the bromination of the dye at the ortho positions to the hydroxyl group (-OH) and at the meta positions to the sulphonic acid group ($-SO_3H$).

The present study describes one titrimetric and two spectrophotometric procedures for the determination of DOTH using bromine generated *in situ* as an eco-friendly agent and avoiding the use of highly toxic and hazardous liquid bromine. The proposed methods are based on the addition of a measured excess of bromate-bromide mixture in acid medium to DOTH followed by determination of the residual bromine after

the reaction between the drug and bromine is judged to be complete. In titrimetry, the reaction was followed by back titration of the unreacted bromine iodometrically, whereas in spectrophotometry, the reaction was followed by measuring the absorbance at 540 nm for method A or at 460 nm for method B (Figure 1).

In all methods, the amount of reacted bromate (*in situ* bromine) corresponded to the amount of DOTH which formed the basis of the assay. The possible reaction schemes are proposed and illustrated in Scheme 1.

Method development

The reaction variables as well as the various experimental conditions providing accurate and precise results and affecting the color development of the measured species in the spectrophotometric methods were carefully optimized.

Titrimetry

The proposed titrimetric procedure is based on the oxidation and bromination reactions between DOTH and bromine generated *in situ.* A (3-10) mg of DOTH were treated with known excess of bromate-bromide mixture in acid medium, and back titrating the unreacted bromine iodometrically after ensuring the completion of the reaction. Hydrochloric acid medium was found to be an ideal and at optimum concentration (2 mL of 3 M HCl in a total volume of 25 mL), the reaction goes to completion within 10 min. At lower acid concentration (≤ 0.5 mL of 3 M HCl) the reaction stoichiometry was slightly less than 1 and at higher acid concentration (≥ 4.0 mL of 3 M HCl) the reaction stoichiometry showed slightly higher than 1. Hence, 2 mL of 3 M HCl was found adequate to be used in the titrimetric study. The reaction was found to be complete in 10 min and contact time up to 20 min had no effect on the stoichiometry or the results. Ten milliliters volume of 5 mM $KBrO_3$-50 mM KBr was found adequate for a quantitative oxidation and bromination of DOTH in the range investigated.

Spectrophotometry

The proposed spectrophotometric methods are based on the oxidation and bromination reactions of DOTH by a measured excess of bromate-bromide mixture in HCl medium. After a predetermined time, the unreacted bromine was determined by treating it with a fixed amount of meta-cresol purple dye, and measuring the absorbance either at 540 or 460 nm. The absorbance was found to be linearly dependent on the DOTH concentration. DOTH, when added in increasing concentrations to a fixed concentration of *in situ* generated bromine, consumes the latter and there will be a concomitant decrease in the concentration of bromine. When a fixed concentration

