INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 January, 2014; received in revised form, 18 March, 2014; accepted, 06 April, 2014; published 01 July, 2014

MS/MS AND HPLC CHARACTERIZATION OF FORCED DEGRADATION PRODUCTS OF DUTASTERIDE AND TAMSULOSIN HYDROCHLORIDE

Rohit Chaudhari, Krishnapriya Mohanraj and Vaishali Shirsat*

Bombay College of Pharmacy, Kalina, Santacruz (E), Mumbai-400098, Maharashtra, India

Keywords:
RP-HPLC, Mass Spectrometry, Dutasteride, Tamsulosin hydrochloride, Structural Elucidation and Forced degradation products.

Correspondence to Author:

Vaishali A. Shirsat

Associate Professor,
Pharmaceutical Analysis
Bombay College of Pharmacy,
Kalina, Santacruz (E),Mumbai
– 400 098, Maharashtra, India

E-mail: vashirsat@gmail.com

ABSTRACT: A new simple and sensitive reverse phase high performance liquid chromatographic method was developed for simultaneous estimation of Dutasteride and Tamsulosin hydrochloride following forced degradation studies as per ICH guidelines. The degradation products were observed in acid and alkaline hydrolysis for Dutasteride; whereas Tamsulosin hydrochloride showed degradation on exposure to light. All the degradants were separated by preparative thin layer chromatography and subjected to structural elucidation by mass spectrometry using electron spray ionization technique. The base peak of DTS with m/z of 529.1 was formed in positive ESI mode. The daughter ion of DTS was observed at m/z of 461.2. Two degradants with m/z of 546.2 and m/z of 527.5 were formed by acid hydrolysis to give a carboxylic acid derivative and another by dehydrogenation respectively. Degradation product with m/z of 573.3 was di-sodium adduct of DTS and was formed only in alkaline hydrolytic condition. The base peak of photo degradant of TMS with m/z of 409.1 was formed in positive ESI mode showed same m/z value of the base peak of TMS. This indicated that under photolytic degradation only structural modification occurred in TMS molecule. The mechanisms for the formation of degradation products for DTS and TMS are presented in the paper. A linear gradient mode of elution was used for separation of DTS, TMS and their degradants on a Hi Q Sil C18HS column using a UV detector. The developed method was validated and used for the analysis of marketed tablets of DTS and TMS in combination.

INTRODUCTION: Forced degradation studies are an important part of the drug development process. With the advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishment of stability-indicating assay method (SIAM) has become more clearly mandated. The guidelines explicitly require conduct of forced decomposition studies under a variety of conditions, such as light, heat, humidity, acid/base, dry heat and oxidation, and separation of drug from degradation products [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(7).2791-06

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(7).2791-06

These experiments play an important role in the drug development process to facilitate: stability indicating method development, drug formulation design, selection of packaging and storage conditions, better understanding of the potential liabilities of the drug molecule chemistry, and the resolution of stability related problems [2].

Accordingly, the aims of the present study were to establish inherent stability of Dutasteride (DTS) and Tamsulosin Hydrochloride (TMS) through forced degradation studies under a variety of ICH recommended test conditions, with the structural identification of their degradation products and to develop a stability-indicating assay method. DTS chemically known as (17b-N-(2, 5-bis-(trifluoro methyl) phenylcarbamoyl)-4-aza-5-androst1-en-3-one), $C_{27}H_{30}F_6N_2O_2$, 528.53, **Fig 1A**) is a synthetic 4-azasteroid compound which is a competitive and specific inhibitor of both the type 1 and type 2

isoforms of an intracellular enzyme 5 alpha-reductase (5AR).

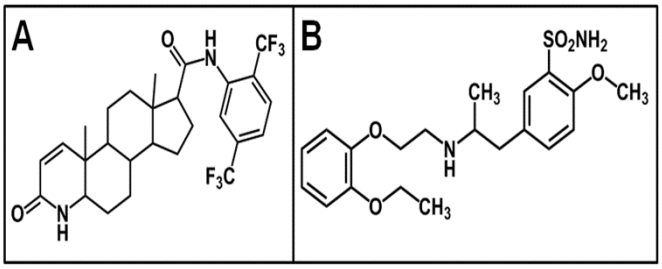

FIG. 1A): STANDARD DUTASTERIDE B): STANDARD TAMSULOSIN HYDROCHLORIDE

DTS is a white to pale yellow powder and is soluble in ethanol and methanol, but is practically insoluble in water. It is used mainly to improve symptoms, reduce the risk of acute urinary retention and reduce the risk of the need for benign prostatic hyperplasia (BPH)-related surgery.

TMS is known as (−) - (R) -5-[2-[[2-(O-ethoxyphenoxy) ethyl] amino] propyl] -2-methoxybenzenesulfonamide hydrochloride, $C_{20}H_{28}N_2O_5S$, 408.5, **Fig. 1B**). TMS is commonly used to treat benign prostatic hyperplasia (BPH) symptomatically. TMS is a white or almost white powder that is slightly soluble in water, anhydrous ethanol, and freely soluble in formic acid. Literature survey reveals that DTS has been analyzed in pure and pharmaceutical formulations by spectroscopic technique [3, 4], for Stress Degradation Studies and Stability- Indicating assay method by HPLC [5, 6], determination of DTS in tablet dosage form[7] and impurity profiling by RP-HPLC [8], determination of DTS by LC-MS/MS analysis [9, 10, 11], assay by thin layer chromatography (TLC) [12, 13, 14] and simultaneous determination of DTS and TMS by spectrophotometric method [15], RP-HPLC in tablet dosage form [16, 17], by Vierordt's method in tablet dosage form [18], by LC–MS–MS in human plasma [19].

TMS is an official drug and is included in European pharmacopoeia 6.0 (EP) wherein the assay procedure for TMS includes non-aqueous titration [20]. Literature survey reveals several bioanalytical methods reported for the analysis of TMS. These include spectrophotometric determination of TMS in pharmaceutical dosage form [21 - 25], Stress Degradation Studies and Stability- Indicating assay method by HPLC [26 – 29] and by HPTLC [30], determination of TMS in tablet dosage form [31, 32], HPLC determination of TMS in human plasma [33] and urine [34], human aqueous humor and serum using liquid chromatography electro spray ionization tandem mass spectrometry (LC/ESI-MS–MS) [35, 36], LC–MS [37] or LC–ESI–MS– MS [38] for determination of TMS and related substances in bulk drugs and formulations [39]. Other methods included the use of capillary electrophoresis [40] and HPLC for chiral separation [41], and stability indicating assay method by HPTLC [42].

Simultaneous determination of DTS and TMS by spectrophotometric method in tablet and capsule[15], by RP-HPLC method for tablets [16, 17, 18], by LC–MS–MS in human plasma [19] was the reported method for quantization of both the drugs in combination. The implementation of HPTLC procedures is commonly found in product development and analytical laboratories [43, 44, 45]. No stress degradation studies and stability indicating assay method for simultaneous estimation of both the drugs were found to be reported. Thus, there was a need to develop a suitable validated analytical method for the simultaneous determination of DTS and TMS. This paper describes a simple and sensitive RP-HPLC method for the simultaneous analysis of DTS and TMS with the MS-MS characterization of forced degradation products. The proposed methods were validated as per the guidelines of the ICH [46].

EXPERIMENTAL:

Chemicals and Reagents: DTS and TMS were obtained as a generous gift sample from Cipla Pvt. Ltd, Vikhroli MIDC, Mumbai, India. Marketed tablets of DTS and TMS in combination (VELTAM PLUS, Intas Pharma Pvt. Ltd., India, containing 0.5 mg of DTS and 0.4 mg of TMS) were obtained from local pharmacy. Double distilled water was obtained from in-house Quartz double distillation plant of Lab-Sil instruments, India, Model No. LQD-5, and was filtered through 0.45 µm nylon filter for the HPLC analysis. Acetonitrile (HPLC grade) and methanol (HPLC grade) was purchased from Qualigens Fine Chemicals, Mumbai, India. Buffers and all other chemicals of analytical grade were acquired from S.D. Fine Chem Ltd, Mumbai, India.

Instrumentation: The HPLC chromatograms were acquired on Borwin Software version 1.5 on JASCO HPLC SYSTEM with the JASCO–PU 2080 plus intelligent HPLC pump with Varian star 800 MODULE INTERFACE, and Rheodyne injector of 20 µL loop size, JASCO UV–2075 plus Intelligent UV-VIS detector at the sensitivity of 0.01. The column used for the study was Hi Q Sil C18HS (250 × 4.6 mm, 5 µm).

The purity of the drugs and their degradants were obtained on DIONEX HPLC with P680 HPLC pump, ASI 100 Automated sample injector with the use of a UVD340U PDA detector. The PDA chromatograms were obtained by using Chromeleon Client software version 6.80 SP2 Build 2284.

The characterization of degradation products were done by using MS-MS spectrometry. An electron spray ionization (ESI) was used as ionization source. Mass spectra were acquired using Varian 500 MS, IT Mass Spectrophotometer. Electron spray ionization parameters used were: Capillary voltage – 80 eV, source temperature – 200 ºC, sample introduction by direct insertion probe, nebulizing gas used was nitrogen in positive ESI and air in negative ESI, nebulizing gas pressure 10 psi, scan range – m/z 50 to 2000, approx run time 0.25 seconds. Mass spectra were acquired using Costar software. All the samples were prepared in methanol.

