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[Research article]

Analytical Method Development And Validation of Dutasteride and Tamsulosin Hcl in Combination And Its Stress Degradation Studies

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ABSTRACT

Asimple,specific, sensitive,precise and reproducible Reverse Phase High Performance liquid Chromatography method has been developed for simultaneous estimation of Dutasteride and Tamsulosin Hcl. Dutasteride and Tamsulosin is Anti-hyperplasia and Anti-hypertensive drug.The determinationwas carried out byusingsymmetryC-18columnwith Methanol:0.1M Monobasic potassiumdihydrogenphosphate buffer(75:25) Adjusted the pH to 2.5 with Ortho phosporic acid as the mobile phase and with the detection wavelength of274 nmrespectively.The flow rate is 0.7 ml/min.TheRetentiontime of Dutasteride,Tamsulosin Hcl was 2.218 minand 6.599 min respectively.Linearityforthe Dutasteride and Tamsulosin Hcl were found inthe rangeof 25-75µgmand 20-60µgm respectively.The limitof quantificationforbothdrugs wasfound to be30,24µg respectively.The recoveries of Tamsulosin and Dutasteride were found to be inthe range of 99.81-99.90 %and98.00-102.00%, respectively. The proposed methodwas validated suitablyand canbeused forroutine analysis. The degradation studies indicated Dutasteride andTamsulosin Hcl to besusceptible to neutralhydrolysis, while Dutasteride and Tamsulosin Hcl showed degradation inacid, H2O2,photolytic and inpresenceof UV radiation.The degradation productsof Dutasteride andTamsulosin Hcl inacidic and photolytic conditions were well resolved from the pure drugwithsignificant differences in the irretention time values. This method can be successfully employed forsimultaneous quantitative analysis of Dutasteride and Tamsulosin Hcl in formulations.

Keywords: HPLC, simultaneous estimation, Dutasteride and Tamsulosin Hcl, stress degradation studies.

INTRODUCTION

Dutasteride (DUT) and Tamsulosin (TAM) combination tablets are newly marketed and both areused as Alpha-blockers help relieve BPH symptoms agents. DUT is chemically (5α, 17β)-N-{2, 5bis (trifluoromethyl) phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide.Dutasteride is 5-alphareductase enzyme inhibitors. Tamsulosin is a (*R*)-5-(2-{[2-(2-ethoxyphenoxy) ethyl] amino} propyl)-2-methoxybenzene-1-sulfonamide, mono hydrochloride. Tamsulosin is an Alpha-blockers help relieve BPH symptoms agents two compounds are active in the metabolism of body.In recent times, there is an increased tendency towards the development of stability indicating assays, using the approach of stress testing as enshrined in the

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International Conference on Harmonization ICH)guideline[1,2]. Even this approach is being extended to drug combinations inderivative spectroscopy[3]. There is no stability-indicating assay method reported yet for this combination developed using the ICH approach of stress testing[4-6]. There are several LC-/MSMS procedures known for the analysis individualy[7-9].There is several RP-HPLC, TLC procedures known for the analysis individualy[10-13].It is needed to develop a method without any drawbacks and only very few methods have been reported for estimation of DUT and for TAM by simultaneous estimation individually and by HPLC method.

MATERIALS AND METHODS

Pharmaceutical grade DUT and TAM were supplied by Orchid chemicals, Chennai, India, with the purity of 99.70% and 99.50% respectively on dried basis. Methanol HPLC grade of merck, NaOH AR grade, Potassium di hydrogen ortho phosphate AR grade, ortho phosphoric acid AR grade, Hydrochloric acid AR grade, Purified Water milli-Q water, were used for the analytical purpose. Waters HPLC System 2965, with Symmetry C_{18} column (150x4.6 mm, 0.5 µm) and Dual λ absorbance Detector 2996 worked in room temperature.

CHROMATOGRAPHIC CONDITIONS

The separation the drugs were performed by using symmetry C_{18} (150 × 4.6 mm, 5µm particle size) column. Mobile Phase consisted of a mixture of Methanol: water (75: 25 v/v) with a Flow rate of 0.7 ml/minute. Volume of injection is 20:l and the detection wavelength was 274 nm.