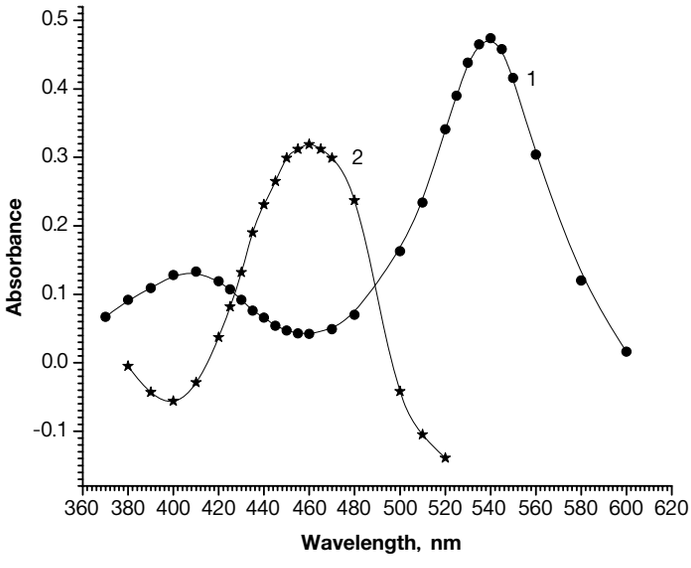
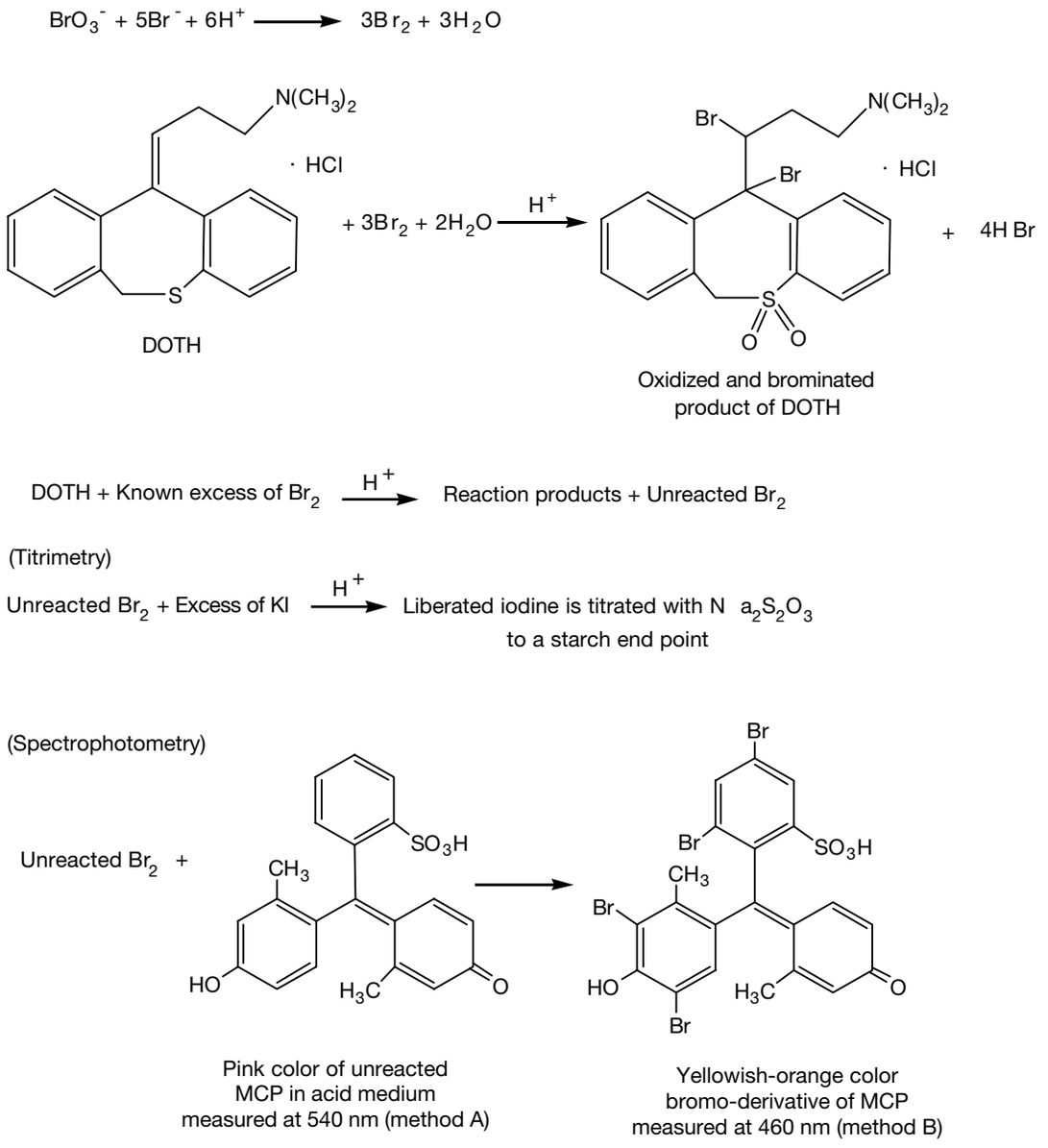


