SPECTROPHOTOMETRIC SIMULTANEOUS DETERMINATION OF DUTASTERIDE AND TAMSULOSIN IN COMBINED TABLET DOSAGE FORM BY FIRST ORDER DERIVATIVE SPECTROSCOPY AND AREA UNDER CURVE (AUC) SPECTROPHOTOMETRIC METHODS AND ITS APPLICATION TO UNIFORMITY OF CONTENT IN TABLET AND CAPSULE.

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ABSTRACT

A simple, economical, precise and accurate method for simultaneous determination of Dutasteride (DUTA) and Tamsulosin (TAM) in combined dosage form has been developed. The first method is first order Derivative spectroscopy method (Method A) in which derivative amplitudes were measured at selected wavelengths. Second method is Area Under Curve Spectrophotometry (Method B). The amplitudes at 247.67 nm and 232.60 nm in the first order derivative spectra were selected to determine DUTA and TAM, respectively and wavelength ranges 237.13-238.25 nm and 222.50-223.62 nm were selected to determine DUTA and TAM, respectively by AUC method in methanol. Beer's law is obeyed in the concentration ranges of 10-50 µg mL^{-1} and 8-40 µg mL^{-1} for DUTA and TAM, respectively in method A while 5-25 µg mL^{-1} and 4-20 µg mL^{-1} by DUTA and TAM, respectively in method B. The % assay for commercial formulation was found to be in the range 99.24 – 100.09 % for DUTA and 99.89 – 101.12 % for TAM by the proposed methods. Recovery was found in the range of 99.60 – 99.99 % for DUTA and 100.02 – 101.09% for TAM by first order derivative spectroscopic method and 99.30 – 101.12% for DUTA and 99.52-101.25% for TAM by AUC method for both the Formulations. The results of analysis have been validated statistically and recovery studies confirmed the accuracy and reproducibility of the proposed methods which were carried out by following ICH guidelines. The proposed methods were applied successfully to determine uniformity of contents in commercial capsule and tablet formulations.

Keywords: Dutasteride, Tamsulosin, First order Derivative Spectroscopy, Area under Curve, Uniformity of Content.

INTRODUCTION

Dutasteride is a synthetic 4-azasteroid compound, chemically it is (5α, 17β)-*N*-{2, 5 bis (trifluoromethyl) phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide is a 5-alpha-reductase inhibitor that inhibits the conversion of testosterone into dihydrotestosterone (DHT). Dutasteride belongs to a class of drugs called 5-alpha-reductase inhibitors, which block the action of the 5-alpha-reductase enzymes that convert testosterone into dihydrotestosterone (DHT) and is used treat benign prostatic hyperplasia(BPH). Dutasteride inhibits both isoforms of 5-alpha reductase, Type I and Type II (1, 2). Tamsulosin is an α_{1a}-selective alpha blocker used in the symptomatic treatment of benign prostatic hyperplasia (BPH) (1- 3). Chemically, Tamsulosin is (*R*)-5-(2-(2-(2-ethoxyphenoxy) ethylamino) propyl)-2-methoxybenzene sulfonamide and is official in USP volume 3 pharmacopoeia.

Review of the literature revealed that there is one Spectrophotometric simultaneous equation method & LC-MS-MS method has been reported for simultaneous determination of this combination (4, 5). HPTLC, Tandem mass & LC-ESI-MS/MS methods with high sensitivity and selectivity for the estimation of Tamsulosin in pharmaceutical dosage forms and human plasma has been reported (6 - 8). One LC method for estimation of Dutasteride has been reported (9).

Therefore the aim of the study was to develop simple, rapid, accurate, reproducible and economical derivative and AUC spectroscopic methods for both the titled drugs in combined dosage forms. The proposed method were optimized and validated as per the International Conference on Harmonization (ICH) analytical method validation guidelines (13).

MATERIALS AND METHODS:

Instrumentation

An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10 mm matched quartz cells was used. All weighing were done on electronic balance (Model Shimadzu AUW-220D), High Speed Centrifuge Research Centrifuge, Biolab-(BL-165D), Ultrasonicator model 5.5L150H were used.