Preparation of Phosphate buffer

Weighed 7.0 grams of KH_2PO_4 into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH to 2.5 with Ortho phosporic acid.
Mobile Phase was prepared by mixing 750 ml of Methanol HPLC Grade with 250 ml of Potassium di hydrogen ortho Phosphate buffer in a 1000 ml standard flask to get the proportion of 75:25 v/v. The mobile phase was filtered through 0.45 micron membrane filter and degassed by Ultrasonication for 15 min. The standard stock solutions of 50 mg of DUT and 40 mg of TAM were prepared by dissolving in mobile phase, in a 10 ml volumetric flask and made up to the volume. Final dilutions were made to 50 µg/ml of DUT, 40 µg/ml of TAM were prepared by dissolving in mobile phase, in a 10 ml volumetric flask and made up to the volume were stored under refrigeration. From the above solutions the dilutions of working standards were made from 25-75 µg/ml DUT and 20-60 µg/ml for TAM respectively.

CALIBRATION CURVE

The calibration curves were constructed for the determination of the linearity and the curves were plotted with the concentration range verses area must obey Beer's law. The linearity was evaluated by analysis of the serially diluted sample in the range of 25-75 µg/ml and 20-60 µg/ml for DUT and TAM respectively. An aliquot was injected using mixture of Methanol and Buffer solution in the ratio of 75:25 v/v. The retention times were 2.218 min and 6.599 min for DUT and TAM respectively with a good resolution of 12.76.

ANALYSIS OF FORMULATIONS

Analysis of the tablets was conducted in only one brand. Twenty tablets of the brand were weighed and powdered separately. A quantity equivalent to 480 mg of DUT and TAM were transferred to 10 ml volumetric flask and dissolved 10 ml of Mobile phase.

STRESS DEGRADATION STUDIES

Forced degradation studies of both the drugs were carried out under conditions of Acid hydrolysis, Alkali hydrolysis, Peroxide oxidation, UV light and photolysis. DUT and TAM were weighed (50 mg and 40mg) and transferred into 10 ml volumetric flasks and diluted up to the mark with mobile phase.

Acid hydrolysis

To 10 ml of the drug solution, 10 ml of 1 m hydrochloric acid was added and the mixture was refluxed on a water bath for 60 minutes at 90º. The forced degradation in acidic media was performed in the dark in order to exclude the possible degrative effect of light. The resulting solution was neutralized by the base, to avoid any interference of acid or base.

20 µl of the resulting solution was injected into HPLC and the chromatograms were recorded.

Alkali hydrolysis

To 10 ml of the drug solution, 10 ml of 0.1 m sodium hydroxide was added and the mixture was refluxed on a water bath for 60 minutes at 90º. The forced degradation in basic media was performed in the dark in order to exclude the possible degrative effect of light. The resulting solution was neutralized by the acid, to avoid any interference of acid or base. 20 µl of the resulting solution was injected into HPLC and the chromatograms were recorded.

Peroxide oxidation

To 10 ml of the drug solution, 10 ml of 3% v/v hydrogen peroxide was added and the solution was kept aside for an hour. After an hour, 20 µl of the resulting solution was injected and the chromatogram was recorded.

UV treatment

10 ml of the drug solution was taken in a beaker and the solution was kept in an up chamber at shorter wavelength region for an hour. After an hour, 20 µl of the resulting solution was injected and the chromatogram was recorded.

Sun light treatment

10 ml of the drug solution was taken in a beaker and the solution was kept in sunlight for an hour. After an hour, 20 µl of the resulting solution was injected and the chromatogram was recorded.

