



SIMPLE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF DUTASTERIDE USING CHLORANIL IN THE PRESENCE OF ACETALDEHYDE

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ABSTRACT

Dutasteride is a synthetic 4-azasteroid compound belonging to the class of 5- α -reductase inhibitors used in the treatment of benign prostatic hyperplasia in men and male pattern baldness. This study is aimed at developing and validating a simple, sensitive and cost-effective visible spectrophotometric method for determination of dutasteride in tablet dosage forms. The method is based on the condensation of dutasteride with chloranil in presence of acetaldehyde producing a blue colored product, which is measured spectrophotometrically at 640 nm. The standard curve was linear ($r^2 = 0.9992$) over the concentration range of 2–40 $\mu\text{g mL}^{-1}$ with a detection limit of 0.116 mg mL^{-1} and a quantification limit of 0.353 mg mL^{-1} . The %RSD value was below 2.0 for intraday precision and recovery was found in the range of 99.30-100.90 %. Recovery studies gave satisfactory results indicating that none of the major excipients interfered with the assay method.

Keywords: Dutasteride, Chloranil, Spectrophotometer, Analysis.

INTRODUCTION

Dutasteride (DSE)¹⁻⁴, chemically known as (5 α , 17 β)-N-{2, 5 bis(trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide (Fig. 1), is a synthetic 4-azasteroid compound with antiandrogenic activity. DSE is used for the treatment of benign prostatic hyperplasia in men with an enlarged prostate gland and for 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, and hair follicles as well as 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.

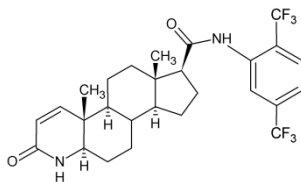


Figure 1: Structure of dutasteride

Various analytical techniques have been reported for determination of DSE in pharmaceutical preparations and human plasma when present alone or in combination with other drugs (Alfuzosin and Tamsulosin). They are LC-MS⁵⁻⁷, HPTLC⁸, Enzyme-linked immunosorbent assay⁹, HPLC¹⁰⁻¹² and stability indicating RP-HPLC^{13,14}. Though the above mentioned techniques are sensitive, they are

found to be relatively complicated, expensive and are not accessible to many laboratories in developing and under developed 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. According to the best of our knowledge, no visible spectrophotometric methods have been reported for the quantitation of the DSE in tablet dosage forms. Kamila¹⁵ *et al.*, and Amin¹⁶ *et al.*, has reported UV spectrophotometric method for the quantification of DSE in pharmaceutical formulations.

The present manuscript describes a visible spectrophotometric procedure for the determination of DSE in both pure form and in tablet dosage form using chloranil as analytical reagent. The proposed method is based on the condensation of *N*-alkylvinylamine formed from the interaction of the free secondary amino group in the DSE and acetaldehyde with chloranil to give blue colored vinylamino-substituted chloranil, which is measured spectrophotometrically at 640 nm. The proposed method was validated according to the ICH guidelines¹⁷ (Validation of Analytical Procedures ICH, 2005).

MATERIALS AND METHODS

Instrumentation

All spectrophotometric measurements were carried out using an ELICO double beam model SL 159 digital spectrophotometer. The cells used for absorbance measurements were 1-cm matched quartz cells.



Reagents

All chemicals used were of analytical reagent grade and used as received. Doubly distilled water was used in the preparation of all solutions. All the solutions were prepared a fresh daily.

1. 0.5% Chloranil (CRL): Prepared by dissolving 500 mg of CRL (Merck, Mumbai) in 100 mL of acetonitrile (Merck, Mumbai).
2. 2% Acetaldehyde: Prepared by diluting 2 mL of CH₃CHO (Sdfine-Chem limited, Mumbai) 100 mL with methanol (Merck, Mumbai).

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 200 µg mL⁻¹ of DSE was obtained by appropriate dilution of stock solution with methanol.

