

RESEARCH ARTICLE

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Tablet Dosage Forms**

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Spectrophotometric Analysis of Dutasteride in Pure and Tablet Dosage Forms

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Abstract

Four simple and sensitive visible spectrophotometric methods have been developed for the assay of dutasteride. Method I describes the interaction of dutasteride, as an *n*-electron donor, with chloranil, as a *p*-acceptor, in acetonitrile to give reddish orange colored chloranil radical anion with absorption maxima at 525 nm. Method II involves the oxidative coupling reaction of dutasteride with 3-methyl-2-benzothiazolinone hydrazone hydrochloride in the presence of ferric chloride in an acidic medium, which results in a green colored product with absorption maxima at 550 nm. Methods III and IV are based on the formation of ion-pairs of dutasteride with bromothymol blue and bromophenol blue, which are extracted into chloroform and exhibit absorption maxima at 425 (bromothymol blue) and 435 nm (bromophenol blue). Regression analysis of Beer's law plot showed good correlation in the concentration range of 2–40, 1–20, 5–50, and 2–20 $\mu\text{g mL}^{-1}$ for Methods I, II, III, and IV, respectively. Different variables affecting the reaction were studied and the experimental conditions were optimized. The proposed methods were applied successfully to analyze dutasteride in tablets dosage forms. No interference was observed from common pharmaceutical excipients.

Keywords: Dutasteride; Chloranil; MBTH; Bromothymol blue; Bromophenol blue.

1. Introduction

Dutasteride (DSE) [1–8], chemically known as (5 α , 17 β)-N-{2,5 bis(trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide (Figure 1), is a synthetic 4-azasteroid compound with antiandrogenic activity. DSE is used to treat benign prostatic hyperplasia in men having an enlarged prostate gland and in the treatment of male pattern baldness. It belongs to a class of drugs called 5 α -reductase inhibitors, which competitively and specifically inhibits type 1 (active in the sebaceous glands of most regions of skin and liver) and type 2 (primarily active in the reproductive tissues like prostate, seminal vesicles, epididymides, hair follicles and liver) isoforms of 5 α -reductase, an intracellular enzyme that converts testosterone to 5 α -dihydrotestosterone. The decrease in dihydrotestosterone levels may mitigate or prevent enlargement of the prostate gland. DSE does not bind to the human androgen receptor.

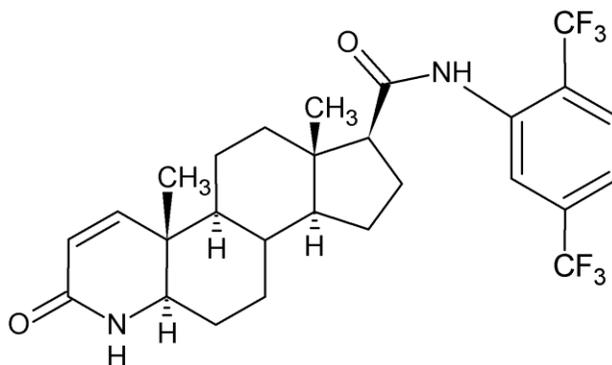


Figure 1: Structure of dutasteride.

A limited number of analytical techniques have been reported for the quantitative determination of DSE in pharmaceutical preparations and human plasma when present alone or in combination with other drugs (Alfuzosin and Tamsulosin). These techniques are LC–MS [9–11], HPTLC [12], Enzyme-linked immunosorbent assay [13], HPLC and stability-indicating RP-HPLC [14–18]. Though the above-mentioned techniques are sensitive, they are found to be relatively complicated, expensive and are not accessible for many laboratories in developing and underdeveloped countries. Visible spectrophotometry is considered as the most convenient analytical technique because of its inherent simplicity, enhanced sensitivity, reasonable accuracy and precision, inexpensive and wide availability in most of the quality control laboratories. To the best of our knowledge, no visible spectrophotometric methods have been reported for the quantitation of the DSE in tablet dosage forms. Kamila *et al.* and Ruhul *et al.* have reported UV spectrophotometric method for the quantification of DSE in pharmaceutical formulations [19, 20].

Charge-transfer complex results from a donor–acceptor mechanism of Lewis acid–base reaction between two or more different chemical constituents. The formation of charge-transfer complex can be rapidly assessed for its validity as a simple quantitative analytical method for many drug substances, which can act as electron donors. Chloranil (π acceptor) has been investigated spectrophotometrically and has been successfully utilized in the determination of a variety of electron-donating basic compounds [21–28].

3-Methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) is one of the widely used chromogenic reagents for spectrophotometric analysis of phenols [29]. It undergoes oxidative coupling reaction with phenolic, amino, ketonic and aldehydic compounds in the presence of an oxidizing agent such as H_2O_2 , cerium(IV), iron(III), chromium(VI) yielding a highly colored reaction products [29]. The MBTH had been used for the spectrophotometric determination of many pharmaceutical compounds [30–37].

The ion-pair complex is a special form of molecular complex resulting from two oppositely charged ions extractable into organic solvents from aqueous phase at suitable pH. The ion-pair extractive spectrophotometry has been applied for the estimation of numerous compounds; possessing basic moieties (secondary or tertiary amino group) by using an anionic dye as a reagent and organic solvent as an extractant. Bromophenol blue and bromothymol blue being anionic dyes have been reported to form ion-pair complexes thus offering simple and rapid spectrophotometric determination of therapeutically significant pharmaceutical compounds [38–50].

This paper, for the first time, deals with the use of the above four reagents for the spectrophotometric quantification of DSE in bulk and tablet dosage forms. Method I is based on the formation of charge-transfer complex of the DSE as n -electron donor and with chloranil as π -acceptor, to give colored species. Method II involves the oxidative coupling reaction of DSE with 3-methyl-2-benzothiazolinone hydrazone hydrochloride in the presence of Fe(III) in an acidic medium to form colored product. Methods III and IV are based on the formation of ion-pair association complexes of DSE with bromothymol blue and bromophenol blue respectively, in chloroform medium. The proposed methods are validated according to the ICH Guidelines with respect to linearity, limit of detection, limit of quantification, accuracy, precision and recovery [51]. These proposed methods can be used in laboratories of underdeveloped and developing nations that cannot afford modern expensive instrumental set ups like LC–MS, HPTLC or HPLC.