RESULT AND DISCUSSION

In method development phase, initially both the drugs showing asymmetry factor more than 2 in Methanol: water, with a run time of more than 8.0 min. Then the mobile phase was shifted to Methanol: Phosphate buffer, showed a good result. At the reported Mobile phase proportion of 75:25, DUT and TAM showed a retention time of 2.219 min and 6.607 min respectively at the flow rate of 0.7 ml/min. The wavelength for the determination was selected at 274 nm for both the drugs. The tailing factor, resolution and peak shape were found to be good in the finally reported condition for both the drugs. The peaks are shown in Fig 1. As per ICH guidelines, system suitability tests were carried out by five replicate injections, with a constant concentration 50 µg/ml, 40 µg/ml DUT and TAM. The % Relative standard deviation of peak area and the retention time were within the limit of ±2%. This indicates that the method was system suitable. The linearity of DUT and TAM were determined by calibration curves and the linearity based on the area observed in the range of 25-75 µg/ml and 20-60 µg/ml respectively. The regression co-efficient value (r^2) for DUT and TAM is 0.9999 and 0.9997 respectively The limit of quantification was determined by injecting minimum concentration of the drugs .The limit of quantification was found to be 30, 24 µg/ml for DUT and TAM respectively the results are tabulated in Table 1. Precision was measured for both inter and intra-day, and checked with repeatability and the %RSD for the repeatability was found to be 0.73% and 0.80% for DUT and TAM respectively. The RSD was found to within the limit and tabulated in Table 2.Since both the drugs were stable for 24 hr only even under refrigeration condition; only intra-day precision studies were conducted the results are tabulated in Table 2. Analysis of the tablets was performed in one brand containing 500 mg and 400 mg of the drugs as label claim. An average quantity of DUT and TAM were 500.943±0.004 and 400.037±0.002 respectively and has conformation with the label claim. The results are tabulated in Table 3. The accuracy was studied by the recovery studies. The recovery studies are usually made by spiking the known amount of pure drug with the formulation. It is usually done by adding 50%,80%,100 %, 120 % and 150 % of the pure drug with the formulation taken for analysis. The average % recovery for DUT and TAM was found to be 99.88 % and 99.99 % for Brand drug.The results are tabulated below in Table 4. Forced degradation studies of both the drugs DUT and TAM were carried out under conditions of Acid hydrolysis RT 2.218 min, 6.605min, Alkali hydrolysis RT 2.222 min, 6.573 min, Peroxide oxidation RT 2.221 min, 6.608 min, UV light RT 2.220 min, 6.573 min and photolysis RT 2.209 min, 6.531 min. All the results of accelerated degradation study for Dutasteride and were Tamsulosin given in Table.5. The peaks are shown in Fig 2-6.

TABLE 1: SYSTEM SUITABILITY PARAMETERS

PARAMETERS	DUT	TAM
Calibration Range (mcg/ml)	25-75	20-60
Correlation Coefficient(r2)	0.9999	0.9997
Retention time(Min)	2.219±0.2	6.607±0.2
Regression equation(y=mx+c)		
Slope (m)	12591	9665
Intercept(c)	-6215	-9883
Theoretical Plates	2659	3496
Resolution factor	12.76	--
Tailing Factor	1.7	1.03
Selectivity	1.78	--
Repeatability %RSD (n=5)	0.73%	0.80%
Limit of quantification	25	20

System suitability parameters are the data's performed to check the system testing and the data's are based on the ICH Guidelines

TABLE: 2 PRECISION STUDY

DUT		TAM	
Conc µg/ml	**% RSD**	**Conc µg/ml**	**%RSD**
50	0.73	40	0.80

Precision studies are done to confirm the repeatability and the stability in a day.
RSD stands for Relative standard deviation taken for three readings.

TABLE 3: ANALYSIS OF MARKETED FORMULATION

Formulation	DUT		TAM	
	Label claim mg/tab	% assay ± RSD	Label claim mg/tab	% assay ± RSD
Brand	500	99.88±0.08	400	99.99±0.02

Formulation analysis was done in brand. * stands for the average reading taken in three readings.

TABLE 4: RECOVERY STUDIES OF TAM AND DUT COMBINED DOSAGE FORM

Formulation	DUT		TAM	
	% added	% recovery ± RSD	% added	% recovery ± RSD
Brand	50 100 150	99.81±0.085 99.93±0.090 99.90±0.105	50 100 150	100.15±0.014 100.09±0.012 100.22±0.045

Recovery experiment data for Tamsulosin and Dutasteride showing the amount of drug recovered from sample solution at each level (n=3), percentage recovery and the average percentage recovery.