Standard Solutions Preparation: A DTS stock solution (1000 µg mL^{-1}) and TMS (1000 µg mL^{-1}) was prepared by accurately weighing 10 mg of each in a 10 mL amber color volumetric flask. The volume was made up to the mark with methanol. Working solutions for HPLC injections were prepared on a daily basis from the stock solution in the mobile phase having a composition of 10 mM ammonium acetate buffer (pH 4.5) and methanol (50:50, v/v). Solutions were filtered through 0.45 µm nylon filters (Millipore, India) before injection.

Test solution preparation (Assay of marketed tablets): Twenty tablets were weighed and crushed to give a fine powder. Tablet powder equivalent to 1.00 mg of DTS and 0.8 mg of TMS was weighed and transferred to 10 mL amber color volumetric flask. Methanol (7 mL) was added to this and sonicated for complete extraction of drugs in methanol. This solution was filtered through Whatman filter paper into another 10 mL amber color volumetric flask and the volume was made up to the mark with methanol. Tablet solution (2 mL) was diluted to 10 mL with mobile phase to give working test solution of 20 µg mL^{-1} of DTS and 16 µg mL^{-1} of TMS.

Method Validation: The proposed HPLC method has been validated for precision, limits of detection and quantitation, linearity, specificity/selectivity, accuracy, and robustness. Method validation was performed as per ICH guidelines. System suitability tests were performed as per the general chapter <621> chromatography in USP 32 NF-27 to confirm the suitability and reproducibility of the system.

Precision: System precision was evaluated by analyzing the standard solutions of DTS and TMS (20 µg mL^{-1}) six times on the same day. The acceptance criterion was ± 2 % for the % RSD of the peak area and retention time (RT). The precision of the assay method was evaluated by carrying out six independent assays of a test sample of DTS and TMS at a concentration of 20 µg mL^{-1} and 16 µg mL^{-1} respectively on the same day. The % RSD of six obtained assay values was calculated. The intermediate precision of the method was evaluated on three different days.

Limits of detection (LOD) and quantitation (LOQ): The limit of detection and limit of quantitation were obtained from signal to noise ratio. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantitation limit was defined as the lowest concentration level that provided a peak area with a signal to noise ratio not less than 10.

Linearity: A stock solution (1000 µg mL^{-1}) of mixture of both drugs (DTS and TMS) was prepared in methanol. Linearity for both the drugs were evaluated at six concentration levels (10, 20, 30, 40, 50, 60 µg mL^{-1}) by serial dilutions of the above stock solution. Each concentration level solution was injected thrice. The analytical curve was evaluated on three different days.

The peak area versus concentration data was analysed with least squares linear regression. The slope and y-intercept of the calibration curve was recorded.

Specificity / Selectivity: The specificity / selectivity of the proposed method were evaluated by determining the concentrations of DTS and TMS in the presence of degradants in order to verify the non-interference of degradation products with the quantitation of drugs.

Accuracy: Accuracy was assessed by the determination of recovery of the method at three different concentrations (corresponding to 50%, 100%, and 150% of the test solution concentration for DTS and TMS) by addition of known amount of standard to the test solutions. Each solution was injected three times and the percentage recoveries were calculated.

Robustness: The robustness of study was carried out to evaluate the influence of small but deliberate variation in the chromatographic conditions. The robustness was tested by introducing small changes in the flow rate and linear gradient time for the mobile phase. The resolution between closely eluting degradation products and drug peaks was measured. The effect of flow rate was studied by decrease in the flow rate from 1.2 mL min^{-1} to 1.0 mL min^{-1} for the optimum mobile phase composition. The other method conditions were kept constant and the chromatograms were obtained. The resolution between closely eluting peaks were determined.

Solution Stability: The stability of solution for test preparation was evaluated. The solution was stored in a tightly capped volumetric flask at room temperature and tested at interval of 12, 24, 48 and 72 h. The responses for these solutions were evaluated using a freshly prepared standard solution.

System suitability: The standard solution of DTS and TMS each having concentration of 20 µg mL^{-1} and their forced degradation solutions in various conditions were injected five times. The parameters measured were peak area, retention time, resolution, peak purity, tailing factor, capacity factor and number of theoretical plates.

Forced degradation studies / Specificity: Both drugs (DTS and TMS) were subjected to forced degradation conditions individually as well as in combination as per ICH guidelines. All solutions for use in forced degradation studies were prepared at an initial concentration of 1 mg mL^{-1} of DTS and TMS. All samples were then diluted in mobile phase prior to the injection to give a final concentration of 10 µg mL^{-1}.

The conditions of the study were as follows:

1. **Acid, Alkaline and Neutral degradation studies:** Acid and alkaline decomposition was carried out in 1M HCl and 1M NaOH respectively at a concentration of 1.0 mg ml^{-1} of DTS and TMS. The acid and alkaline decomposition was carried under reflux conditions at temperature of 80°C for 8 h and 4 h respectively. Neutral degradation studies were also carried out in water under reflux conditions at temperature 80°C for 8 h.

2. **Oxidation:** Solutions for oxidative forced degradation studies were prepared in 3.0 % v/v solution of hydrogen peroxide at a concentration of 1000 µg mL^{-1} of DTS and TMS. The solutions were stored in dark at room temperature for 48 h.

3. **Thermal degradation studies:** For thermal degradation testing the drug powder was placed in a closed vial in an oven at 60 °C for 30 days.

4. **Photostability:** DTS and TMS were exposed to sunlight for 15 days in powder form and in methanolic solution having a concentration of 1 mg ml^{-1} of each drug. Suitable controls for photostability were also kept under dark condition.

5. **Isolation of Degradation products:** Preparative TLC was used for separation of degradation products from the reaction mixture using ethyl acetate, hexane and methanol in different compositions. The isolated degradation products were then characterized by MS-MS spectrometry.

RESULTS AND DISCUSSION:

HPLC Method development and Optimization: Isocratic mode of elution by HPLC analysis of all the degradation conditions was carried out initially on individual drugs to identify the degradation pathways. In Isocratic mode, mobile phase composition required for separation of DTS from its degradants was 10 mM ammonium acetate buffer (pH 4.5): methanol (20:80, v/v), and that for TMS was 10 mM ammonium acetate buffer (pH 4.5): methanol (50:50, v/v). When these mobile phases were tried for simultaneous estimation of DTS and TMS with their degradation products, merging of the peaks was observed.

Hence Gradient mode of elution was tried. Mobile phase in a linear gradient mode with composition of 10 mM ammonium acetate (pH 4.5): methanol (0-2 min, 50:50; 2-20 min, 20:80; 20-30 min, 50:50; 30-40 min, 50:50) with a flow rate of 1.2 mL min^{-1} and UV detection wavelength of 225 nm was found to give good resolution between the degradation products and standard drugs (DTS and TMS). The elution time for TMS and DTS was found to be at 4.585 min and 27.438 min respectively. The typical chromatograms obtained for standard drug and their degradants are shown in **Fig. 2(A & B).** Good resolution was observed between the drugs and their degradation products.

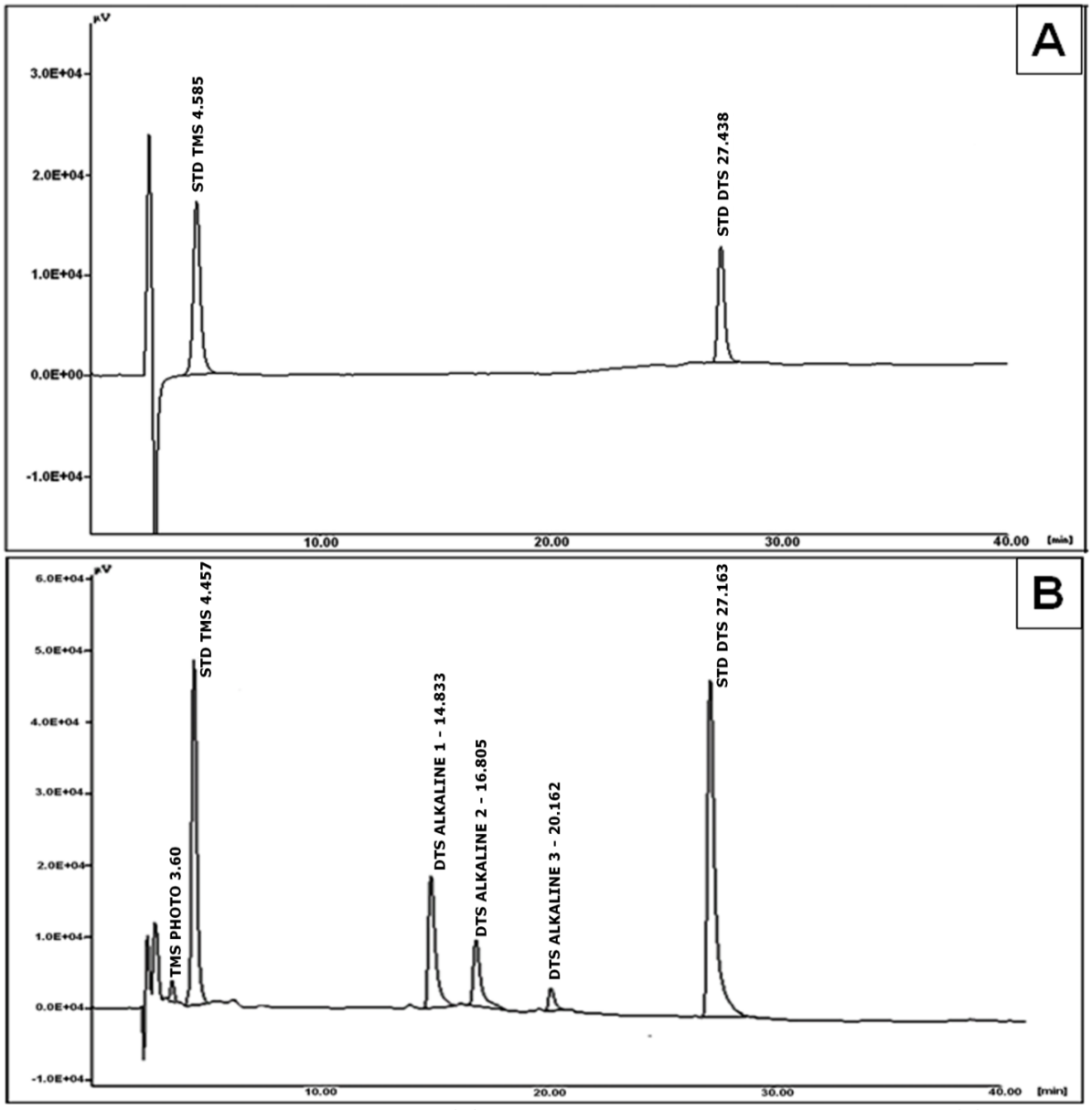

FIG. 2: HPLC CHROMATOGRAM OF (A) STANDARD DUTASTERIDE AND (B) TAMSULOSIN HYDROCHLORIDE

Method Validation:

Precision: The % RSD for the system precision was found to be 0.106 % and 1.248 % for RT and peak area of DTS, and 0.516 % and 0.326 % for RT and peak area of TMS on the same day (intra-day) respectively. The % RSD for method precision was found to be 0.520 % (intra-day) and 0.522 % (inter-day) for peak area of DTS, and 0.325 % (intraday) and 0.77 % (inter day) for peak area of TMS respectively. The % RSD for intra-day and inter-day assays values obtained was found to be 1.54 % for DTS and 0.76 % for TMS. The values obtained for system and method precision are shown in **Table 1**.

TABLE 1: SYSTEM AND METHOD PRECISION DATA (n = 6)

	System Precision				Method Precision			
	DTS		TMS		DTS		TMS	
	RT (min)	Peak Area	RT (min)	Peak Area	Concentration (µg mL^{-1})			
					Intra day	Inter day	Intra day	Inter day
Mean	26.268	20678	4.153	645433	19.989	20.057	20.502	20.592
SD	0.028	2581.537	0.021	2107.263	0.104	0.105	0.067	0.158
% RSD	0.106	1.248	0.516	0.326	0.520	0.522	0.325	0.770

LOQ and LOD: The LOD for DTS and TMS was found to be 0.02 µg mL^{-1} and 0.01 µg mL^{-1} respectively. The LOQ for DTS and TMS was found to be 0.2 µg mL^{-1} and 0.1 µg mL^{-1} respectively.

Linearity: The calibration curve was linear over the concentration range of 5 µg mL^{-1} to 70 µg mL^{-1} for DTS and TMS. The data for the peak area of DTS and TMS versus their concentration were treated by linear regression analysis with following equations, y = 32847.28x + 7879.603 for DTS and y = 18342.53 x + 4917.918 for TMS. The correlation coefficient (r^2) of 0.99897 for DTS and 0.99923 for TMS were obtained. The results revealed an excellent correlation between the peak area and analyte concentration. The data for linear regression equation is as shown in **Table 2**.

TABLE 2 LINEARITY DATA

Analyte		DTS	TMS
Slope ± SD (n=3)		32847.28 ± 158.37	18342.53 ± 34.37
Intercept ± SD		7879.603 ± 841.85	4917.918 ± 466.59
R^2 ± SD		0.99897 ± 0.00032	0.99923 ± 0.00015
Confidence interval of slope at 95%	**Lower**	33240.68	5788.33
	Upper	32453.88	9970.88
Confidence interval of intercept at 95%	**Lower**	18257.40	779.65
	Upper	18427.67	9056.19

Selectivity / Specificity: The specificity of the developed method was evaluated by checking the peak purity index and threshold for DTS and TMS observed in forced degradation samples. The spectral purity of drug peaks was evaluated using photodiode array detector and Chromeleon software to verify that the drug peaks were well resolved from the degradation peaks. The peak purity of DTS and TMS under different forced degradation conditions was found to be in the range of 993-1000, which is satisfactory and indicates that there was no interference with the analyte peak from degradation products. The average result for specificity and system suitability parameters are as shown in **Table 3**.

Accuracy: The method accuracy was proven by the recovery test. A known amount of DTS and TMS standards were added to the aliquots of test solution and diluted to concentrations as given in **Table 4.** The results obtained for accuracy are also shown in **Table 4**. Each level was injected thrice on same day. The assay was repeated for 3 consecutive days (n=9). The mean % RSD for this study was found to be 0.875 % for DTS and 0.68 % for TMS. The percentage recovery values for DTS and TMS was found to be 100.41 % and 99.85 % respectively.

TABLE 3: SPECIFICITY AND SYSTEM SUITABILITY OF DTS AND TMS IN DIFFERENT FORCED DEGRADATION STUDIES

Stress degradation	RT (min)	Peak area	% Assay	Peak Purity	Resolution	Asymmetry	Capacity Factor	Theoretical Plates
				DTS				
Standard	27.438	17406	100	995	18.12	0.97	1.62	3249
Acid	27.102	15162	87.33	999	3.96	1.39	2.85	2890
Alkaline	27.164	13821	79.40	994	4.64	1.10	2.92	4371
Neutral	27.082	16897	99.87	994	18.07	0.95	1.65	3985
Oxidation	27.077	17085	99.72	998	17.99	0.99	1.65	3256
Photo stability	27.129	16985	99.90	995	18.54	1.05	1.66	3996
Temp (60^{o}C)	27.304	16542	99.30	993	18.15	1.12	1.70	3105
				TMS				
Standard TMS	4.585	58867	100	998	2.015	1.19	0.53	4490
Acid	4.509	55559	99.53	996	2.401	1.18	0.71	3360
Alkaline	4.457	56641	99.93	1000	2.432	1.06	0.72	3888
Neutral	4.498	57853	100	997	2.389	0.93	0.70	3171
Oxidation	4.514	57992	100	997	2.2	0.95	0.61	3636
Photo stability	4.533	33161	88.46	999	1.33	1.14	0.62	2640
Temp (60^{o}C)	4.425	56248	100	998	2.202	1.07	0.61	3967

TABLE 4: ACCURACY (n = 9)

Level %	Concentration of drug added (µg mL^{-1})	Total concentration of drug (µg mL^{-1})	Mean Recovery %	% RSD
		DTS		
50	10	30	100.75	1.37
100	20	40	100.76	0.44
150	30	50	99.72	0.52
		Mean	**100.41 %**	**0.875 %**
		TMS		
50	10	26	100.46	0.56
100	20	36	99.61	0.21
150	30	46	99.49	0.44
		Mean	**99.85 %**	**0.68 %**

Robustness: The robustness of the method was assessed by assaying forced degradation sample solutions under different analytical conditions, and the resolution between closely eluting degradation products and drug was measured. The results for robustness studies are tabulated in **Table 5**.

The small change in flow rate of the mobile phase did not significantly affect the resolution between degradation products, DTS and TMS.

The % RSD of assay was found to be less than 1%. The different rates of change of buffer composition showed insignificant change in RT of TMS. A significant change in RT of DTS was seen, however the resolution between DTS and its degradation product was not affected much.

Hence, it can be said that the developed HPLC method was robust for the simultaneous determination of DTS and TMS in presence of their degradation products.

TABLE 5: ROBUSTNESS OF THE METHOD

Change in flow rate for the Mobile Phase: 10 mM ammonium acetate, pH 4.5 : methanol (0-2 min, 50:50; 2-20 min, 20:80; 20-30 min, 50:50; 30-40 min, 50:50)								
	RT						**% Assay**	
Flow rate (mL min^{-1})	**Photo TMS**	**STD TMS**	**DTS Deg 1**	**DTS Deg 2**	**DTS Deg 3**	**STD DTS**	**TMS**	**DTS**
1	4.980	5.193	18.126	21.524	26.987	34.667	99.85	98.99
1.2	3.60	4.457	14.833	16.805	20.162	27.163	100.21	99.84
Change in linear gradient time of the mobile phase; 10 mM ammonium acetate (pH 4.5) : methanol at a flow rate of 1.2 mL min^{-1}								
	RT						**% Assay**	
Time min	**Photo TMS**	**STD TMS**	**DTS Deg 1**	**DTS Deg 2**	**DTS Deg 3**	**STD DTS**	**TMS**	**DTS**
0 – 1.5 1.5 – 35 35 – 45 45 – 50	3.872	4.591	17.284	19.926	25.347	37.043	100.26	100.05
0 – 2 2 – 30 30 – 40 40 – 50	3.522	4.455	13.204	14.912	18.597	26.559	101.80	99.19
0 – 2 2 – 20 20 – 30 30 – 40	3.500	4.461	14.883	16.857	20.162	27.168	99.60	99.62
0 – 3 3 – 20 20 – 26 26 –32 32 – 45	3.419	4.272	14.027	16.942	19.013	24.525	98.50	100.84
0 – 2 2 – 15 15 – 25 25 – 30 30 – 40	3.174	4.091	12.742	15.034	16.745	21.547	101.90	99.54

TMS – Tamsulosin hydrochloride, STD – Standard, DTS – Dutasteride, Deg – Degradant

Solution Stability: The solutions of standard drugs and test preparations for assay determination were found to be stable up to 72 h. Assay values obtained were statistically identical with % RSD less than 2.