Figure 1 Absorption spectra of the measured colored species: 1. MCP in HCl (5 μg mL^{-1} of MCP) for method A, 2. Brominated product of MCP (10 μg mL^{-1} of MCP and 4.5 μg mL^{-1} of $KBrO_3$) for method B



Scheme 1 The possible reaction schemes for the proposed methods

of MCP dye is added to decreasing concentrations of bromine, a concomitant increase in the absorbance of the pink color of MCP in acid medium at 540 nm (method A) and at the same time a decrease in the absorbance of the yellowish-orange color of the bromo-derivative of MCP at 460 nm (method B) resulted, serving as basis for spectrophotometric assay.

Preliminary experiments were performed to fix the upper Beer's law limits for MCP that could be determined spectrophotometrically at 540 and 460 nm; and they were found to be 10.0 and 80.0 μg mL^{-1} MCP for method A and method B, respectively. A bromate concentration of 4.5 μg mL^{-1} in the presence of large excess of bromide was found optimum to change the pink colour of 10.0 μg mL^{-1} MCP in acid medium in method A whereas 13.5 μg mL^{-1} bromate produced a reasonable maximum absorbance at 460 nm in method B. Hence, different concentrations of DOTH were reacted with 1.0 mL each of 45 and 135 μg mL^{-1} bromate in method A and method B, respectively, in acid medium and in the presence of large excess of bromide, followed by determination of the residual bromine as described under the respective procedures. Hydrochloric acid was found to be a convenient medium for both spectrophotometric methods. In method A, the effect of (1-5 mL of 3 M HCl) was studied and the results showed that 2 mL of 3 M HCl

was optimum for the reaction between the drug and the bromine as well as the reaction between the bromine and MCP. Taking into account the maximum absorbance of the MCP color in acid medium and the minimum absorbance of the blank, 2 mL of 3 M HCl was fixed (Figure 2). In method B, 3 mL of 3 M HCl was found optimum and any excess of the acid up to 5 mL would not affect the absorbance of the bromo-derivative of MCP at the respective wavelength (Figure 2).

The reaction time between DOTH and the bromine generated *in situ* was found to be 15 min for both methods. Also, a 5 min standing time was found to be necessary for the complete bromination of MCP by the residual bromine. The absorbance of the measured species was constant for more than 24 h in both the methods, even in the presence of the reaction product.

Method validation

Quantitative data

The titrimetric procedure is applicable over the range of 3-10 mg of DOTH and the reaction stoichiometry was calculated to be 1:1 (DOTH: $KBrO_3$). In spectrophotometric procedures, linear relations were found between absorbance and concentration of DOTH in the concentration ranges given in Table 2. The calibration graphs are described by the following regression equation:

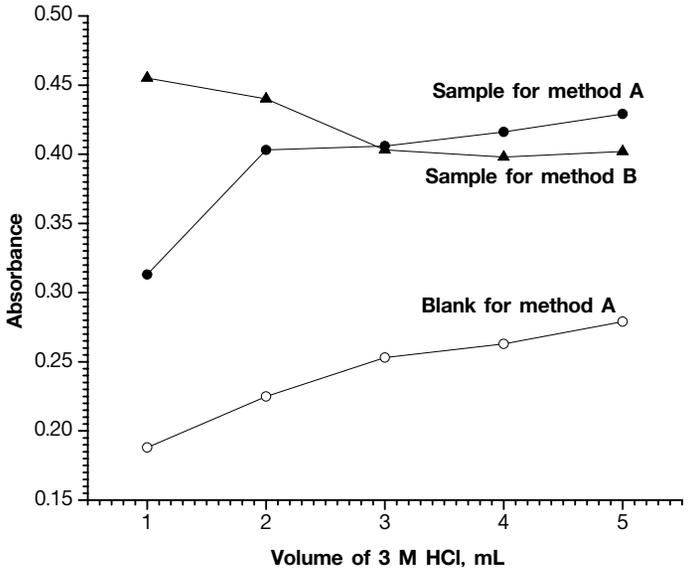


Figure 2 Effect of acid on the color development of the measured species

Table 2 Analytical and regression parameters of the spectrophotometric methods

Parameter	Method A	Method B
λ_{max}, nm	540	460
Beer's law limits, μg mL^{-1}	0.5-7.0	1.0-16.0
Molar absorptivity, l mol^{-1} cm^{-1}	3.65×10^4	1.65×10^4
Sandell sensitivity*, μg cm^{-2}	0.0091	0.0201
Limit of detection, μg mL^{-1}	0.27	0.75
Limit of quantification, μg mL^{-1}	0.83	2.27
Regression equation, Y**		
Intercept, (a)	0.0017	0.8240
Slope, (b)	0.1110	-0.0447
Correlation coefficient, (r)	0.9991	-0.9993
Standard deviation of intercept (S_a)	0.0098	0.0076
Standard deviation of slope (S_b)	0.0023	0.0008

*Limit of determination as the weight in μg per mL of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm., **$Y = a + bX$, where Y is the absorbance and X concentration in μg mL^{-1}

$$Y = a + bX \quad (2)$$

where Y is an absorbance, a is an intercept, b is a slope and X is a concentration in μg mL^{-1}, obtained by the method of least squares.

Correlation coefficients, intercepts and slopes for the calibration data are summarized in Table 2. Sensitivity parameters such as molar absorptivity, Sandell sensitivity values, the limits of detection and quantification are also presented in Table 2 and speak of the excellent sensitivity of the proposed methods.

Precision and accuracy

In order to determine the precision of the proposed methods, solutions containing three different concentrations of DOTH were prepared and analyzed in seven replicates and the analytical results are summarized in Table 3.

The low values of the relative standard deviation (% RSD ≤ 2.66) and percentage relative error (% RE ≤ 3.25) also indicated the high precision and the good accuracy of the proposed methods. RSD (%) and RE (%) values were obtained within the same day to evaluate repeatability (intra-day precision) and over five days to evaluate intermediate precision (inter-day precision).

Selectivity

The selectivity of the proposed methods for the analysis of DOTH was evaluated by analysis of placebo blank solution as shown under "Procedure for tablets" and the titrant value as well as the measured absorbance with respect to the blank were same as reagent blank, inferring no interference from the placebo. A separate test was performed by applying the proposed methods to the determination of DOTH in a synthetic mixture and the percent recoveries of DOTH were 101.42 ± 1.24, 99.04 ± 2.16 and 98.77 ± 2.65 (n=5) by titrimetric, spectrophotometric methods A and B, respectively, suggesting that no significant interference by the excipients in the assay of DOTH under the described optimum conditions.

Robustness and ruggedness

The evaluation of the robustness of the methods was done by making small incremental changes in two experimental variables, volume of HCl and reaction time, and performing the analysis under the altered experimental conditions. The effect of the changes on the titrant value in titrimetric method and the absorbance reading in the spectrophotometric methods was studied and found to be negligible confirming the robustness of the proposed methods (Table 4). Ruggedness of the proposed methods was expressed as % RSD of the same procedure applied by three different analysts and also by a single analyst performing the analysis on three different burettes (titrimetric method) or cuvettes (spectrophotometric methods). The results presented in Table 4 showed that no statistical differences between different analysts and