2. Methods

2.1. Instrumentation

All absorbance measurements were carried out using an ELICO double-beam model SL 159 digital spectrophotometer (Hyderabad, India) with 1-cm matched quartz cells.

2.2. Reagents and chemicals

All the chemicals used were of analytical reagent grade and used as received. Double-distilled water was used in the preparation of all solutions. All the solutions were prepared afresh daily.

2.2.1. Method I

2.033×10^{-2} M Chloranil (CRL): Prepared by dissolving 500 mg of chloranil (Merck, Mumbai, India) in 100 mL of acetonitrile (Merck, Mumbai, India).

2.2.2. Method II

- 9.271×10^{-3} M 3-Methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH): Prepared by dissolving 200 mg of 3-methyl-2-benzothiazolinone hydrazone hydrochloride (Merck, Mumbai, India) in 100 mL of water.
- 0.5 N HCl: Prepared by diluting 1.825 mL of HCl (Fisher Scientific, Mumbai, India) to 100 mL of distilled water.
- 4.315×10^{-2} M Ferric chloride: Prepared by dissolving 700 mg of ferric chloride (SD Fine-Chem Limited, Mumbai, India) in 100 mL of water.

2.2.3. Methods III and IV

- 3.094×10^{-3} M Bromothymol blue (BTB): Prepared by dissolving 200 mg of bromothymol blue (SD Fine-Chem Limited, Mumbai, India) in 100 mL of distilled water (Method III).
- 2.890×10^{-3} M Bromophenol blue (BPB): Prepared by dissolving 200 mg of bromophenol blue (SD Fine-Chem Limited, Mumbai, India) in 100 mL of distilled water (Method IV).
- 0.1 N HCl: Prepared by diluting 0.365 mL of HCl to 100 mL of distilled water.
- Chloroform (SD Fine-Chem Limited, Mumbai, India).

2.3. Standard solutions of dutasteride

Pharmaceutical grade DSE was kindly gifted by local pharmaceutical company. A stock standard solution containing 1 mg mL^{-1} of DSE was prepared in methanol. Working standard solution equivalent to $100 \text{ } \mu\text{g mL}^{-1}$ (for Methods II and IV), $200 \text{ } \mu\text{g mL}^{-1}$ (for Method I) and $250 \text{ } \mu\text{g mL}^{-1}$ (for Method III) of DSE was obtained by appropriate dilution of stock solution with methanol (Methods I and II) and water (Methods III and IV).

2.4. Tablet dosage forms of dutasteride

Tablet dosage forms of DSE such as Duprost (0.5 mg/tablet, Cipla Ltd., Mumbai, India), Dutas (0.5 mg/tablet, Dr. Reddy's Lab. Ltd., Hyderabad, India) and Sterdu (0.5 mg/tablet, Alkem Lab. Ltd., Mumbai, India) were purchased from local pharmacy market.

2.5. General analytical procedures

2.5.1. Method I (Charge-transfer complexation with CRL)

Aliquots of (0.1–2.0 mL) standard drug solution ($200 \text{ } \mu\text{g mL}^{-1}$) of DSE were pipetted into a series of 10 mL standard volumetric flasks and the volume in each flask was brought to 2 mL by adding acetonitrile. Then 2 mL of 2.033×10^{-2} M CRL was added to each flask. The contents of each flask were mixed well and allowed to stand at room temperature ($25 \pm 1^\circ\text{C}$) for 10 min. The volume was made up to the mark with acetonitrile. The absorbance of the reddish orange colored species was measured at 525 nm against the reagent blank prepared similarly omitting the drug.

2.5.2. Method II (Oxidative coupling with MBTH)

Aliquots of (0.1–2.0 mL) standard drug solution ($100 \text{ } \mu\text{g mL}^{-1}$) of DSE were pipetted into a series of 10 mL standard volumetric flasks and the volume in each flask was brought to 2 mL by adding methanol. To each flask, 1.5 mL of 9.271×10^{-3} M MBTH, 1 mL of 0.5 N HCl and 1 mL of 4.315×10^{-2} M FeCl_3 were added. The contents of each flask were mixed well and allowed to stand at room temperature ($25 \pm 1^\circ\text{C}$) for 5 min. The volume was made up to the mark with methanol and the absorbance of the green colored species was measured at 550 nm against the reagent blank prepared similarly omitting the drug.

2.5.3. Method III (Ion-pair association with BTB)

Aliquots of (0.2–2.0 mL) standard drug solution ($250 \text{ } \mu\text{g mL}^{-1}$) of DSE were transferred into a series of 100 mL separating funnels. The volume in each separating funnel was adjusted to 2 mL with distilled water. Now, 1 mL of 0.1 N HCl and 2 mL of 3.094×10^{-3} M BTB were added to each separating funnel. The funnels were shaken vigorously with 10 mL of chloroform for 2 min, and then allowed to stand at room temperature ($25 \pm 1^\circ\text{C}$) for clear separation of the two phases. The separated organic phase was transferred to a 10 mL volumetric flask. Then the extract was made up to the mark with chloroform and mixed well. The absorbance of the yellow colored complex was measured at 425 nm against a reagent blank prepared similarly omitting the drug.

2.5.4. Method IV (Ion-pair association with BPB)

Aliquots of (0.2–2.0 mL) standard drug solution ($100 \mu\text{g mL}^{-1}$) of DSE were transferred into a series of 100 mL separating funnels. The volume in each separating funnel was adjusted to 2 mL with distilled water. Now, 1 mL of 0.1 N HCl and 2 mL of 2.890×10^{-3} M BPB were added to each separating funnel. The funnels were shaken vigorously with 10 mL of chloroform for 2 min, and then allowed to stand at room temperature ($25 \pm 1^\circ\text{C}$) for clear separation of the two phases. The separated organic phase was transferred to a 10 mL volumetric flask. Then the extract was made up to the mark with chloroform and mixed well. The absorbance of the yellow colored complex was measured at 435 nm against a reagent blank prepared similarly omitting the drug.