Assay of marketed tablets: The marketed tablets when assayed by the proposed method were found to contain 0.4915 mg of DTS (98.30 % of the label claim) and 0.3979 mg of TMS (99.48% of the label claim) per tablet. The % RSD for the assay of DTS and TMS was found to be 1.54 % and 0.76 % respectively.

Forced degradation studies: Two degradation products with 12.67 % degradation was observed when DTS was subjected to forced degradation in acidic condition, whereas additional one degradation product with 33.55% degradation was observed with forced degradation in alkaline condition. DTS did not show any degradation in neutral hydrolysis, oxidation, photolysis and thermal conditions.

The methanolic solution of TMS when exposed to sunlight showed 10 % degradation with the presence of one degradation product. No degradation for TMS was observed in acidic, alkaline and neutral hydrolysis, oxidation and thermal conditions.

Structure elucidation of degradation products: Alkaline degradants of DTS and photolytic degradant of TMS were isolated using preparative thin layer chromatography (TLC). Mobile phase used for separation of alkaline degradants of DTS was ethyl acetate: hexane: methanol in the ratio of 60:40:0.4 v/v/v.

Mobile phase used for separation of photo degradant of TMS was ethyl acetate: hexane: methanol in the ratio of 50:50:0.5 v/v/v. All the degradants were characterized by Mass spectrometry.

LC-PDA studies: The HPLC chromatogram and PDA-UV spectra of DTS and its acid and alkaline degradants are shown in **Fig. 3.** The PDA absorption spectra of acid degradation product **I** of DTS (RT-5.3 min, λ_{max} – 218.5 nm) and alkaline degradation product **I** (RT-5.3 min, λ_{max} – 219.7) of DTS indicate that both the degradation products have similar UV absorbance spectra. Also acid degradation product **II** (RT-8.9 min, λ_{max} – 218.5 nm, 240.0 nm, 275.1 nm) and alkaline degradation product **III** (RT-9.0 min, λ_{max} – 219.7 nm, 240.1 nm, 274.1nm) of DTS showed similar absorbance spectra. However, alkaline degradation product **I** and **III** had different absorbance spectra with λ_{max} of 219.7 nm and 202.3 nm respectively, which indicated that alkaline degradation product **III** has a different chromophore than the alkaline degradation product **I** as well as parent drug. This suggested that the drug chromophore was lost or altered during conversion of DTS to alkaline degradation products **I** and **III**. The alkaline degradation product **II** of DTS had different retention time (RT-6.9 min) than the standard DTS (RT-12.2 min). However the UV absorbance spectrum of alkaline degradation product **II** of DTS was found to match with the UV absorbance spectrum of standard DTS. The results obtained in MS-MS fragmentation studies shows that the alkaline degradation product II of DTS is sodium adduct of DTS.

The HPLC chromatogram and PDA-UV spectra of TMS and its photo degradant are shown in **Fig. 4** The PDA absorption spectra of TMS photo degradation product (RT-4.881 min) were found to be different than the standard TMS (RT-5.804 min). The standard TMS showed two wavelengths of maxima at 225.2 nm and 279.7 nm, whereas the photo degradation product of TMS showed absorption maxima at 254.5 nm and 279.9 nm**.** This indicates that TMS undergoes photo degradation. The results obtained in MS-MS fragmentation studies of TMS suggest that some structural rearrangement has taken place.