Table 3 Intra-day and inter-day precision and accuracy studies

Method*	DOTH taken	Intra-day (n=7)			Inter-day (n=5)		
		DOTH found[a]	% RSD[b]	% RE[c]	DOTH found[a]	% RSD[b]	% RE[c]
Titrimetry	5.00	5.04	1.02	0.80	5.06	0.93	1.20
	7.00	7.08	1.36	1.14	7.10	1.76	1.43
	9.00	9.16	2.01	1.78	9.19	1.98	2.11
Spectrophotometric	2.00	2.05	1.07	2.50	2.06	1.96	3.00
(method A)	4.00	4.11	1.61	2.75	4.13	2.04	3.25
	6.00	6.10	2.10	1.67	6.17	1.48	2.83
Spectrophotometric	4.00	4.08	1.86	2.00	4.11	1.93	2.75
(method B)	8.00	7.95	2.13	0.62	7.90	2.09	1.25
	12.00	12.25	2.36	2.08	12.29	2.66	2.42

* DOTH taken/found in titrimetric method is in mg and in spectrophotometric methods are in μg mL^{-1}

[a]Mean value of five determinations, [b]Relative standard deviation (%), [c]Bias % : [(found-taken)/taken)] × 100

Table 4 Robustness and ruggedness of the proposed methods

Method	DOTH taken*	Robustness (% RSD)		Ruggedness (% RSD)	
		Volume of 3 M HCl**	Reaction time, min***	Inter analysts (n=3)	Inter Instruments (n=3)
Titrimetric method	5.00	1.45	1.02	1.32	2.55
	7.00	1.03	0.76	0.94	1.88
	9.00	0.83	1.16	2.17	2.34
Spectrophotometric	2.00	1.66	2.00	1.08	3.11
method A	4.00	2.04	1.45	2.21	2.09
	6.00	1.13	0.95	1.83	2.44
Spectrophotometric	4.00	0.78	1.46	2.66	3.02
method B	8.00	0.96	1.84	1.95	2.61
	12.00	1.37	0.72	2.33	2.19

* In titrimetric method, DOTH taken is in mg and in spectrophotometric methods is in $\mu g\ mL^{-1}$

** The volume of 3 M HCl was 1.8, 2.0 and 2.2 mL for titrimetric method and spectrophotometric method A and 2.8, 3.0 and 3.2 mL for the spectrophotometric method B

*** The reaction time was 9, 10 and 11 min for titrimetric method and was 14, 15 and 16 min for both spectrophotometric methods

Table 5 Results of assay of DOTH formulations using the proposed methods and statistical evaluation

Tablet brand name	Nominal amount	Found (% of nominal amount ± SD)*			
		Official method**	Proposed methods		
			Titrimetric method	Spectrophotometric methods	
				Method A	Method B
Prothiaden	75 mg	99.54 ± 0.97	101.8 ± 1.34	99.07 ± 1.53	98.80 ± 1.26
			t = 3.05	**t** = 0.58	**t** = 1.04
			F = 1.91	**F** = 2.49	**F** = 1.69
Dothip	50 mg	99.26 ± 0.67	100.6 ± 1.07	97.94 ± 1.62	98.26 ± 0.95
			t = 2.37	**t** = 1.68	**t** = 1.92
			F = 2.55	**F** = 5.85	**F** = 2.01

* Mean value of five determinations

** This method is the official method for DOTH raw material

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

instruments suggesting the ruggedness of the proposed methods.

Application to tablets analysis

The proposed methods were applied to determine DOTH in two brands of tablets with two different doses. The results of this study were presented in Table 5 and statically compared with those obtained by the official British Pharmacopoeial method [3] for accuracy and precision by applying the Student's t-test and variance ratio F-test. The official method [3] described a non-aqueous titration of the drug with acetous perchloric acid and determining the end-point potentiometrically. The calculated t and F-values (Table 5) did not exceed the tabulated values of 2.78 and 6.39, respectively, indicating no significant difference between the proposed methods and the official method in terms of accuracy and precision.