TABLE 5: ACCELERATED DEGRADATION STUDIES

5.1. DUTASTERIDE:

Test No	Unstressed	Acid stress	Alkali Stress	Peroxide Stress	Heat stress	Photolytic Stress
Average weight	480mg					
Weight Taken(in mg)	480.2mg	481.2mg	480.8mg	482.0mg	481.5mg	479.8mg
Area	546148	505212	489264	492754	482926	502814
Assay (in mg)	50	46.19	44.73	45.05	44.15	45.97
Assay (in %)	101.5	92.38	89.46	90.10	88.30	91.94
%Degradation	NA	3.81	5.27	4.95	5.85	4.03

5.2 TAMSULOSIN HCL

Test No	Unstressed	Acid stress	Alkali Stress	Peroxide stress	Heat stress	Photolytic Stress
Average Weight	480mg					
Weight Taken(in mg)	480.2	481.2	480.8	482mg	481.5mg	479.8mg
Area	363218	303182	331830	298214	320818	299830
Assay (in mg)	40	33.488	36.6526	33.071	33.456	30.098
Assay (in %)	100	83.7206	91.6315	82.6791	88.59	82.882
%Degradation	NA	16.2794	8.3685	17.3209	11.41	17.118

Fig 1: A Typical Chromatogram for Dutasteride and Tamsulosin (Concentration of 50 mcg DUT and 40 mcg TAM)