2.5.5. Reference method [19]

Absorption maximum of DSE ($25 \mu\text{g mL}^{-1}$) in methanol was determined by scanning the drug solution from 200 to 400 nm and was found to be at 240 nm. Into a series of 10 mL volumetric flasks, different volumes (0.2–2.0 mL) of DSE standard solution ($250 \mu\text{g mL}^{-1}$) equivalent to 5–50 $\mu\text{g mL}^{-1}$ of the drug were transferred and diluted to the mark with methanol. The absorbance of the solution was measured at 240 nm against the blank prepared similarly omitting the drug

In all the above procedures, the calibration curves were constructed by plotting the absorbance *versus* final concentration of DSE. The concentration of the drug was read from the standard graph or computed from the respective regression equation.

2.6. Analysis of dutasteride in tablet dosage forms

Fifty tablets were weighed accurately and ground into a fine powder. An amount of powder equivalent to 25 mg of DSE was weighed into a 25 mL volumetric flask, 15 mL of methanol was added and shaken thoroughly for about 10 min, then the volume was diluted up to the mark with the same solvent, mixed well and filtered using a quantitative filter paper. The filtered solution was appropriately diluted with methanol (Methods I, II, and reference) or water (Methods III and IV). Convenient aliquots were subjected to analysis by the procedures described in Methods I–IV and reference method.

3. Results and Discussion

3.1. Absorption maxima of the colored products

The absorption maxima of the colored products produced in Methods I, II, III, and IV were determined by scanning them in the wavelength region of 380–760 nm against a corresponding reagent blank. The absorption maxima of the colored products were found to be 525, 550, 425, and 435 nm for Methods I, II, III, and IV, respectively (Figure 2). Under the optimized experimental conditions, reagent blank of Methods I–IV showed negligible absorbance at the corresponding optimum wavelength.

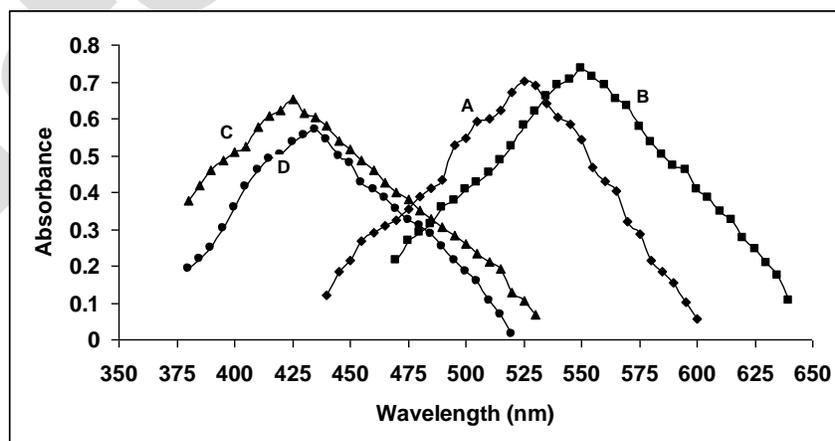


Figure 2: A: Absorption spectra of chloranil radical anion ($\lambda_{\text{max}} = 525 \text{ nm}$, DSE $20 \mu\text{g mL}^{-1}$). B: MBTH-DSE complex ($\lambda_{\text{max}} = 550$, DSE $10 \mu\text{g mL}^{-1}$ nm). C: BTB-DSE ion-pair complex ($\lambda_{\text{max}} = 425 \text{ nm}$, DSE $25 \mu\text{g mL}^{-1}$). D: BPB-DSE ion-pair complex ($\lambda_{\text{max}} = 435 \text{ nm}$, DSE $10 \mu\text{g mL}^{-1}$).

3.2. Optimization of reaction conditions

The optimum reaction conditions for the quantitative determination of the dutasteride were achieved through a number of preliminary experiments. The optimum conditions for color development of each method were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species. The optimum values of the reaction conditions were maintained throughout the experiment.

3.2.1. Method I (Charge-transfer complexation with CRL)

3.2.1.1. Effect of concentration of chloranil

The influence of the volume of 2.033×10^{-2} M CRL on the intensity of the color developed at constant DSE concentration ($20 \mu\text{g mL}^{-1}$) was examined in the range 0.5–5 mL of CRL. The maximum absorbance was obtained with 2 mL of CRL; above this volume the absorbance remained unchanged. Therefore, 2 mL of 2.033×10^{-2} M CRL was used in all further measurements.

3.2.1.2. Effect of time and stability of the colored species

To optimize the reaction time for color development, 2 mL of 2.033×10^{-2} M CRL and 1 mL of DSE ($20 \mu\text{g}$) were added and kept at room temperature for varied time. The maximum intensity of color was obtained at 10 min and remained constant up to 4.5 hr. Therefore, 10 min of reaction time was used throughout the determination process.

3.2.1.3. Effect of diluting solvent

Different diluting solvents like dichloromethane, methanol, ethanol, propanol, butanol, acetonitrile and chloroform were tested for appropriate dilution. The highest color intensity was attained when acetonitrile was used as the diluting solvent.

3.2.2. (II) Method II (Oxidative coupling with MBTH)

3.2.2.1. Effect of concentration of 3-methyl-2-benzothiazolinone hydrazone hydrochloride

The effect of the concentration of MBTH on the color development was studied by adding different volumes (0.5–5.0 mL) of 9.271×10^{-3} M MBTH to 1 mL of DSE ($10 \mu\text{g mL}^{-1}$). It was found that the maximum absorbance of the color was reached with 1.5 mL of the MBTH and remained constant with higher volumes. Therefore, 1.5 mL of the 9.271×10^{-3} M MBTH was chosen as an optimum value.