Reagents and chemicals

Spectroscopic grade Methanol was purchased form LOBA Chemie Pvt. Ltd., Mumbai. Tablet used for analysis were VELTAM – PLUS (Batch No. DK 3183) manufactured by Intas Pharmaceuticals, Selaqui, Dehradhun, India and Capsule TAMDURA (Batch No.SK 80967) manufactured by Sun pharmaceutical, Silvasa India ltd containing DUTA 0.5mg and TAM 0.4 mg per tablet/capsule. DUTA and TAM are available in the ratio of 5:4, respectively in formulation and were used in same ration for preparation of calibration curves. Pharmaceutical grade of Dutasteride (% purity 99.78) was kindly supplied as a gift sample by Dr. Reddys Pharmaceutical Ltd., Hyderabad; Andhra Pradesh. TAM (% purity 99.92) was gifted by Aarti drugs

Pvt. Ltd. Mumbai. These samples were used without further purification.

Preparation of Standard Stock Solutions and calibration Curve

Standard stock solution of pure drug containing 1000 µg mL^{-1} of DUTA and 1000 µg mL^{-1} of TAM were prepared separately in the methanol. These stock solutions were used to prepare series of solutions with conc. 10 – 50 µg mL^{-1} and 5 – 25 µg mL^{-1} of DUTA (TAM solutions having concentrations in the ratio as per the formulation were used) for method A and B, respectively and were used to prepare calibration curve.

Method A: First Order Derivative Spectroscopy

To determine derivative amplitude for DUTA and TAM solution of increasing and decreasing concentrations of DUTA and TAM were prepared in combination and scanned in uv spectrum in the range 200 - 350 nm at 0.2 band width and 200 nm/min scan speed parameter (Fig.1). These spectrums were converted to first order derivative spectra by using instrument mode with filter size 5 and interval 1.0 (Fig.2). After observing the derivative amplitude of first order derivative spectra, it was observed that there is proportionate increase in amplitude of DUTA with increase in its concentrations by calibrations method and it was found that at wavelength 247.67 nm and amplitude of TAM decreased with decreased in its concentrations at 232.60 nm. Therefore λmax 247.67 nm and λmax 232.60 nm were assigned to DUTA and TAM, respectively for the study of tablet and capsule.

Method B: Area Under Curve

For the simultaneous determination using the area under curve (AUC) method, suitable dilutions of the standard stock solutions (1000 µg/ml) of DUTA and TAM were prepared separately in methanol. The solutions of drugs were scanned in the range of 200-350 nm (Fig.3). For Area Under Curve method, caliberation curve was plotted and the sampling wavelength ranges selected for estimation of DUTA and TAM are 237.13-238.25 nm (λ1-λ2) and 222.50-223.62 nm (λ3-λ4) and area were integrated between these selected wavelength ranges for both drugs, which showed linear response with increasing concentration hence the same wavelength range were used for estimation of tablet and capsule formulations. By using integrated areas two simultaneous equations were constructed and solved to determine concentrations of analytes. Concentration of analytes in mixed standard and the sample solution were calculated using equation (1) and (2).

$$C_{DUTA} = A_2 \times a_{X2} - A_1 \times a_{y2} / a_{X2} \times a_{Y1} - a_{X1} \times a_{Y2} \ldots\ldots (1)$$

$$C_{TAM} = A_2 - a_{X2} \times C_{DUTA} / a_{Y2} \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where,

a_{X1} (222) and a_{X2} (242.66) are absorptivities of DUTA at (λ1-λ2) and (λ3-λ4) respectively.

a_{Y1} (114.16) and a_{y2} (566.66) are absorptivities of TAM at (λ1-λ2) and (λ3-λ4) respectively.