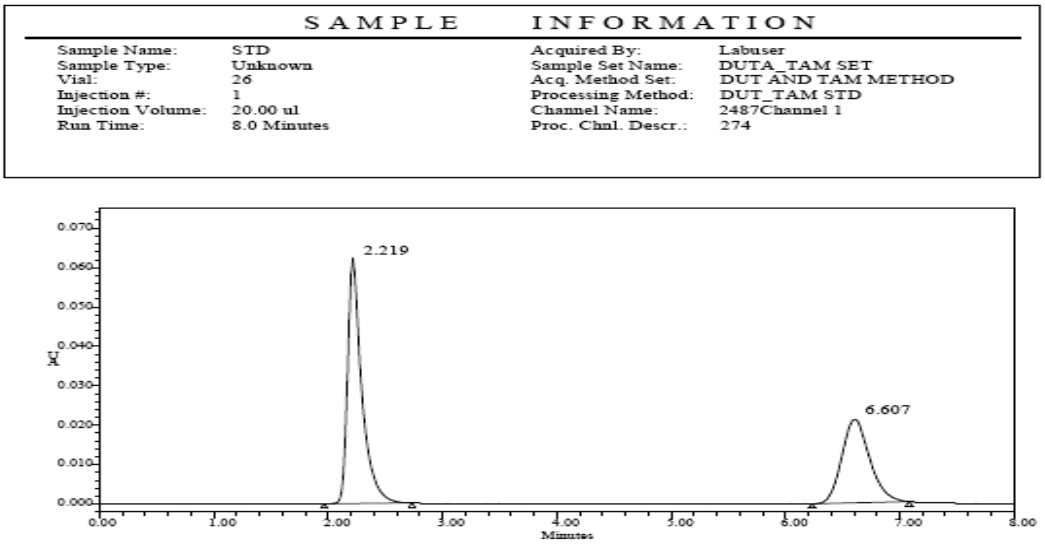


Fig 2: A Typical Chromatogram for Dutasteride and Tamsulosin - Acid stress

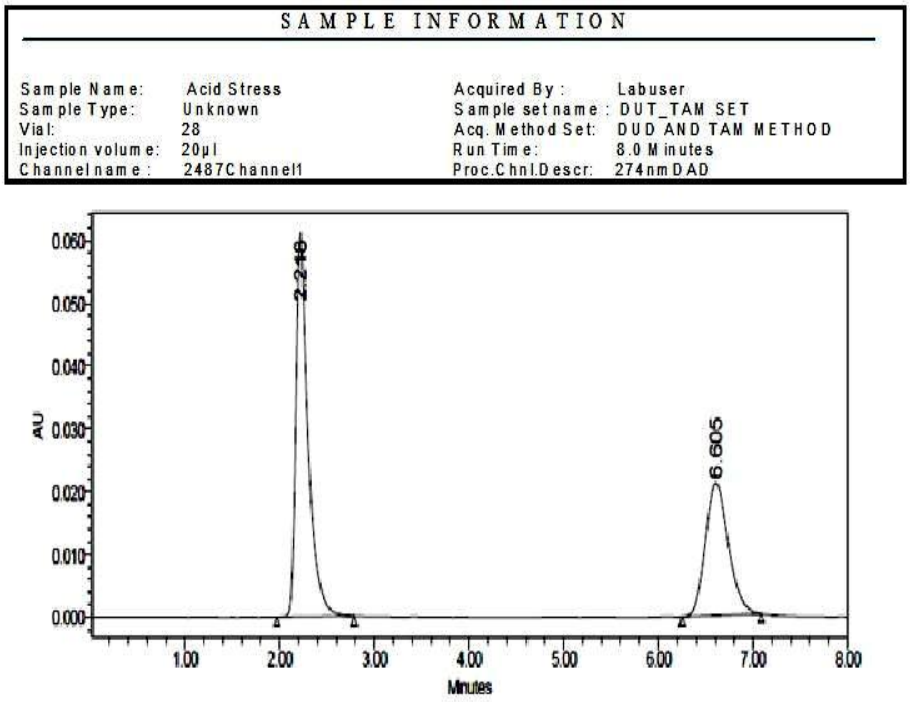


Fig 3: A Typical Chromatogram for Dutasteride and Tamsulosin - Alkali stress