3.2.2.2. Effect of concentration of Ferric chloride

The influence of the volume of 4.315×10^{-2} M FeCl_3 on the formation of color was studied. This was performed by adding different volumes (0.2–2.0 mL) of 4.315×10^{-2} M FeCl_3 to 1 mL of DSE ($10 \mu\text{g}$). The maximum absorbance was obtained with 1 mL of 4.315×10^{-2} M FeCl_3 . Above this volume, the absorbance remained constant. Therefore, the above said volume was used for all the measurements.

3.2.2.3. Effect of HCl

The influence of acidity on the reaction of MBTH with dutasteride in the presence of FeCl_3 was studied by adding different volumes (0.2–1.0 mL) of 0.5 N HCl to a fixed concentration of DSE ($10 \mu\text{g mL}^{-1}$). It was observed that the maximum absorbance was found with 1 mL of 0.5 N HCl, beyond which the absorbance was decreased. Thus, 1 mL of 0.1 N HCl was chosen for the procedure.

3.2.2.4. Effect of time and stability of the colored species

To optimize the reaction time for color development, 1.5 mL of 9.271×10^{-3} M MBTH, 1 mL of 4.315×10^{-2} M FeCl_3 , 1 mL of 0.1 N HCl and 1 mL of DSE ($10 \mu\text{g}$) were added and kept at room temperature for varied time. The maximum intensity of color was obtained at 5 min and remained constant up to 15 hr. Therefore, 5 min of reaction time was used throughout the determination process.

3.2.3. (III) Methods III and IV (Ion-pair association with BTB and BPB)

3.2.3.1. Effect of concentration of bromothymol blue

To optimize the concentration of BTB, different volumes (0.5–5.0 mL) of 3.094×10^{-3} M BTB were added to the mixture under study. It was found that 2 mL of BTB was sufficient for maximum and stable ion-pair complex formation. The absorbance decreased at lower concentration of 3.094×10^{-3} M BTB, whereas no change in absorbance was observed at higher concentration.

3.2.3.2. Effect of concentration of bromophenol blue

To study the effect of the volume of the BPB on the absorbance of the ion-pair complex, varying volumes of (0.5–5.0 mL) 2.890×10^{-3} M BPB were mixed with 1 mL of DSE (10 μ g). The results showed that the highest absorbance was obtained with 2 mL, which remained unaffected by further addition of BPB. Hence, 2 mL of 2.890×10^{-3} M BPB was used for the determination.

3.2.3.3. Effect of HCl

The influence of acidity on the development and stability of the color in Methods III and IV using different volumes (0.2–2.0 mL) of 0.1 N HCl was tested in this study. The maximum color intensity was observed with 0.1 N HCl and therefore 1 mL of 0.1 N HCl, where maximum absorbance was achieved, was used throughout the experiment.

3.2.3.4. Effect of extracting solvent

Several organic solvents such as dichloromethane, chloroform, carbon tetrachloride, and butanol were examined for their ability to extract the drug–dye ion-pair in Methods III and IV. The chloroform was found to be the most suitable solvent in terms of higher sensitivity and considerably lower extraction of the dye.

3.2.3.5. Effect of time and stability of the colored species

The reaction time after the addition of BTB (Method III) or BPB (Method IV) was also examined. It was found that 2-min reaction time was sufficient for the complete formation of the ion-pair complex in both the methods. The absorbance of the formed ion-pair complex was observed to be stable for 10 and 13 hr, in Methods III and IV respectively, at room temperature.