A1 and A2 are Absorbances of mixed standard at (λ1-λ2) and (λ3-λ4) respectively. C_{DUTA} and C_{TAM} are the conc. of DUTA & TAM, respectively in g/100mL. (10)

Preparation of Sample Stock Solution and Formulation analysis

A quantity of powder from twenty tablets / capsules equivalent to 5 mg of Duta (4 mg of Tam) was weighed and transferred to 25 ml flask containing 20 mL of methanol and ultrasonicated for 30 min and centrifuged for 10 min at 10000 RPM. Supernatant was transferred to 25 ml volumetric flask and volume was made up to mark. The solution was filtered and suitably diluted with methanol to have 30 µg mL^{-1} and 15 µg mL^{-1} of DUTA for method A and method B, respectively and samples were analysed by the proposed methods. To determine uniformity of content ten tablets were analyzed individually by following above procedure and assay values were calculated.

Precision of the Method

To study intraday precision, method was repeated 5 times in a day and the average % RSD was calculated by method A and B, respectively. Similarly the method was repeated on five different days and average % RSD was calculated (Table 1). These values confirm the intra and inter day precision.

Figure 1: Spectra of Mixture of Dutasteride and Tamsulosin for First Order Derivative method

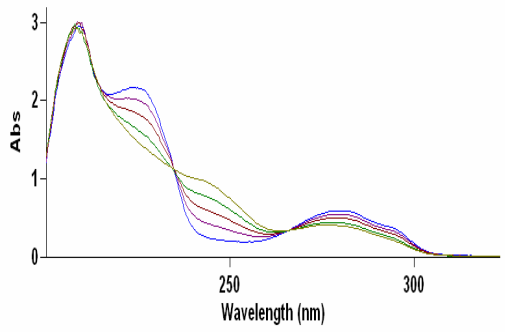

Figure 2: First Order Derivative Spectra of Dutasteride and Tamsulosin

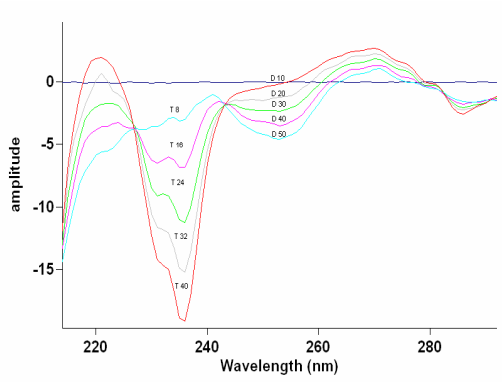

Table 1: Optical characteristics of the proposed methods.

PARAMETER		DUTASTERIDE		TAMSULOSIN	
		Method A	Method B	Method A	Method B
λ (nm)		247.67	237.13-238.25	232.60	222.50-223.62
Beer's law limit (µg mL^{-1})		10-50	5-25	8-40	4-20
Régression Equation (y = mx + c)	Slope (m)	0.0733	----	0.3701	----
	Intercept (c)	-0.023	----	0.107	----
Correlation coefficient		0.999	----	0.999	----
Precision (%R.S.D.)	Repeatability (n=5)	0.56	0.66	1.09	0.72
	Intra-day (3×3 times)	0.48	0.74	1.13	0.85
	Inter-day(3×5 days)	0.35	0.52	1.06	0.63
	Analyst	0.54	0.72	1.01	0.75
Formulation Analysis (% Assay, %RSD) n=6	Tablet	99.98, 0.84	99.91, 1.01	100.89,1.16	101.12, 1.24
	Capsule	100.09, 0.67	99.24, 0.43	99.89, 0.53	101.05, 0.35

Recovery studies

The accuracy of the proposed methods was checked by recovery study, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (50 %, 100 % and 150 %) within the range of linearity for both the drugs (Table 2). The basic concentration level of sample solution selected for spiking of the drugs standard solution was 15 µg mL^{-1} of DUTA and 12 µg mL^{-1} of TAM for both the methods.

Uniformity of Contents test

Individually 10 tablets were powdered and transferred to 10 ml of methanol separately, sonicated for 30 min, centrifuged for 5 min at 10000 rpm, and supernatant was diluted to 20 ml with methanol. It was then suitably diluted to contain 25 µg/ml of DUTA and 20 µg/ml TAM and proposed methods were followed to determine % content in each unit (Fig.4 (A), Fig.4 (B)).