Tablet dosage forms of dutasteride

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

Recommended procedure

Aliquots of (0.1–2.0 mL) standard drug solution (200 µg 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, 1 mL of 2% CH₃CHO and 1 mL of 0.5 % CRL were added to each flask. The contents of each flask was mixed well and allowed to stand at room temperature (25±1°C) for 10 minutes. The

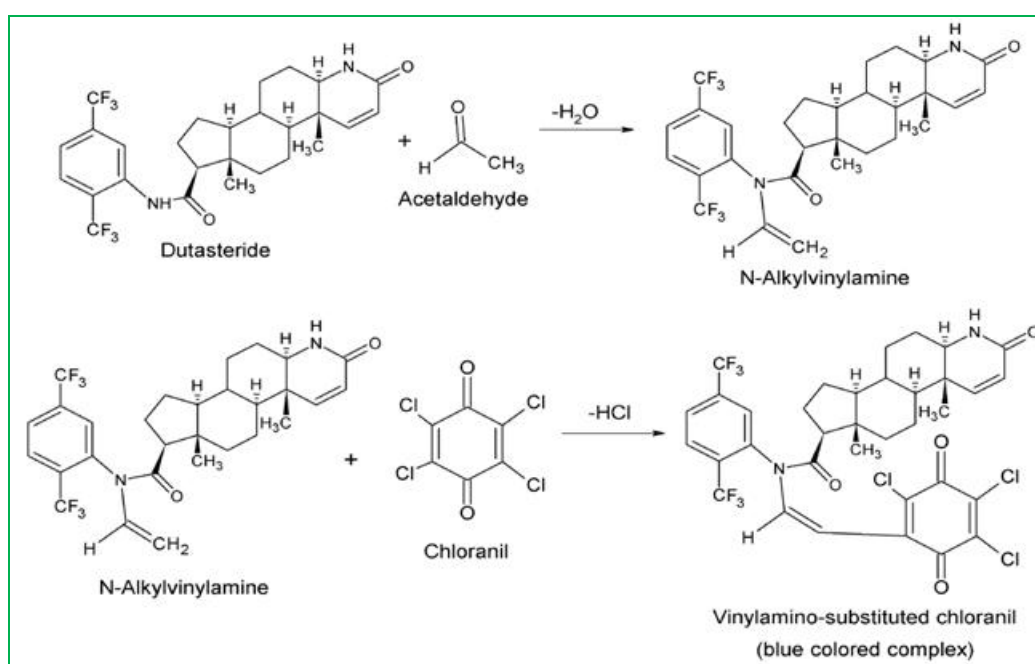
volume was made up to the mark with acetonitrile. The absorbance of the colored species was measured at 640 nm against the reagent blank prepared similarly omitting the drug. The calibration curve was constructed by plotting the absorbance *versus* final concentration of DSE. The concentration of the drug was read from the standard graph or computed from the respective regression equation.

Reference method¹⁵

Absorption maximum of DSE (25 µg mL⁻¹) in methanol was determined by scanning the drug solution from 200–400 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 µg mL⁻¹) equivalent to 5–50 µg 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. The calibration curve was 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.

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 dutasteride was weighed into a 25 mL volumetric flask, 15 mL of the 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. Convenient aliquots were subjected to analysis by the recommended procedure and reference method.



Scheme 1: Proposed reaction mechanism for condensation of dutasteride with chloranil in presence of acetaldehyde

RESULTS AND DISCUSSION

Determination of Absorption Maxima (λ_{\max})

To determine the λ_{\max} 20 $\mu\text{g mL}^{-1}$ of the DSE was added to 10 mL volumetric flask. Then, 1 mL of 2% CH_3CHO in methanol and 1 mL of 0.2% CRL in acetonitrile reagents were added. The contents of each flask was mixed well and allowed to stand at room temperature ($25 \pm 1^\circ\text{C}$) for 10 minutes and diluted to 10 mL with acetonitrile. The absorbance was measured against reagent blank in the range of 400-700 nm. λ_{\max} for DSE was found to be 640 nm. Absorption spectrum of the proposed method was shown in Fig. 2. Under the experimental conditions reagent blank showed a negligible absorbance at 640 nm.