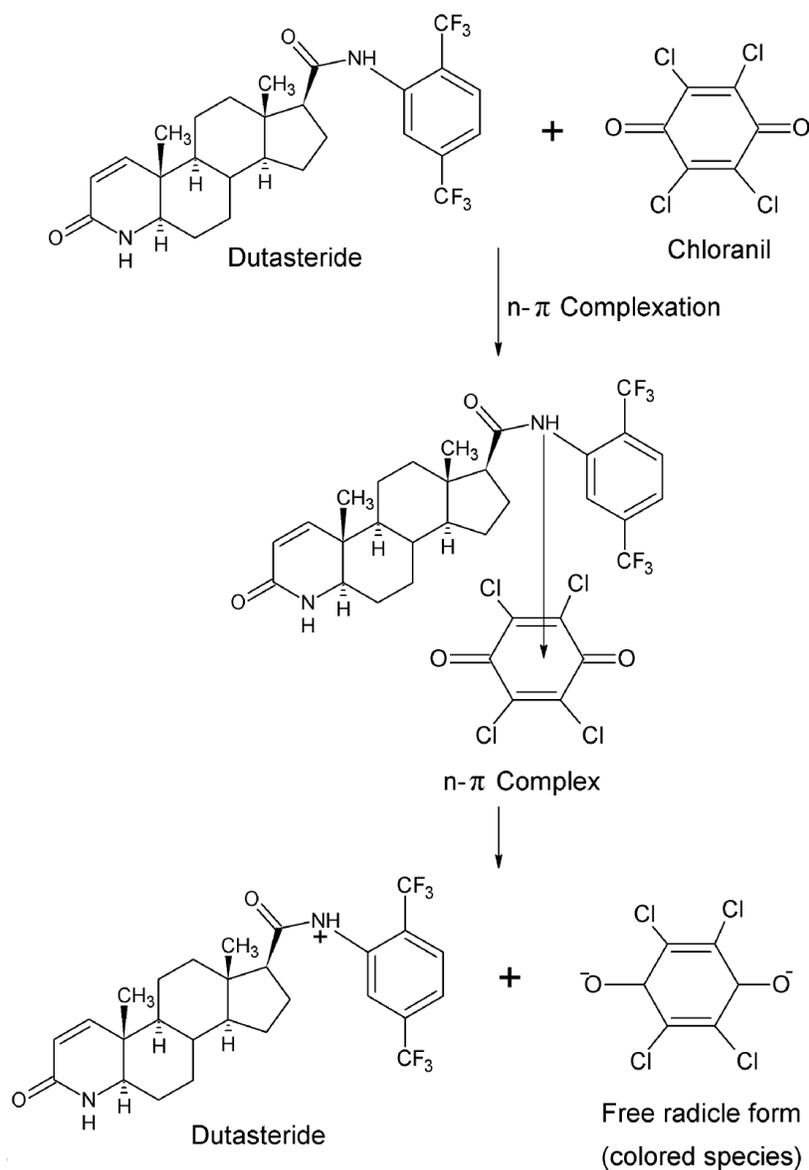
3.3. Reaction mechanism

3.3.1. Method I (Charge-transfer complexation with CRL)

The results obtained in Method I were due to the charge-transfer reaction between the DSE and CRL to yield a reddish orange colored product having maximum absorption at a wavelength of 525 nm against the corresponding reagent blank. Chloranil is a π electron acceptor as a result of the strong electron withdrawing halo- and cyano-groups conjugated with the π -system. The DSE has secondary amino group, which act as n -electron donor. Therefore, the DSE reacts with electron acceptor to form charge-transfer complex or radical anion. The charge-transfer complex is formed through the lone pair of electrons donated by the DSE as n -donor and the CRL as an electron acceptor in which a partial ionic bond was assumed to be formed. This interaction was particularly strong on using CRL so that it involves a complete transfer of electronic charge with the formation of a free radical anion. These radical anions formed were the predominant chromogens in the reaction. The high-ionizing power of acetonitrile promotes the dissociation of the donor–acceptor complex in these reactions. A general reaction mechanism is proposed in Scheme 1.

3.3.2. Method II (Oxidative coupling with MBTH)

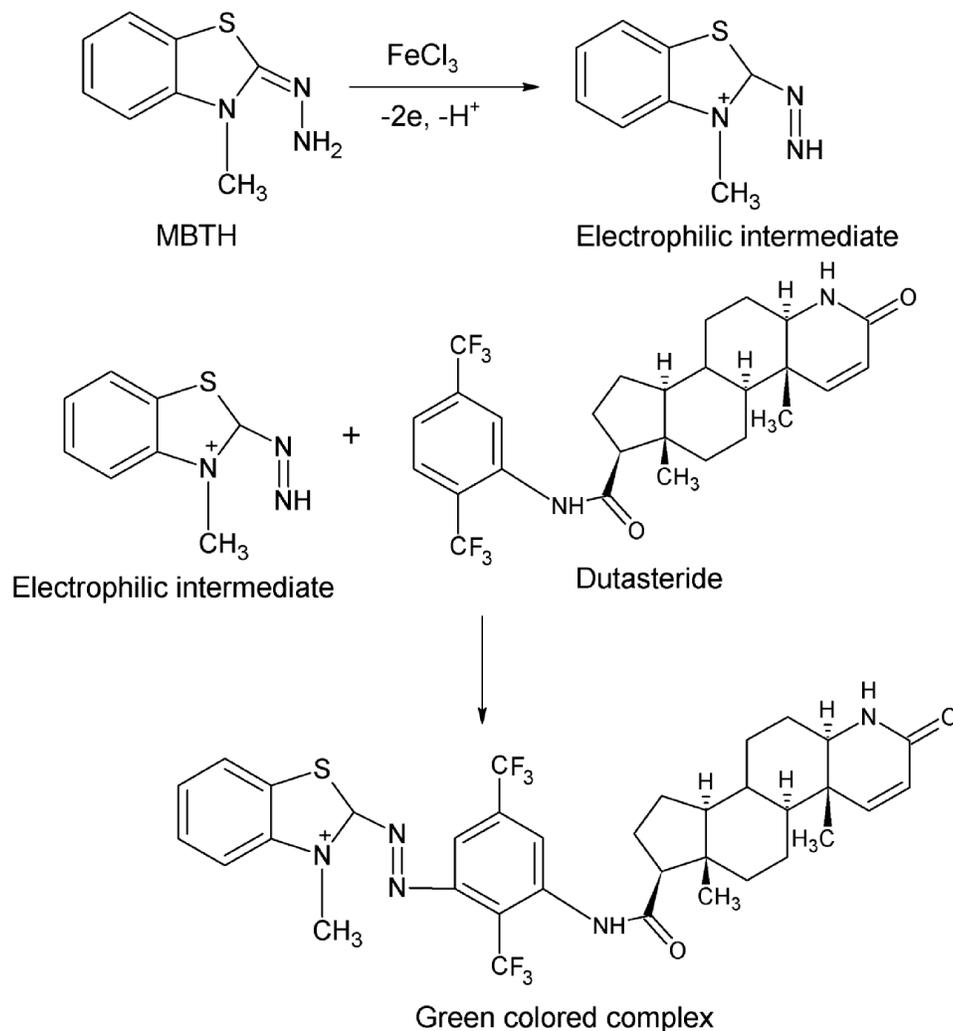
The results obtained in Method II were based on the oxidative coupling reaction of DSE with MBTH in the presence of FeCl_3 in acidic medium, yields a green colored product having maximum absorption at a wavelength of 550 nm against the corresponding reagent blank. Actually, this is an iron catalyzed oxidative coupling reaction of MBTH with the drug. Under the reaction conditions, on oxidation, MBTH loses two electrons and one proton forming an electrophilic intermediate, which is the active coupling species. This intermediate undergoes electrophilic substitution with the DSE to form the colored product. The proposed reaction mechanism is presented in Scheme 2.



Scheme 1: Proposed reaction mechanism for charge-transfer complexation of dutasteride with chloranil.

3.3.3. Methods III and IV (Ion-pair association with BTB and BPB)

The results obtained in Methods III and IV were based on extractive spectrophotometry. The DSE exhibits basic character essentially because of the presence of secondary amino group. In acidic media, the secondary amino group of DSE is protonated, while sulphonic group present in BTB and BPB undergoes dissociation. Dutasteride involves an ion-pair association with BTB (Method III) and BPB (Method IV) under acidic conditions, which is extractable with chloroform from the aqueous phase, resulting in the formation of a yellow colored complex exhibiting maximum absorption at 425 and 435 nm against the corresponding reagent blank, respectively. The proposed reaction mechanisms of DSE with BTB and BPB have been given in Schemes 3 and 4 respectively.