Recovery study

Accuracy of the proposed methods was further confirmed by standard-addition procedure. Pre-analyzed tablet powder (Prothiaden 75 mg and Dothip 50 mg) was spiked with pure DOTH at three different concentration levels (50, 100 and 150% of the quantity present in the tablet powder) and the total was measured by the proposed methods. The determination with each level was repeated three times and the

Table 6 Results of recovery experiments using the standard addition method

Method	Tablet studied	DOTH in tablet[a]	Pure DOTH added[a]	Total found[a]	Pure DOTH recovered[b], (Percent ± SD)
Titrimetric method	Prothiaden	3.05	1.50	4.60	103.33 ± 1.03
	(75 mg)	3.05	3.00	6.10	101.67 ± 2.15
		3.05	4.50	7.66	102.44 ± 2.26
	Dothip	4.02	2.00	5.97	97.50 ± 1.64
	(50 mg)	4.02	4.00	7.99	99.25 ± 1.99
		4.02	6.00	9.93	98.50 ± 2.43
Spectrophotometric	Prothiaden	1.98	1.00	2.97	99.00 ± 2.77
(method A)	(75 mg)	1.98	2.00	4.07	104.50 ± 2.06
		1.98	3.00	5.09	103.67 ± 1.88
	Dothip	1.96	1.00	2.97	101.00 ± 1.16
	(50 mg)	1.96	2.00	4.01	102.50 ± 1.22
		1.96	3.00	4.94	99.33 ± 1.49
Spectrophotometric	Prothiaden	3.95	2.00	5.92	98.50 ± 2.22
(method B)	(75 mg)	3.95	4.00	7.96	100.25 ± 1.61
		3.95	6.00	9.79	97.33 ± 2.40
	Dothip	5.90	3.00	8.94	101.33 ± 1.65
	(50 mg)	5.90	6.00	11.75	97.50 ± 1.37
		5.90	9.00	14.73	98.11 ± 2.01

[a]mg in titrimetry and μg mL^{-1} in spectrophotometric methods

[b]Mean value of three measurements

results presented in Table 6 indicated that the commonly excipients present in the formulations did not interfere in the assay.

Conclusion

The present paper describes one titrimetric and two spectrophotometric methods for the determination of DOTH in pharmaceuticals. The assay results demonstrated that it is possible to use bromate-bromide mixture as an environmentally friendly reagent and meta-cresol purple for the direct titrimetric and indirect spectrophotometric determination of DOTH in authentic samples. The titrimetric method is much simpler method and it is applicable over a micro range (3-10 mg DOTH), yet provides very accurate and precise results. Unlike most of the existing spectrophotometric methods, the proposed spectrophotometric procedures are sensitive, simple, use eco-friendly chemicals, free from organic solvents and unwelcome steps such as heating or extraction and also from critical pH conditions. The spectrophotometric method A is more sensitive than the spectrophotometric method B as can be seen from the molar absorptivity values of both methods. The proposed methods rely on the use of cheap and readily available chemicals, and inexpensive techniques and have the advantages of simplicity, cost-effectiveness and easily accessible technique in under-developed and developing countries. These advantages coupled with good accuracy and precision make the proposed methods highly suitable for routine use in laboratories as a part of industrial quality control.

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References

[1] The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 14th Ed., Merck & Co., Inc., Whitehouse Station, NJ, 2006, p. 580.

[2] N. T. Abdel-Ghani, R.M. El-Nashar, and A.A. Bioumy. Flow injection potentiometric determination of dothiepin hydrochloride, *Anal. Lett.* 37: 3237-3254 (2004).

[3] The British Pharmacopoeia, Vol. I, Her Majesty Stationery office, London, 2003, p. 671.

[4] R. T. Sane, R. V. Tendolkar, D. P. Gangal, K. D. Ladage, and R. M. Kothurkar. High-performance liquid-chromatographic determination of dothiepin hydrochloride from pharmaceutical preparations, *Indian J. Pharm. Sci.* 51: 61-62 (1989).

[5] K. Šlais, and J. Šubert. Determination of cis-and trans-isomers of dosulepine and dithiadene by high-performance liquid chromatography, *J. Chromatogr. A* 191: 137-143 (1980).