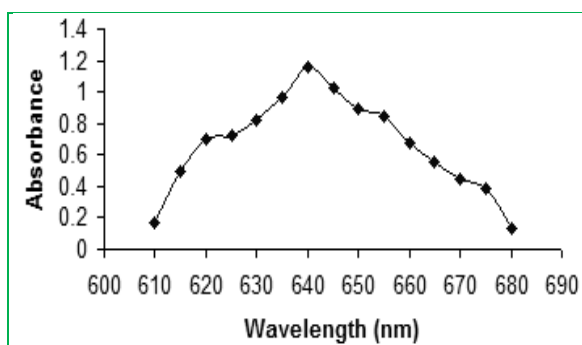


Figure 2: Absorption spectra of N-vinyl chlorobenzoquinone derivative of dutasteride ($20 \mu\text{g mL}^{-1}$ Dutasteride)

Optimization of reaction conditions

The factors affecting reaction conditions (concentration of CRL, CH_3CHO , solvent used for dilution and reaction time) were evaluated by altering each variable in turn while keeping the others constant and observing the effect produced on the absorbance of the colored species. The optimum values of the variables were maintained throughout the experiment to determine the concentration of DSE.

Effect of concentration of chloranil

The influence of the volume of 0.5 % CRL on the intensity of the color developed at constant DSE concentration ($20 \mu\text{g mL}^{-1}$) was examined in the range 0.2–2 mL of CRL. The maximum absorbance was obtained with 1 mL of CRL; above this volume the absorbance remained unchanged (Fig. 3). Therefore, 1 mL of 0.5 % CRL was used in all further measurements.

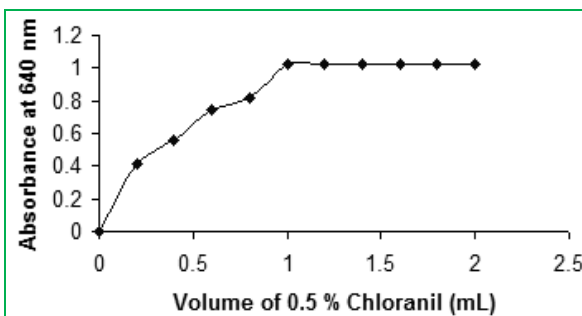


Figure 3: Effect of concentration of chloranil on the absorbance at 640 nm ($20 \mu\text{g mL}^{-1}$ Dutasteride)

Effect of concentration of acetaldehyde

The effect of the concentration of CH_3CHO on the color development was studied by adding different volumes (0.2–2.0 mL) of 2% CH_3CHO in methanol to 1 mL of DSE ($20 \mu\text{g mL}^{-1}$). It was found that the maximum absorbance of the color was reached with 1 mL of the CH_3CHO , and remained constant with higher volumes (Fig. 4). Therefore, 1 mL of the 2% CH_3CHO in methanol was chosen as an optimum value.

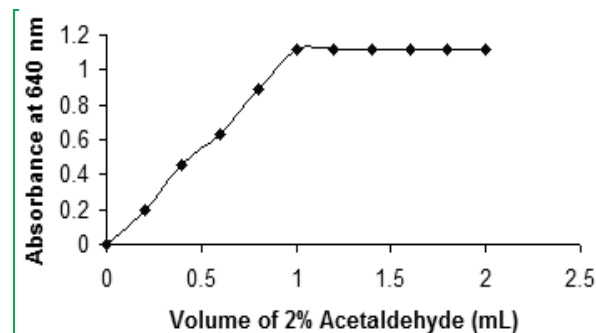


Figure 4: Effect of concentration of acetaldehyde on the absorbance at 640 nm ($20 \mu\text{g mL}^{-1}$ Dutasteride)

Effect of reaction time

To optimize the reaction time for color development, 1 mL of 2% CH_3CHO , 1 mL of 0.5 % CRL and 1 mL of DSE ($20 \mu\text{g}$) were added and kept at room temperature for varied time. The results obtained from optimization of the reaction time indicated that complete color development was attained after 10 min at room temperature (Fig. 5). A longer reaction time had no effect on the color development at room temperature. Therefore, 10 min of reaction time was used throughout the determination process.