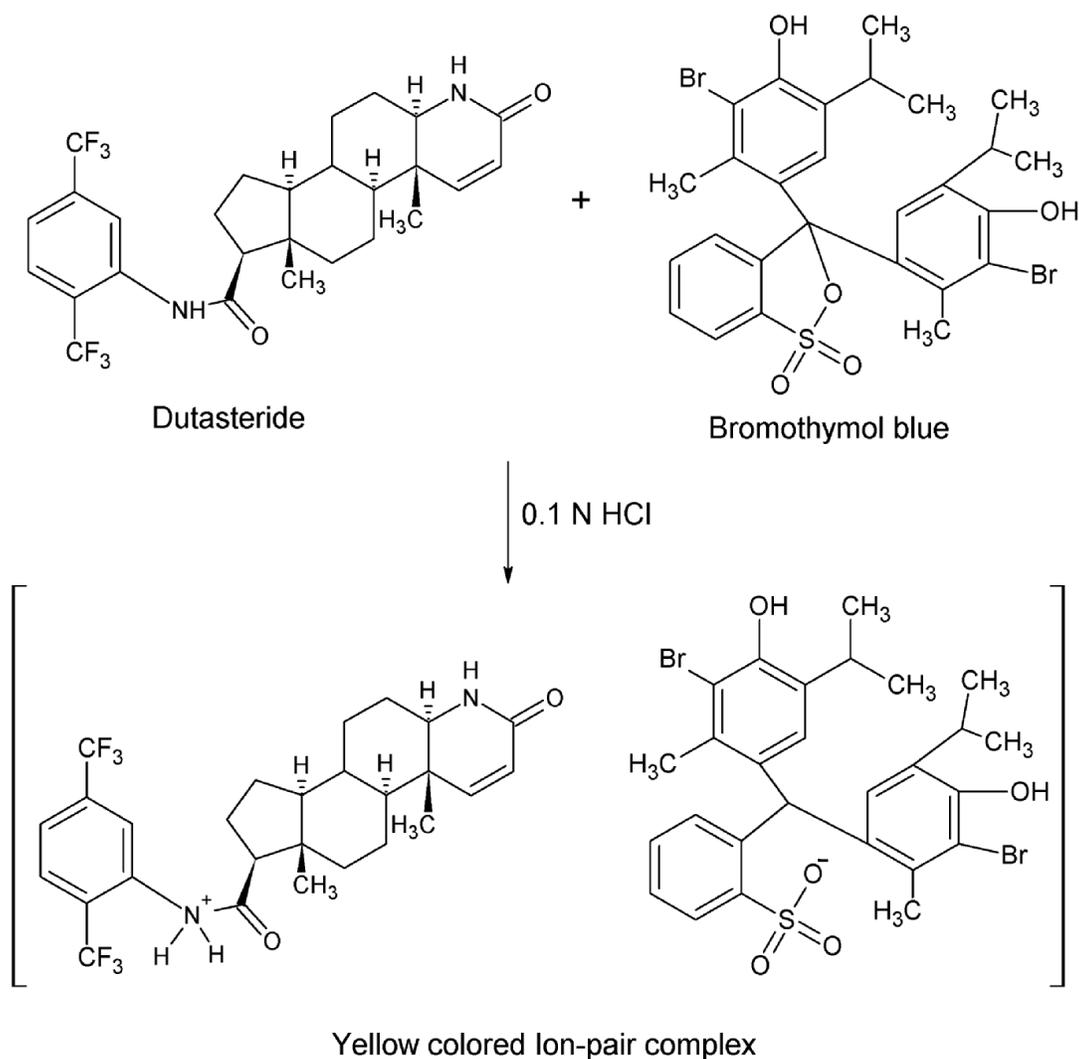
Scheme 2: Proposed reaction mechanism for oxidative coupling of dutasteride with MBTH.

3.4. Validation of the proposed methods

Validation of analytical procedures is a vital aspect not only just for regulatory purposes, but also for their efficient and reliable long-term application. The proposed methods are validated according to ICH Guidelines [51].

3.4.1. Linearity and optical characteristics

Under the optimized reaction conditions, the calibration curves for the dutasteride with the four analytical reagents (CRL, MBTH, BTB, and BPB) used in this work were constructed by analyzing a series of concentrations of the standard solutions of the DSE. The assay was performed according to the general analytical procedure previously described. The linearity was evaluated by linear regression analysis of the Beer's law data by least-square regression method, which was used to calculate the correlation coefficient, intercept and slope of the regression line and the values are presented in Table 1. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity values of the proposed methods were calculated and are summarized in Table 1.



Scheme 3: Proposed reaction mechanism for ion-association complex of dutasteride with bromothymol blue.