[6] W. P. A. Li, and W. J. Irwin. A high performance liquid chromatographic assay of cis-and trans-isomers of tricyclic neuroleptic drugs, *J. Pharm. Pharmacol.* 31: 512-516 (1979).

[7] Z. Pawlak, D. Kay, and B. J. Clark. Assay of dothiepin hydrochloride and its isomers by liquid chromatography, *Anal. Proc. (London)* 27: 16-18 (1990).

[8] Z. Pawlak, and B. J. Clark. Assay of dothiepin hydrochloride and its geometric isomers by liquid chromatography, *J. Pharm. Biomed. Anal.* 7: 1903-1907 (1989).

[9] B. J. Clark, P. Barker, and T. Large. The determination of the geometric isomers and related impurities of dothiepin in a pharmaceutical preparation by capillary electrophoresis, *J. Pharm. Biomed. Anal.* 10: 723-726 (1992).

[10] E. Bishop, and W. Hussein. Electroanalytical study of tricyclic antidepressants, *Analyst* 109: 73-80 (1984).

[11] M. M. Hosny. Quantitative analysis of dothiepin HCl by ion selective electrode, *Taiwan Pharm. J.* 59: 25-30 (2007).

[12] A. F. A. Youssef. Application of ion associate formation for conductimetric determination of dothiepin hydrochloride in pharmaceutical formulations by using trioxalatocomplexes, *Sci. Pharm.* 73: 1-15 (2005).

[13] N. T. Abdel-Ghani, R. M. El-Nashar, and A. A. Bioumy. Conductimetric determination of the antidepressants amitriptyline and dothiepin hydrochlorides and tranylcypromine hemisulphate in their pharmaceutical formulations, *FABAD J. Pharm. Sci.* 29: 195-201 (2004).

[14] H. E. Abdellatef, M. M. El-Henawee, H. M. El-Sayed, and M. M. Ayad. Spectrophotometric and spectrofluorimetric methods for analysis of tramadol, acebutolol and dothisepin in pharmaceutical preparations, *Spectrochim. Acta Part A* 65: 1087-1092 (2006).

[15] M. I. Walash, F. Belal, N. El-Enany, and H. Elmansi. Spectrophotometric and spectrofluorimetric methods for the determination of dothiepin hydrochloride in its pure and dosage forms using eosin, *Int. J. Biomed. Sci.* 6: 327-334 (2010).

[16] E. A. Taha. Kinetic spectrophotometric methods for the determination of dothiepin hydrochloride in bulk and in drug formulation, *Anal. Bioanal. Chem.* 376: 1131-1136 (2003).

[17] R. T. Sane, R. M. Kothurkar, K. D. Ladage, R. V. Tendolkar, and D. P. Gangal. An extractive colorimetric method for the determination of dothiepin hydrochloride from pharmaceutical preparations, *Indian J. Pharm. Sci.* 50: 345-346 (1988).

[18] E. A. Taha, S. M. Soliman, H. E. Abdellatef, and M. M. Ayad. Colorimetric methods for the determination of some tricyclic antidepressant drugs in their pure and dosage forms, *Microchim. Acta* 140: 175-182 (2002).

[19] W. E. Hassan. Extractive colorimetric method for the determination of dothiepin hydrochloride and risperidone in pure and in dosage forms, *Chem. Pharm. Bull.* 56: 1092-1096 (2008).

[20] M. M. Hosny. Spectrophotometric determination of diltiazem, fluphenazine and dothiepin hydrochlorides using chromotrope 2R and rose bengal, *Mansoura J. Pharm. Sci.* 20: 64-77 (2004).

[21] K. Basavaiah, K. Tharpa, S.G. Hiriyanna, and K.B. Vinay. Spectrophotometric determination of lisinopril in pharmaceuticals using ninhydrin-a modified approach, *J. Food Drug Anal.* 17: 93-99 (2009).

[22] F.A. Carroll, Perspectives on structure and mechanism in organic chemistry, 2nd Ed., Wiley-Interscience, 2010, p. 553.