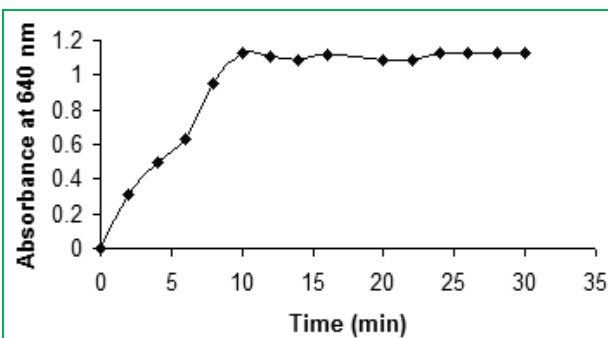


Figure 5: Effect of time on the absorbance at 640 nm ($20 \mu\text{g mL}^{-1}$ Dutasteride)

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.

Stability of the colored species

After diluting the reaction solution, it was found that the absorbance of the chromogen formed in the proposed method remained stable for at least 5 hours. This allowed

the processing of large batches of samples, and their comfortable measurements with convenience. This is increasing the convenience of the method as well as making the method applicable for large number of samples.

Reaction mechanism

The condensation of *N*-alkylvinylamine formed from the interaction of the free secondary amino group and CH_3CHO with haloquinones to give colored vinylamino-substituted quinone, which can be measured spectrophotometrically, has been reported in the literature. This reaction was applied for the determination of amantadine hydrochloride¹⁸, gatifloxacin¹⁹, norfloxacin²⁰, moxifloxacin²¹, Lercanidipine hydrochloride²², salbutamol sulfate²³, ephedrine hydrochloride²⁴ and phenylephrine hydrochloride²⁴. The proposed method for the determination of DSE was based on the formation of a colored *N*-vinyl chlorobenzoquinone derivative of DSE by its reaction with CRL in presence of CH_3CHO . The formed blue color showed maximum absorption at 640 nm (Fig. 3). The probable reaction mechanism was based on the reported methods¹⁸⁻²⁴ is given in Scheme 1.

Validation of the proposed method

Optical characteristics

The optical characteristics such as Beer's law limits (Fig. 6), Sandell's sensitivity and molar absorptivity were calculated for the proposed method and the results are summarized in table 1. Regression analysis of the Beer's law plot at their λ_{max} revealed a good correlation. Graphs of absorbance *versus* concentration showed zero intercept and are described by the regression equation $Y = bx + a$ (where Y is the absorbance, b is the slope, x is the concentration of drug in $\mu\text{g mL}^{-1}$ and a is the intercept) obtained by least squares method. The results were summarized in table 1.

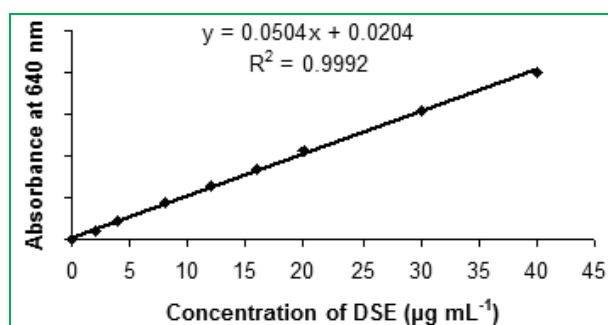


Figure 6: Beer's law curve for the proposed method

Sensitivity

Sensitivity of the proposed method was evaluated by calculating Limit of detection (LOD) and limit of quantification (LOQ). According to the Analytical Methods Committee (Analytical Methods Committee, 1987) the detection limit (LOD) is the concentration of drug corresponding to a signal equal to the blank mean (Y_b) plus three times the standard deviation of the blank

(S_b). Quantification limit (LOQ) is the concentration of drug corresponding to the blank mean plus ten times the standard deviation of the blank. The LOD and LOQ values for DSE are presented in table 1. The results indicating high sensitivity of the proposed method.