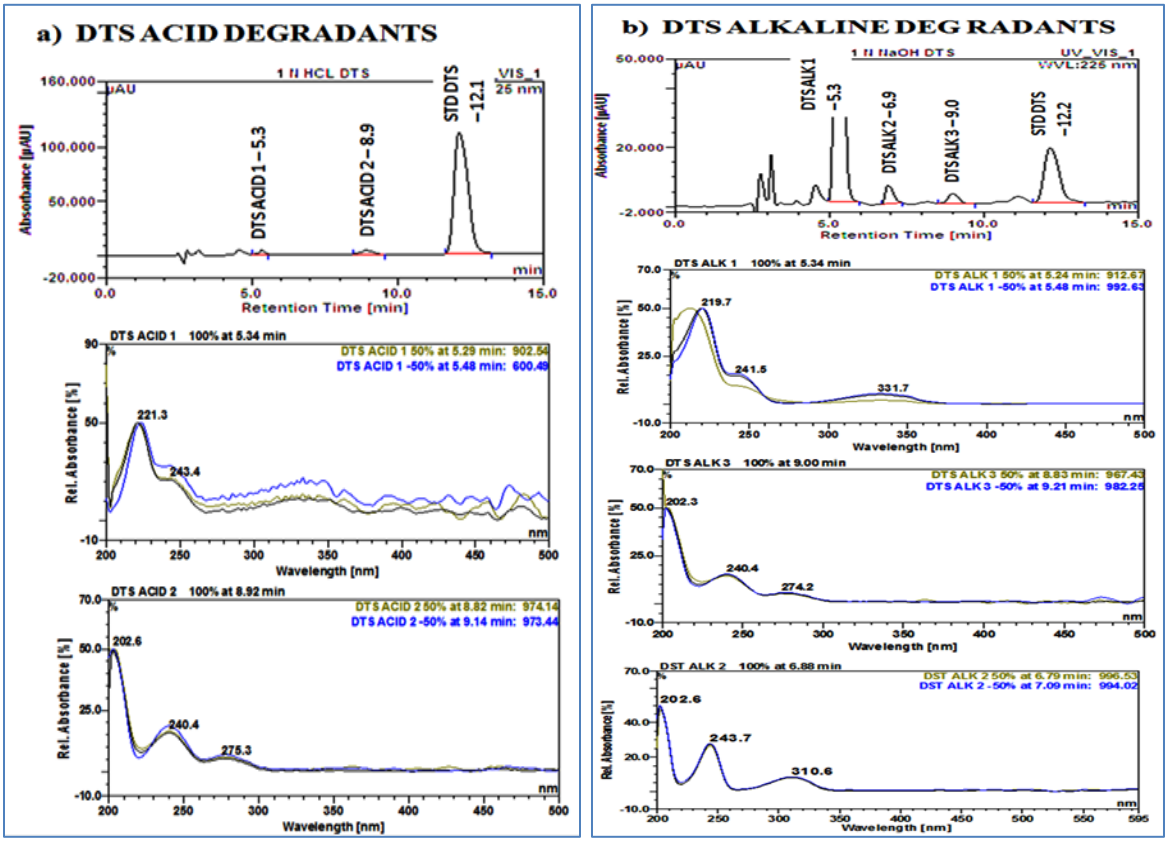

FIG 3: HPLC CHROMATOGRAM AND PDA ABSORPTION SPECTRA OF: a) ACID DEGRADANTS OF DTS & b) ALKALINE DEGRADANTS OF DTS

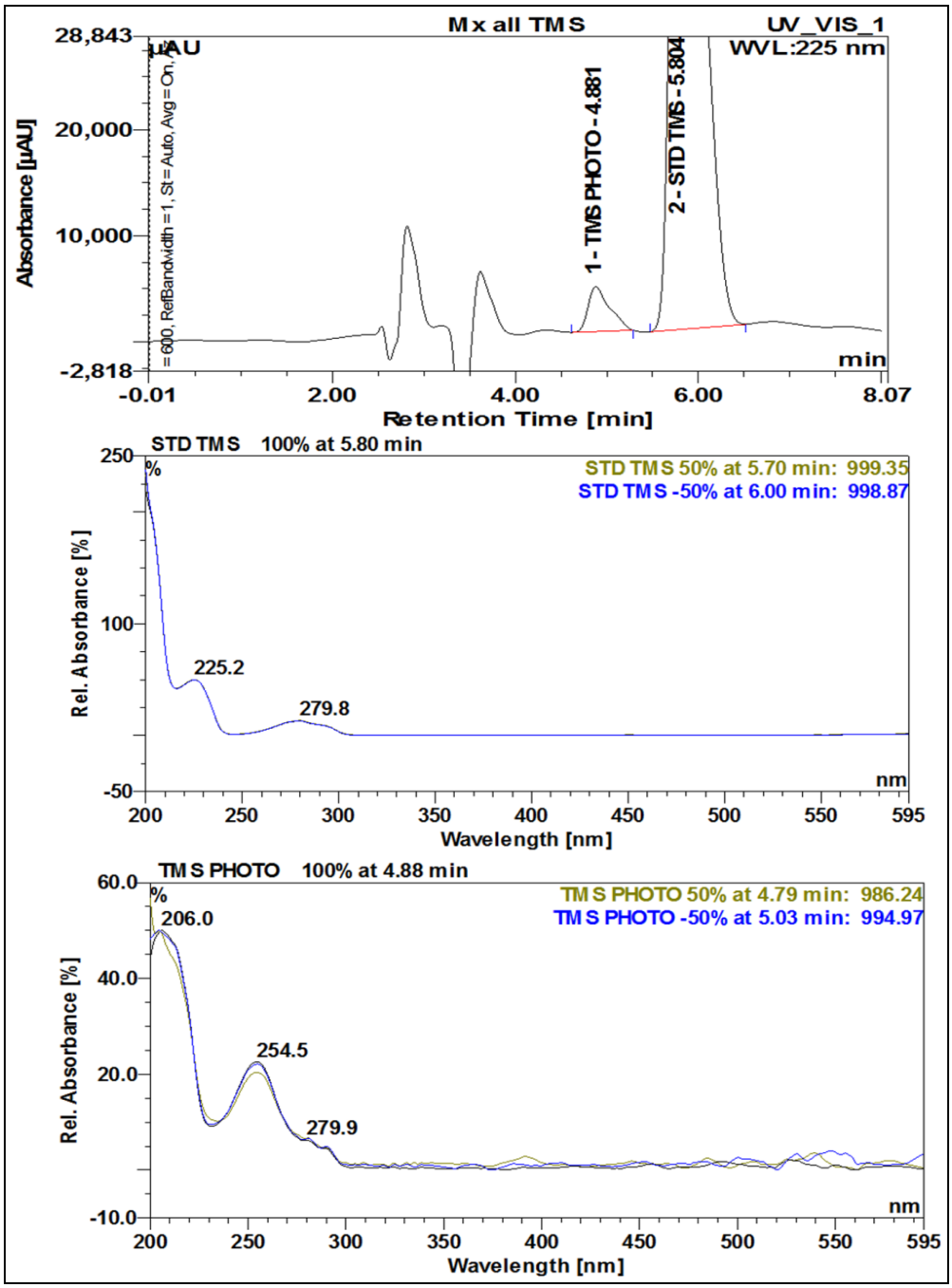

FIG. 4: HPLC CHROMATOGRAM AND PDA ABSORPTION SPECTRA OF: a) STANDARD TMS AND b) PHOTO DEGRADANT OF TMS

MS/MS fragmentation of DTS: The MS spectrum, MS-MS spectrum and fragmentation pattern of DTS is shown in **Fig. 5.** The base peak of DTS $[M+H]^+$ with m/z of 529.1 was formed in positive ESI mode. The daughter ion of DTS was observed at m/z of 461.2, which was same as reported in the literature. The MS spectra, MS-MS spectra, Fragmentation pattern and hydrolysis mechanism of acid hydrolysis degradants of DTS are shown in **Fig. 6** and **Fig. 7** respectively.

Two degradants with m/z of 546.2 and m/z of 527.5 were formed by acid hydrolysis to give carboxylic acid derivative (**I**) and by dehydrogenation to give degradation product (**III)** respectively. Degradation product **I** with m/z of 546.2 was formed by addition of 17 amu $[M+17]^+$ that indicated hydrolysis of the compound. Breaking of DTS via lactam ring was observed, as they are more susceptible to hydrolysis and forms acid and amine group.

Degradation product **III** with m/z of 527.5 was formed by difference of 2 amu $[M-2H]^+$ indicating removal of hydrogen molecule from DTS. The MS spectrum, MS-MS spectrum, fragmentation pattern and hydrolysis pattern of alkaline hydrolysis of DTS is shown in **Fig. 8**. Degradation product **II** with m/z of 573.3 was formed by addition of 44 amu $[M+44]^+$ indicating di-sodium adduct of DTS. Degradation product **II** was formed only in alkaline hydrolytic condition. The degradation product **I** and **III** was formed in both acidic and alkaline hydrolysis. The degradation products for DTS were interpreted and identified by us as (**I**) - 4-[1-(2,5-Bis-trifluoromethyl-phenylcarbamoyl)-4,7a-dimethyl-2,3,3a,6,7,7a - hexahydro-1H-inden-5-yl]-pentanoic acid (MW. 546.2) , (**II**) Sodium adduct of DTS (MW. 573.3) and (**III**) - 4a,6a-Dimethyl-2-oxo-4a,4b,5,6,6a,7,8,9,9a,9b,10,11-dodecahydro-2H-indeno[5,4-f]quinoline-7-carboxylic acid (2,5-bis-trifluoromethyl-phenyl)-amide (MW. 527.5). The possible molecular formulae and major fragments of standard DTS and its degradation products are as given in **Table 6** and **Table 7** respectively.