RESULTS AND DISCUSSION

The proposed methods for simultaneous estimation of DUTA and TAM in combined dosage form were found to be accurate, simple and rapid. Since only one method is reported for simultaneous analysis of the two drugs earlier by simultaneous equation, the developed methods can be used for routine analysis of two drugs in combined dosage forms. Area under curve method involves formation and solving of simultaneous equation. Once the equations are formed, then only measurement of the area of sample solution at two wavelength ranges and simple calculations are required. Practically no interference from tablet excipients was observed in these methods. Both the methods are accurate, simple, rapid, precise, reliable, sensitive, reproducible and economical as per ICH guidelines. The values of % RSD and correlation of coefficient for First order Derivative spectra (Tablet & Capsule) were found to be (% RSD 0.84-1.16 and 0.67-0.53) and correlation coefficient was 0.999 for DUTA and TAM.

Figure 3: Overlay spectrum of DUTA and TAM in Methanol. DUTA (5-25µg mL^{-1}) and TAM (4-20 µg mL^{-1}) for AUC method

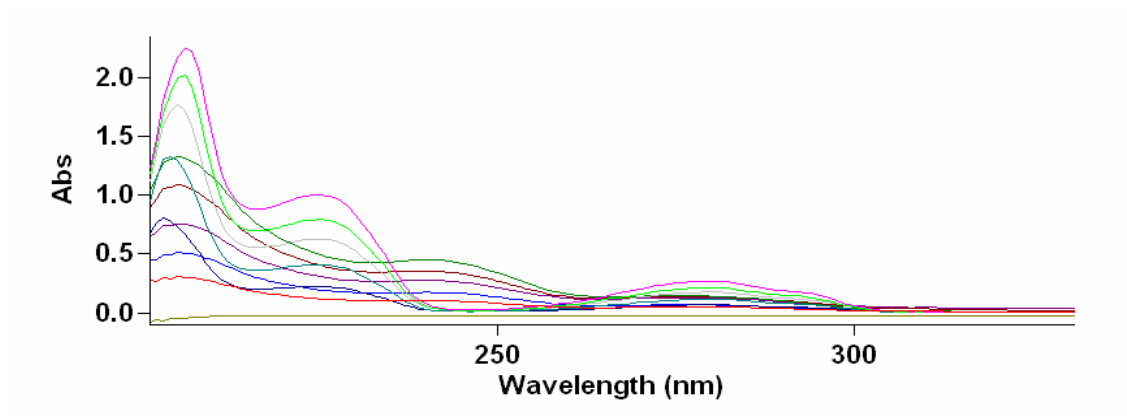

Figure 4 (A): Result of Uniformity of Content Test for Dutasteride.

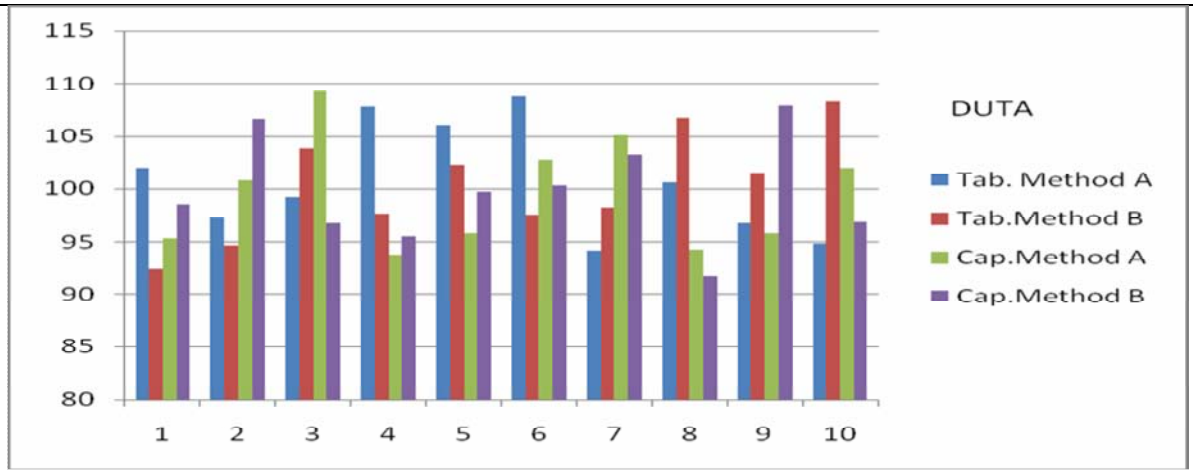

Figure 4 (B): Result of Uniformity of Content Test for Tamsulosin.