Table 1: Spectral and Statistical data for the determination of dutasteride

Parameters	CA
λ_{max} (nm)	640
Beer's Limit ($\mu\text{g mL}^{-1}$)	2-40
Molar Absorptivity ($\text{L mole}^{-1} \text{cm}^{-1}$)	2.801×10^4
Sandell's sensitivity ($\mu\text{g cm}^{-2}/0.001$ Absorbance unit)	0.01886
Stability of colored products (hours)	6.0
Regression equation ($Y = mx + c$)	
Slope (m)	0.0504
Intercept (c)	0.0204
Regression coefficient (r^2)	0.9992
LOD ($\mu\text{g mL}^{-1}$)	0.116
LOQ ($\mu\text{g mL}^{-1}$)	0.351
Standard deviation	0.00178
Relative standard deviation (%)	0.843
% Range of error (Confidence Limits)	
0.05 level	0.704
0.01 level	1.042

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

Accuracy and Precision

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

Recovery studies

The accuracy and validity of the proposed method was further assessed by the recovery studies via standard addition method. The recovery studies were carried out by adding a fixed concentration of bulk sample of DSE to the pre-analyzed formulation and the total concentration was once again determined using the proposed methods. The results (table 2) revealed that any small change in the drug concentration in the solutions could be accurately determined by the proposed analytical method. 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

Dosage form	Declared value (mg)	Spiked value (mg)	Found value (mg) \pm S.D [*]	RSD (%)	Recovery (%)
Duprostat	0.5	0.5	0.993 \pm 0.0062	0.624	99.30
Dutas	0.5	0.5	1.020 \pm 0.0083	0.813	102.00
Sterdu	0.5	0.5	1.009 \pm 0.0056	0.555	100.90

Average of five determinations

Table 3: Determination of dutasteride in dosage forms and statistical comparison with the reference method

Method	Dosage form	Declared value (mg)	Found value (mg) \pm S.D [*]	RSD (%)	Recovery (%)	T Value ^{**}	F Value ^{**}
Reference	Duprostat	0.5	0.502 \pm 0.0048	0.956	100.40	-	-
	Dutas	0.5	0.491 \pm 0.0064	1.303	98.20	-	-
	Sterdu	0.5	0.505 \pm 0.0071	1.405	101.00	-	-
Proposed	Duprostat	0.5	0.498 \pm 0.0042	0.843	99.60	0.986	1.932
	Dutas	0.5	0.503 \pm 0.0059	1.172	100.60	1.065	2.348
	Sterdu	0.5	0.501 \pm 0.0068	1.357	100.20	1.264	2.463

Average of five determinations; ^{*} Tabulated t value at 95 % confidence level = 2.77 and Tabulated F value at 95% confidence level = 6.39.

Application of the proposed method

The proposed method was successfully applied to the determination of DSE in tablet dosage forms of three different brands. The results of the proposed method were compared statistically to those of the reference method. The calculated t- and F-values at 95 % confidence level, shown 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.

CONCLUSION

A new visible spectrophotometric method for the determination of DSE was developed and validated. The proposed method, because they involve measurements in visible region, is more selective than the previously reported spectrophotometric method that involves measurement in ultraviolet region. The proposed method has been successfully applied to assay of DSE in tablet dosage forms. The assay method did not involve any stringent experimental conditions and is also free from common excipients found in the tablet dosage forms. The statistical parameters and recovery data proved that the proposed method has acceptable precision, accuracy, and linearity. Therefore, it is concluded that the proposed method is simple, sensitive, accurate and precise and can be recommended for routine and quality control analysis of DSE.

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