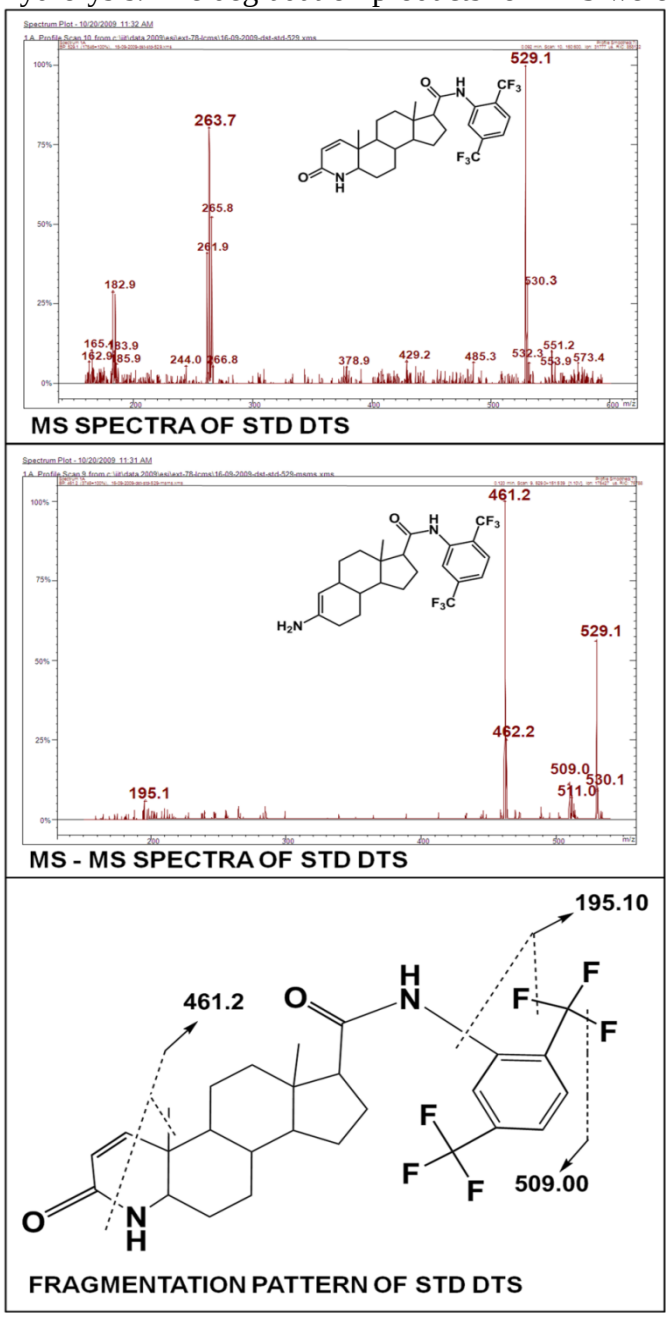

FIG. 5: MASS SPECTRAL DETAILS OF STANDARD DTS

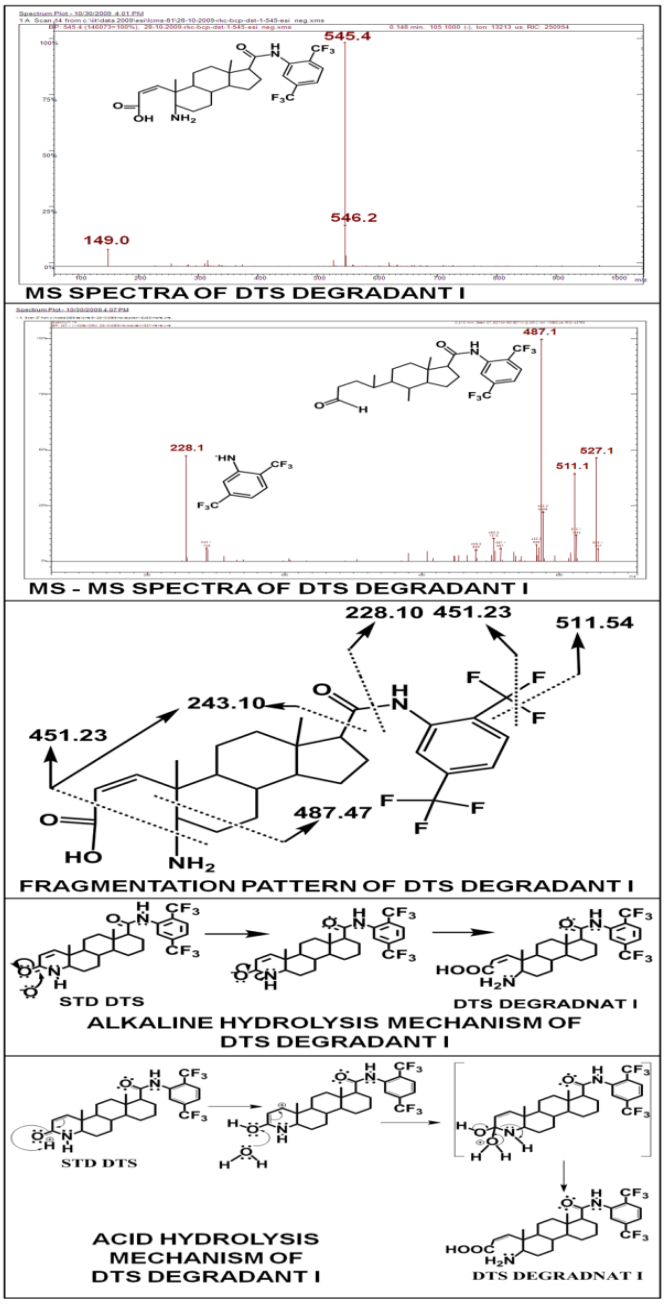

FIG. 6: MASS SPECTRAL DETAILS OF DTS DEGRADANT I

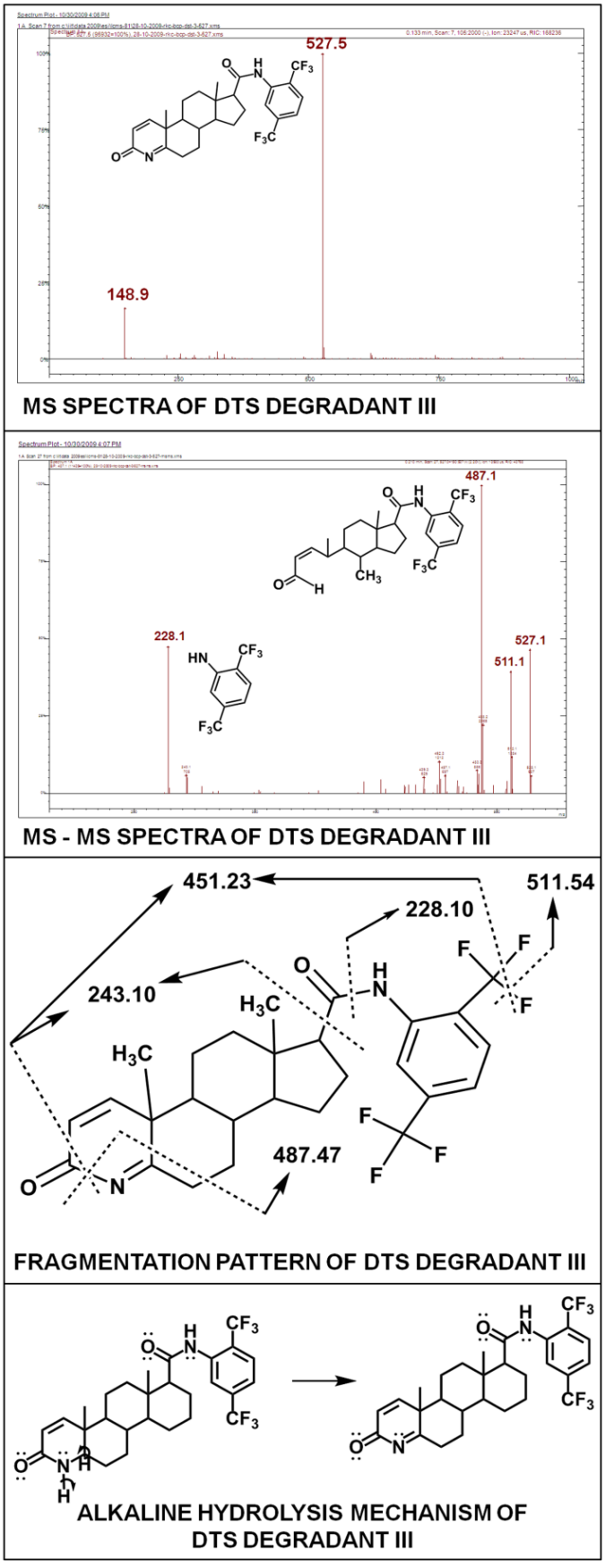

FIG. 7: MASS SPECTRAL DETAILS OF DTS DEGRADANT III

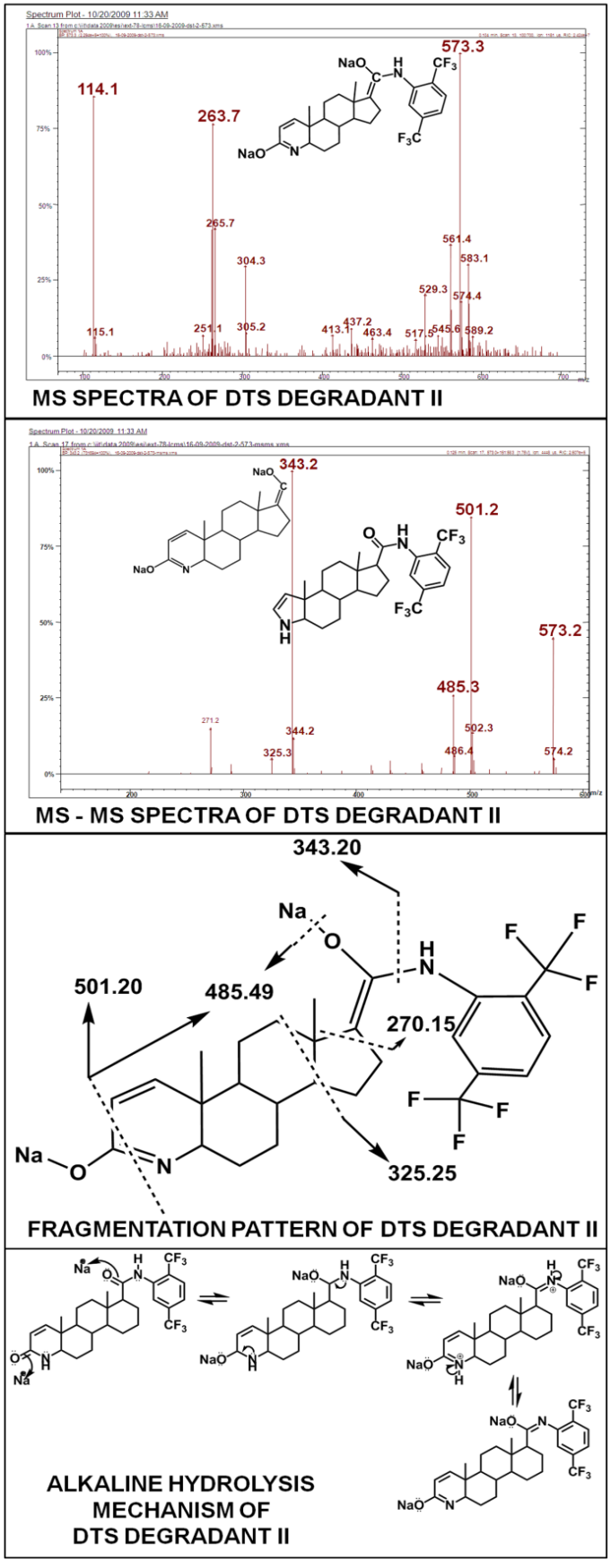

FIG. 8: MASS SPECTRAL DETAILS OF DTS DEGRADANT II

TABLE 6: MS-MS DATA OF STANDARD DTS AND TMS

Sr. No.	Experimental mass	Best possible molecular formula	Theoretical mass	Error in mmu	Difference from parent ion	Possible loss corresponding to difference
			DTS			
1	529.10	$C_{27}H_{30}F_6N_2O_2^+$	528.22	0.880		
2	509.00	$C_{27}H_{30}F_5N_2O_2^+$	509.53	-0.53	20.1	F
3	461.20	$C_{23}H_{26}F_6N_2O^+$	461.46	-0.26	67.9	C_4H_4O
4	195.10	$C_8H_4F_5^+$	19.108	-0.008	334.0	$C_{19}H_{26}FN_2O_2$
			TMS			
1	409.10	$C_{20}H_{28}N_2O_5S^+$	408.17	0.93		
2	271.00	$C_{12}H_{20}N_2O_3S^+$	271.36	-0.36	138.10	$C_8H_8O_2$
3	228.10	$C_{10}H_{14}NO_3S^+$	228.29	-0.19	181.00	$C_{10}H_{14}NO_2$
4	200.10	$C_8H_{10}NO_3S^+$	200.234	-0.134	209.00	$C_{12}H_{18}NO_2$

TABLE 7: MS - MS DATA OF DEGRADATION PRODUCTS OF DTS AND TMS WITH THEIR POSSIBLE MOLECULAR FORMULAE AND MAJOR FRAGMENTS

Degradation products	Experimental mass	Best possible molecular formula	Theoretical Mass	Error in mmu	Major fragments		Error in mmu	Best possible molecular formula
					Experimental mass	Theoretical mass		
				DTS				
Base Degradant I	545.40	$C_{27}H_{31}F_6N_2O_3^-$	545.54	-0.14	511.0	511.54	-0.54	$C_{27}H_{32}F_5N_2O_2^-$
					487.10	487.48	-0.38	$C_{25}H_{27}F_6NO_2^-$
					452.3	451.52	0.78	$C_{25}H_{31}F_4N_2O^-$
					228.10	228.12	-0.02	$C_8H_4F_6N^-$
Base Degradant II	527.50	$C_{27}H_{28}F_6N_2O_2^-$	526.21	1.29	511.0	511.54	-0.54	$C_{27}H_{32}F_5N_2O_2^-$
					487.10	487.48	-0.38	$C_{25}H_{27}F_6NO_2^-$
					452.3	451.52	0.78	$C_{25}H_{31}F_4N_2O^-$
					228.10	228.12	-0.02	$C_8H_4F_6N^-$
Base Degradant III	573.30	$C_{27}H_{28}F_6N_2Na_2O_2^-$	572.49	0.81	501.20	500.23	0.97	$C_{26}H_{30}F_6N_2O^-$
					485.30	485.49	-0.19	$C_{25}H_{27}F_6N_2O^-$
					343.20	344.16	-0.96	$C_{19}H_{24}F_6NNa_2O_2^-$
					271.20	270.15	1.05	$C_{10}H_6F_6NO^-$
				TMS				
Photo Degradant	409.10	$C_{20}H_{28}N_2NO_5S^+$	408.19	0.91	271.00	271.36	-0.36	$C_{12}H_{20}N_2O_3S^+$
					228.10	228.29	-0.19	$C_{10}H_{14}NO_3S^+$
					200.10	200.234	-0.13	$C_8H_{10}NO_3S^+$

MS/MS fragmentation of TMS: The MS spectrum, MS-MS spectrum and fragmentation pattern of TMS is shown in **Fig. 9**. The base peak of TMS $[M+H]^+$ with m/z of 409.1 was formed in positive ESI mode. It showed major degradation in photostability studies forming a compound, relatively polar but showing the same m/z value of 409.1 as that of the base peak of TMS. The MS spectrum, MS-MS spectrum, fragmentation pattern and photo degradation mechanism of TMS is shown in **Fig. 10**. This indicates that under photolytic degradation only structural modification occurred in TMS molecule. The photodegradation product for TMS was identified as -5-{2-[2-(2-Hydroxy-phenoxy)-butylamino]-propyl}-2-methoxy-benzene sulfonamide. The data for possible molecular formulae and major fragments of standard TMS and its photo degradation product is given in **Table 6** and **Table 7** respectively.