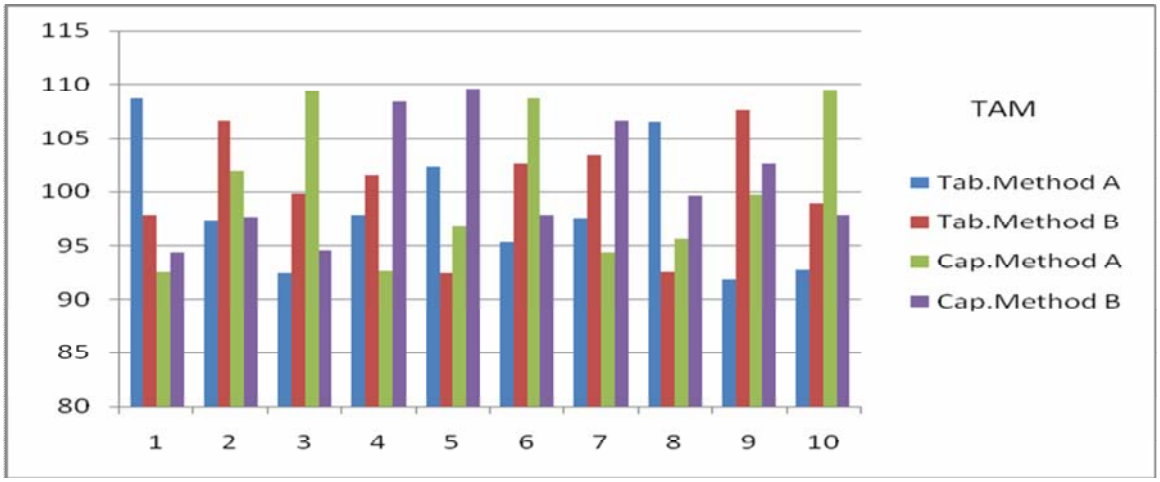

Table 2: Result of Recovery studies.

Formulation studies	Recovery Level	Recovery of	% Mean Recovery, % RSD by using	
			Method A	Method B
Formulation I (Tablet)	50%	DUTA	99.60, 0.67	100.12, 0.32
		TAM	100.02, 0.38	99.52, 0.98
	100%	DUTA	99.98, 0.45	101.12, 1.45
		TAM	101.09, 1.04	101.09, 0.98
	150%	DUTA	99.95, 0.49	99.92, 0.94
		TAM	100.45, 0.76	100.23, 0.26
Formulation II (Capsule)	50%	DUTA	99.20, 0.87	99.30, 0.67
		TAM	100.22, 0.48	101.22, 1.07
	100%	DUTA	99.78, 0.49	99.58, 1.12
		TAM	101.09, 0.95	100.09, 0.55
	150%	DUTA	99.99, 0.19	99.59, 0.79
		TAM	100.25, 0.49	101.25, 1.19

The result of recovery studies for Tablet & Capsule was found to be 99.60-101.06 for method A and 100.01-100.25 for method B, indicates that there is no interference due to excipients present in the formulation. It can be easily and conveniently adopted for routine quality control analysis.

CONCLUSION

The proposed methods are simple, precise, accurate, economical and rapid for the determination of DUTA and TAM in combined tablet dosage forms. Analysis of authentic samples containing DUTA and TAM showed no interference from the common additives and excipients. Hence, recommended procedure is well suited for the assay and evaluation of drugs in commercial tablets and capusules. It can be easily and conveniently adopted for routine quality control analysis.

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