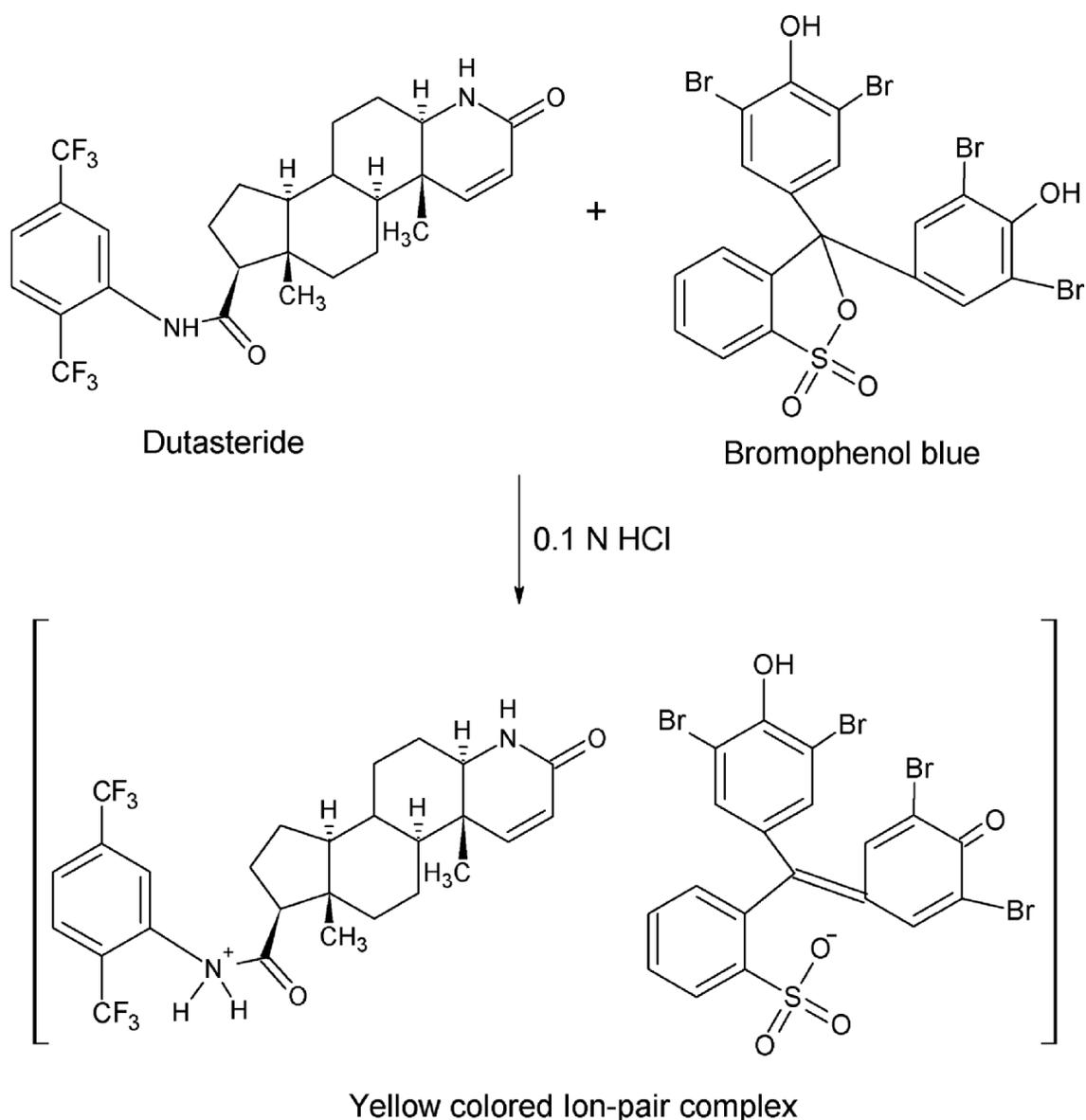
3.4.2. Limit of detection and limit of quantification

Sensitivity of the proposed methods was evaluated by calculating Limit of detection (LOD) and limit of quantification (LOQ). The LOD is the lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. The LOD and LOQ for the proposed methods were calculated using the following equation [52]:

$$\text{LOD} = 3.3 \text{ SD}/m$$

$$\text{LOQ} = 10 \text{ SD}/m$$

where **SD** is the standard deviation of five replicate determination values under the same conditions as done for the sample analysis but in the absence of the analyte and **m** is the sensitivity, namely the slope of the calibration graph. The results are presented in Table 1. The results indicating proposed methods are highly sensitive.



Scheme 4: Proposed reaction mechanism for ion-association complex of dutasteride with bromophenol blue.

3.4.3. Accuracy and precision

In order to determine the accuracy and precision of the proposed Methods (I, II, III, and IV), solution containing fixed concentration (within the working limits) of the drug was prepared and analyzed in six replicates by the proposed methods under the optimized experimental conditions. The standard deviation, relative standard deviation and percentage relative error obtained in the intraday analyses by Methods I, II, III, and IV were calculated and are summarized in Table 1. The relative standard deviation indicates the high precision of corresponding methods. Accuracy was evaluated as percentage relative error between the measured concentrations and concentrations taken for dutasteride. The relative error (Table 1) indicated good accuracy and an agreement between the theoretical value and the real value of concentration. Thus the proposed methods are effective for the determination of dutasteride.

Table 1: Spectral and statistical data for the determination of dutasteride.

Parameters	Method I (CRL)	Method II (MBTH)	Method III (BTB)	Method IV (BPB)
λ_{\max} (nm)	525	550	425	435
Beer's limit ($\mu\text{g mL}^{-1}$)	2–40	1–20	5–50	2–20
Molar absorptivity ($\text{L mole}^{-1} \text{cm}^{-1}$)	2.431×10^4	4.545×10^4	1.775×10^4	2.748×10^4
Sandell's sensitivity ($\mu\text{g cm}^{-2}/0.001$ Absorbance unit)	0.02173	0.01162	0.02976	0.01923
Stability of colored products (hr)	4.5	15	10	13
Regression equation ($Y = mx + c$)*				
Slope (m)	0.0342	0.0729	0.0342	0.0615
Intercept (c)	0.0083	0.0121	0.0067	-0.0069
Correlation coefficient (r)	0.9990	0.9998	0.9993	0.9992
LOD ($\mu\text{g mL}^{-1}$)	0.187	0.075	0.136	0.092
LOQ ($\mu\text{g mL}^{-1}$)	0.567	0.229	0.412	0.281
Standard deviation**	0.00194	0.00167	0.00141	0.00173
Relative standard deviation (%)	1.197	1.037	0.839	1.679
% Range of error (Confidence Limits)				
0.05 level	1.000	0.866	0.701	1.403
0.01 level	1.480	1.282	1.037	2.076

LOD: Limit of detection. LOQ: Limit of quantification.

* $Y = mx + c$, where Y is the absorbance and x is the concentration of drug in $\mu\text{g mL}^{-1}$.