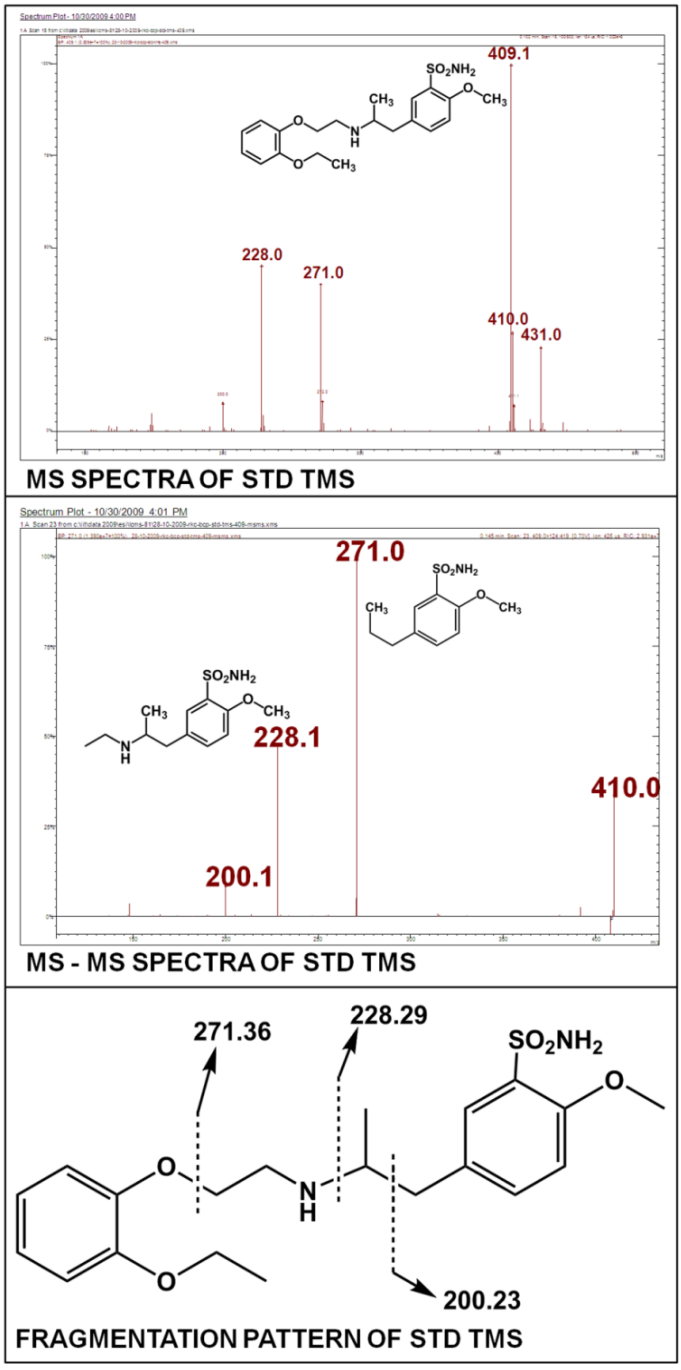

FIG. 9: MASS SPECTRAL DETAILS OF STD TMS

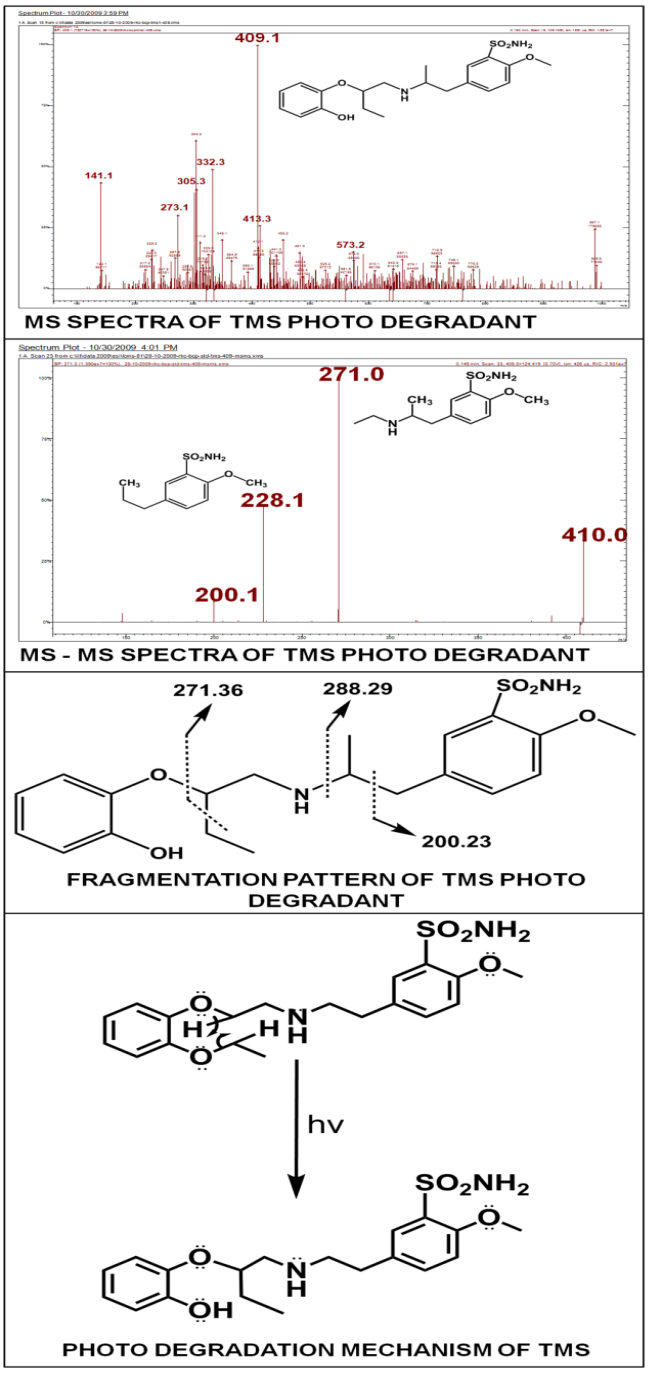

FIG. 10: MASS SPECTRAL DETAILS OF TMS PHOTO DEGRADANT

CONCLUSIONS: The proposed forced degradation studies on Dutasteride (DTS) and Tamsulosin hydrochloride (TMS) as per ICH guidelines helped us in the identification of the degradation pathways for these drugs with the MS-MS structure elucidation of degradation products. The Stability indicating RP-HPLC method for the simultaneous estimation of DTS and TMS was found to be very simple, accurate, precise and rugged. The above studies are reported for the first time and would be useful in stability samples in industry as well as for the routine analysis of the tablets.

ACKNOWLEDGEMENT: We wish to thank Cipla Pvt. Ltd, Mumbai for providing us gift samples of Dutasteride and Tamsulosin hydrochloride.

REFERENCES:

1. Bakshi M and Singh S: Development of validated stability-indicating assay methods critical review. Journal of Pharmaceutical and Biomedical Analysis 2002; 28:1011- 1040.
2. Ruan J, Tattersall P, Lozano R and Shah P: The Role of Forced Degradation Studies in Stability Indicating HPLC Method Development. American Pharmaceutical Review. Analytical Research & Development, Bristol – Myers Squibb. (http://americanpharmaceuticalreview.com/ViewArticle.aspx?ContentID=339)
3. Kamila MM, Mondal N and Ghosh LK: A validated spectrophotometric method for determination of dutasteride in bulk drug and pharmaceutical formulations. International Journal of PharmTech Research 2010, 2: 113 – 117.
4. Kumar AVVNKS, Saradhi SV, Sekaran CB, Reddy TV: Spectrophotometric Analysis of Dutasteride in Pure and Tablet Dosage Forms. Chemical Sciences Journal 2012: CSJ-47.
5. Subba Rao DV and Radhakrishnan and P: Stress Degradation Studies on DTS and Development of a Stability-Indicating HPLC Assay Method. Chromatographia 2008; 67:841 - 845.
6. Kamat SS, Choudhari V, Vele VT and Prabhune SS: Determination of dutasteride by LC: Validation and Application of the Method. Chromatographia 2008; 67:911 – 916.
7. Patel DB, Patel NJ, Patel SK, Prajapati AM and Pate SA: RP-HPLC method for the estimation of dutasteride in tablet dosage form. Indian Journal of Pharmaceutical Sciences 2010; 72: 113-116.
8. Navaneeswari R and Reddy R: Development and Validation of a RPHPLC Method for Dutasteride and its Impurities in Bulk Drug. African Journal of Scientific Research 2011; 6:318 – 324.
9. Ramakrishna NVS, Vishwottam KN, Puran S, Koteshwara M, Manoj S and Santosh M: Selective and rapid liquid chromatography–tandem mass spectrometry assay of dutasteride. Journal of Chromatography B 2004; 809:117 – 124.
10. Burinsky DJ, Williams JD, Thornquest AD and Sides SL: Mass spectral fragmentation reactions of a therapeutic 4-Azasteroid and related compounds. Journal of the American Society for Mass Spectrometry 2001; 12:385 – 398.
11. Varmuza K, Rotter H and Krenmayr P: Interpretation of Steroid Mass Spectra with Pattern Recognition Methods. Chromatographia 1974; 7:522 - 525.
12. Kamat SS, Vele VT, Choudhari VC and Prabhune SS: A thin layer chromatography (TLC) method for determination of dutasteride form bulk and pharmaceutical preparations. Asian Journal of Chemistry 2008; 20:5033 – 5036.
13. Choudhari VP and Nikalje AP: Stability-Indicating TLC Method for the Determination of Dutasteride in Pharmaceutical Dosage Forms. Chromatographia 2009; 70:309 – 313.
14. Patel DB, Patel NJ, Patel SK, and Patel PU: Validated Stability Indicating HPTLC Method for the Determination of Dutasteride in Pharmaceutical Dosage Forms. Chromatography Research International 2011, 1-5
15. Choudhari VP, Gite SR, Raut RP, Hable AA, Parekar SR and Kuchekar BS: 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. International Journal of Pharmaceutical Sciences, Review Research 2010; 2:63 - 67.
16. Sreelakshmi V, Rao VUM, Venkata PM, Pugazhendhy S and Sunitha M: A Validated RP-HPLC Method for Simultaneous Estimation of Tamsulosin and Dutasteride in Tablet Dosage Form. Inventi Rapid: Pharmaceutical Analysis & Quality Assurance 2013.
17. Sowmya Y, Aleti P and Venisetty RK: Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Dutasteride and Tamsulosin in Tablet Dosage Form. International Journal of Pharmacy and Biological Sciences 2013; 3:301 – 316.
18. Giriraj P and Sivakkumar T: Simultaneous estimation of dutasteride and tamsulosin hydrochloride in tablet dosage form by vierordt's method. Arabian Journal of Chemistry 2013; In Press.
19. Agarwal S, Gowda KV, Sarkar AK, Ghosh D, Bhaumik U, Chattaraj TK and Pal TK: Simultaneous determination of DTS and tamsulosin HCl by LC– MS–MS in human plasma. Chromatographia 2008; 67:893 - 903.
20. European Pharmacopeia 6.0; 2008; 2:3016.
21. Nanda RK, Gaikwad J and Prakash A: Simultaneous Spectrophotometric Estimation of Tamsulosin in Pharmaceutical Dosage Form. Asian Journal of Research in Chemistry 2009; 2:63 - 65.
22. Gadhve NA, Sawant SC, Ghante MR and Nikam AD: Spectrophotometric estimation of tamsulosin hydrochloride in tablet dosage form. International Journal of Pharmaceutical Research Development 2011; 3:87 - 92.
23. Bari SB, Bakshi AR, Jain PS and Surana SJ: Application of UV-Spectroscopy and First Order Derivative Method for Determination of Tamsulosin Hydrochloride in Bulk and Tablets. Pharmaceutica Analalytica Acta 2011; 2:120 – 121.
24. Shrivastava A, Saxena P and Gupta VB: Spectrophotometric estimation of tamsulosin hydrochloride by acid-dye method. Pharmaceutical Methods 2011; 2:53 - 60.
25. Chaudhari BG, Patel NU and Patel DB: Spectrophotometric Method for Estimation of Tamsulosin Hydrochloride in Pharmaceutical Dosage Form Using Bromate-Bromide and Methyl Orange Reagent. International J Pharmaceutical Research Scholars 2012; 1:104 - 111.
26. Basniwal PK, Panda S, Jain S and Jain D: Stability-indicating HPLC Assay Method and Degradation Profile of Tamsulosin. American-Eurasian Journal of Scientific Research 2012; 7:193 - 198.
27. Kumar GS and Kumar SP: Stability-Indicating RP-HPLC Method for Determination of Tamsulosin HCL in Pharmaceutical Dosage Form" Journal of Basic and Clinical Pharmacy 2012; 3:255 - 260.
28. Siva RKGV, Janardhan M and Rasool S: Development and validation of stability-indicating RP-HPLC method for estimation of tamsulosin HCl pellets. International Journal of Pharmaceutical Invention 2012; 2:51 - 60.
29. Jain PS, Chaudhari AJ, Bari PR and Surana SJ: Validated stability-indicating RP-HPLC method for tamsulosin hydrochloride in pharmaceutical dosage form according to ICH guidelines: Application to stability studies. Der Pharmacia Lettre 2012; 4:1760 - 1767.

30. Bari SB, Bakhshi AR, Jain PS and Surana SJ: Development and Validation of Stability-Indicating HPTLC Determination of Tamsulosin in Bulk and Pharmaceutical Dosage Form. Chromatography Research International 2011; 1-6.
31. Thimmaraju MK, Rao V, Hemanth K and Siddartha P: RP HPLC Method for the determination of Tamsulosin in bulk and Pharmaceutical formulations. Journal of Applied Pharmaceutical Science 2011; 1:177 - 180.
32. Kumari R, Dash PP, Lal VK, Mishra A, Murthy PN: RP - HPLC method for the estimation of Tamsulosin Hydrochloride in Tablet Dosage Form. Indian Journal of Pharmaceutical Sciences 2010; 72: 785 - 787.
33. Macek J, Klıma J and Ptacek P: Rapid determination of tamsulosin in human plasma by high-performance liquid chromatography using extraction with butyl acetate. Journal of Chromatography B 2004; 809:307 – 311.
34. Matsushima H, Takanuki KI, Kamimura H, Watanabe T and Higuchi S: Highly sensitive method for determination of tamsulosin hydrochloride inhuman plasma dialysate, plasma and urine by high-performance liquid chromatography-electrospray tandem mass spectrometry. Journal of Chromatography B 1997; 695:317 – 327.
35. Pekka KR, Olavi P, Esa LA, Timo M, Marko L and Seppo A: Determination of tamsulosin in human aqueous humor and serum by liquid chromatography–electrospray ionization tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 2007; 43:606 – 412.
36. Choi CI, Lee HI, Bae JW, Lee YJ, Byeon JY, Jang CG and Lee SY: Determination of tamsulosin in human plasma by liquid chromatography/tandem mass spectrometry and its application to a pharmacokinetic study. Journal of Chromatography B: Analytical Technology and Biomedical Life Sciences 2012; 909:65 - 69.
37. Li D, Limin Li, Ping T, Jin Y and Zhengxing Z: Quantitation of tamsulosin in human plasma by liquid chromatography - electrospray ionization mass spectrometry. Journal of Chromatography B 2002; 767:75 – 81.
38. Qi M, Wang P and Liu L: Determination of tamsulosin in dog plasma by liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry. Journal of Chromatography B 2004; 805:7 – 11.
39. Rao RN, Kumartalluri MVN, Narasa RA, Shinde DD and Ramanjaneyulu GS: Development of a validated RP-LC/ESI-MS–MS method for separation, identification and determination of related substances of tamsulosin in bulk drugs and formulations. Journal of Pharmaceutical and Biomedical Analysis 2008; 46:94 – 103.
40. Maier V, Horakova J, Petr J, Tesarova E, Coufal P and Sevcık J: Chiral separation of tamsulosin by capillary electrophoresis. Journal of Pharmaceutical and Biomedical Analysis 2005; 39:691 – 696.
41. Zhefeng Z, Gengliang Y, Guijian L, Haiyan L and Yi C: Chiral separation of Tamsulosin isomers by HPLC using cellulose Tris (3,5- dimethhylphenylcarbamate) as a chiral stationary phase. Journal of Pharmaceutical and Biomedical Analysis 2004; 34:689 – 693.
42. Choudhari VP and Nikalje APG: Stability-Indicating HPTLC Method for the Determination of Tamsulosin in Pharmaceutical Dosage Forms. Chromatographia 2009; 69:1 - 5.
43. Kulkarni SP and Amin PD: Stability indicating HPTLC determination of timolol maleate as bulk drug and in pharmaceutical preparations. Journal of Pharmaceutical and Biomedical Analysis 2000; 23:983 – 987.
44. Thoppil SO, Cardoza RM and Amin PD: Stability indicating HPTLC determination of trimetazidin as bulk drug in pharmaceutical formulation. Journal of Pharmaceutical and Biomedical Analysis 2001; 25:15 - 20.
45. Makhija SN and Vavia PR: Stability indicating HPTLC method for the simultaneous determination of pseudophedrine & cetirizine in pharmaceutical formulation. Journal of Pharmaceutical and Biomedical Analysis 2001; 25:663 – 667.
46. ICH Q1A (R2). Stability testing of new drug substances and products. Geneva, February 2003.

How to cite this article:
R Chaudhari, KP Mohanraj and V Shirsat: MS/MS and HPLC characterization of forced degradation products of dutasteride and tamsulosin hydrochloride. *Int J Pharm Sci Res* 2014; 5(7): 2791-2806.doi: 10.13040/IJPSR.0975-8232.5 (7).2791-2806.