**Average of six determinations.

3.4.4. Recovery studies

The validity and reliability of the proposed methods were assessed by the recovery studies via standard addition method. The recovery studies were carried out by adding a fixed concentration of bulk sample of dutasteride to the pre-analyzed formulation and the total concentration was once again determined using the proposed methods. The % recovery of the added pure drug was calculated as [53]:

$$\% \text{ Recovery} = [(C_t - C_s)/C_a] \times 100$$

where C_t is the total drug concentration measured after standard addition; C_s , the drug concentration in the formulation sample; C_a , the drug concentration added to formulation. The results (Table 2) revealed that any small change in the drug concentration in the solutions could be accurately determined by the proposed analytical methods. The closeness of the recoveries suggests lack of interference from tablet excipients and thereby establishes some degree of selectivity.

Table 2: Application of the standard addition technique for the determination of dutasteride in dosage forms using the proposed methods.

Method	Dosage form	Declared value (mg)	Spiked value (mg)	Found value (mg) \pm SD*	RSD (%)	Recovery (%)
I (CRL)	Duprost	0.5	0.5	0.986 ± 0.0037	0.375	98.6
	Dutas	0.5	0.5	0.998 ± 0.0094	0.941	99.8
	Sterdu	0.5	0.5	1.014 ± 0.0064	0.631	101.4
II (MBTH)	Duprost	0.5	0.5	1.023 ± 0.0051	0.498	102.3
	Dutas	0.5	0.5	0.996 ± 0.0083	0.833	99.6
	Sterdu	0.5	0.5	1.051 ± 0.0099	0.941	105.1
III (BTB)	Duprost	0.5	0.5	0.995 ± 0.0076	0.763	99.5
	Dutas	0.5	0.5	0.992 ± 0.0059	0.594	99.2
	Sterdu	0.5	0.5	1.046 ± 0.0043	0.411	104.6
IV (BPB)	Duprost	0.5	0.5	0.993 ± 0.0036	0.362	99.3
	Dutas	0.5	0.5	1.038 ± 0.0049	0.472	103.8
	Sterdu	0.5	0.5	1.045 ± 0.0085	0.813	104.5

*Average of five determinations.

3.4.5. Application of the proposed methods

The proposed methods (I, II, III, and IV) were successfully applied to the determination of dutasteride in tablet dosage forms of three different brands. The results of the proposed methods were compared statistically to those of the reference method [19]. The calculated *t*- and *F*-values at 95% confidence level presented in Table 3, did not exceed the tabulated values of 2.77 and 6.39 respectively, thus confirming no significant differences between accuracy and precision of the methods compared.

Table 3: Results of determination of dutasteride in dosage forms and statistical comparison with the reference method.

Method	Dosage form	Declared value (mg)	Found value (mg) ± SD*	RSD (%)	Recovery (%)	T-value**	F-value**
Reference	Duprostat	0.5	0.502 ± 0.0048	0.956	100.40	–	–
	Dutas	0.5	0.491 ± 0.0064	1.303	98.20	–	–
	Sterdu	0.5	0.505 ± 0.0071	1.405	101.00	–	–
I (CRL)	Duprostat	0.5	0.496 ± 0.0064	1.290	99.20	1.07	1.51
	Dutas	0.5	0.492 ± 0.0049	0.995	98.40	1.88	4.52
	Sterdu	0.5	0.502 ± 0.0053	1.055	100.40	1.18	2.75
II (MBTH)	Duprostat	0.5	0.497 ± 0.0068	1.370	99.40	1.07	1.51
	Dutas	0.5	0.505 ± 0.0053	1.049	101.00	0.89	2.68
	Sterdu	0.5	0.498 ± 0.0044	0.883	99.60	0.35	1.20
III (BTB)	Duprostat	0.5	0.491 ± 0.0067	1.364	98.20	0.97	1.55
	Dutas	0.5	0.506 ± 0.0033	0.652	101.20	1.26	3.24
	Sterdu	0.5	0.509 ± 0.0069	1.355	101.80	0.74	2.35
IV (BPB)	Duprostat	0.5	0.493 ± 0.0051	1.034	98.60	0.56	1.93
	Dutas	0.5	0.503 ± 0.0073	1.451	100.60	1.06	1.99
	Sterdu	0.5	0.504 ± 0.0081	1.607	100.80	0.79	2.36

*Average of five determinations.

**Tabulated *t*-value at 95% confidence level = 2.77 and Tabulated *F*-value at 95% confidence level = 6.39.

4. Conclusion

In this study, four Vis-spectrophotometric methods were developed and validated for the determination of dutasteride in bulk and tablet dosage forms. Unlike the LC–MS, HPTLC, HPLC and stability-indicating RP–HPLC procedures, the spectrophotometer instrument is simple and not of high cost, on the other hand, in terms of simplicity and expense, the proposed methods could be considered superior in comparison with the previously reported methods. The proposed methods are superior to the existing UV spectrophotometric methods in terms of selectivity as the interferences from many excipients present in formulations will increase in the UV region compared with visible region. The apparatus and reagents used are easily accessible even for the simple laboratories and the procedures do not involve any critical reaction conditions or tedious sample preparation. As seen from the molar absorptivity values, the order of sensitivity of the proposed methods were MBTH > BPB > CRL > BTB. These methods show no interference from the ingredients usually found in the tablet dosage forms. The statistical parameters and recovery data reveal the good accuracy and precision of the proposed methods. Therefore, it is concluded that the proposed methods are simple, sensitive, reproducible, accurate and precise and can be recommended for routine and quality control analysis of dutasteride.

Abbreviations

DSE	: Dutasteride
MBTH	: 3-Methyl-2-benzothiazolinone hydrazone hydrochloride
CRL	: Chloranil
BTB	: Bromothymol blue
BPB	: Bromophenol blue
ICH	: International Conference on Harmonization

LOD : Limit of detection
LOQ : Limit of quantification

Authors' Contributions

AVVNKSK designed the concept and experiments. The optimization of experimental variables was carried out by AVVNKSK and TVR. Studies on the validation parameters like optical characteristics, precision, accuracy and recovery studies were done by SVS and CBS. CBS collected the necessary literature for the proposed method. The final version of the manuscript was verified by SVS and TVR.

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