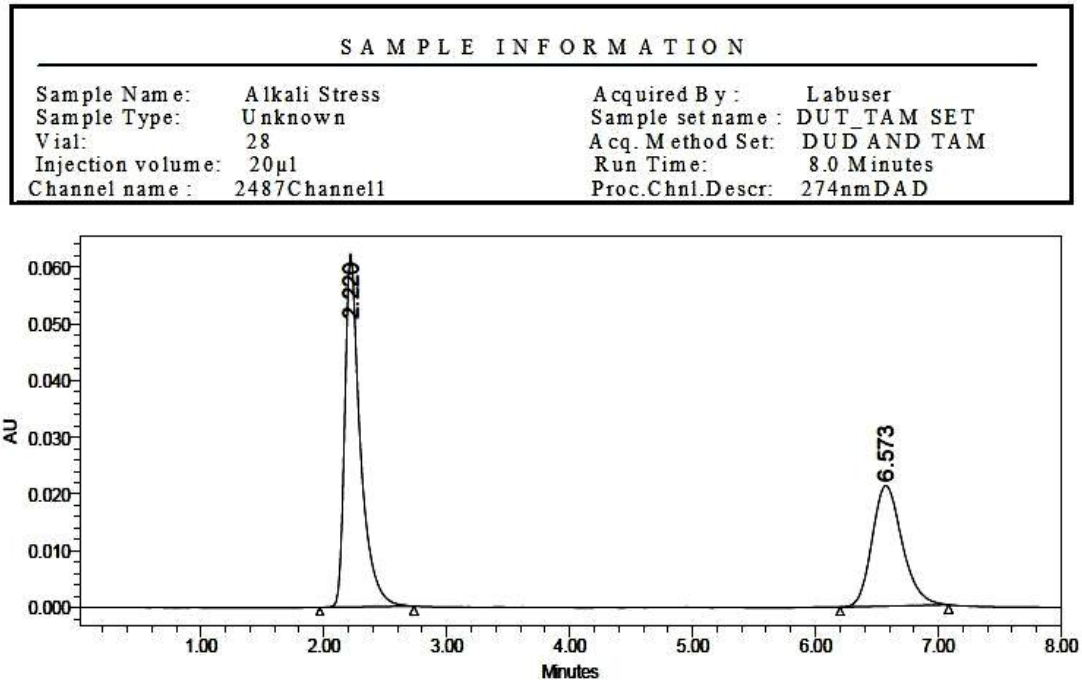


Fig 4: A Typical Chromatogram for Dutasteride and Tamsulosin - Peroxide stress

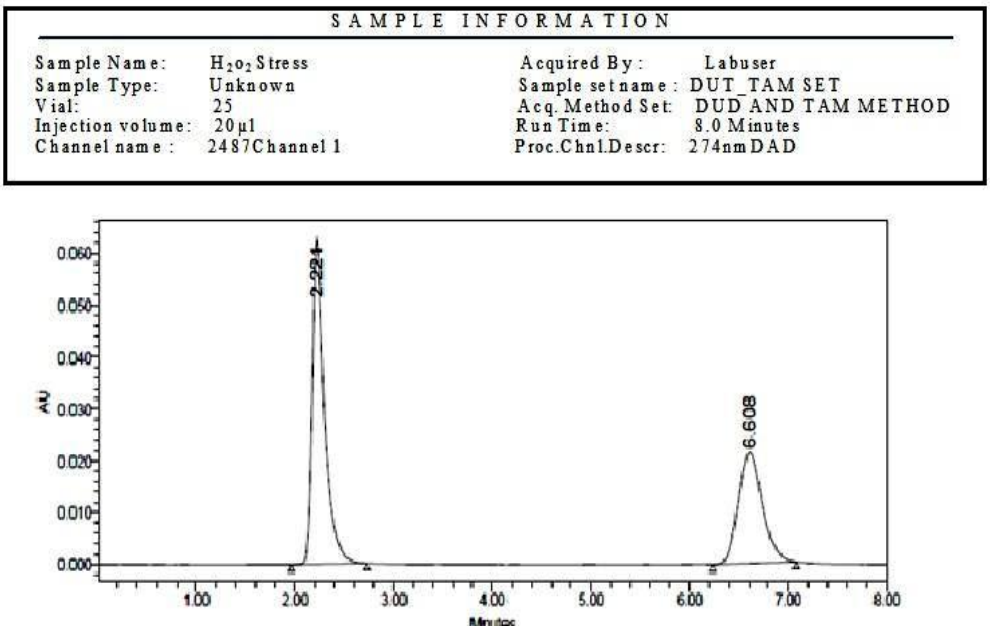


Fig 5: A Typical Chromatogram for Dutasteride and Tamsulosin - UV stress

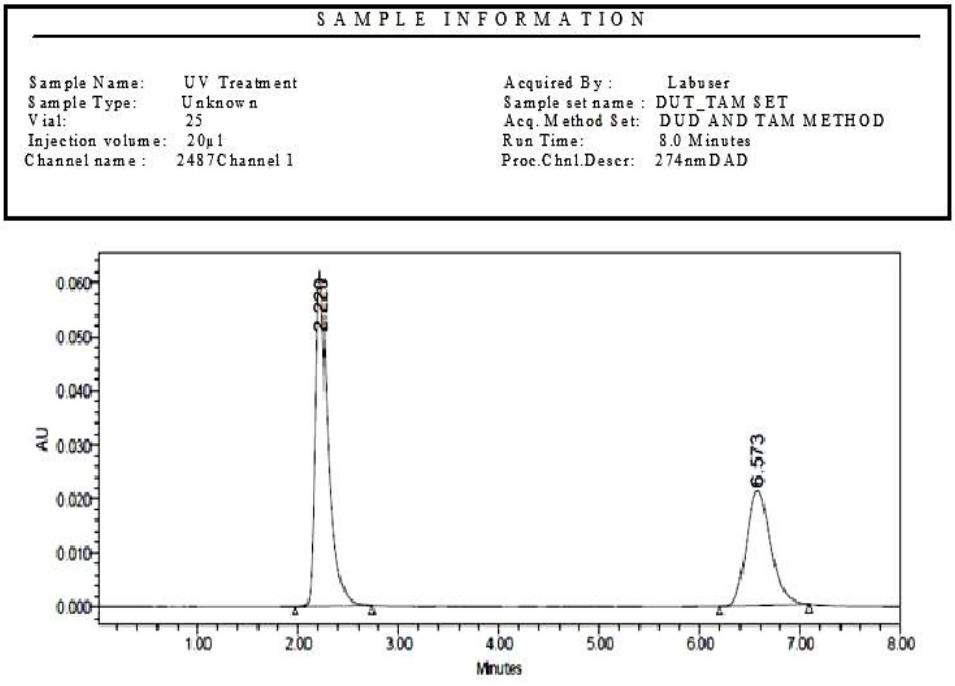
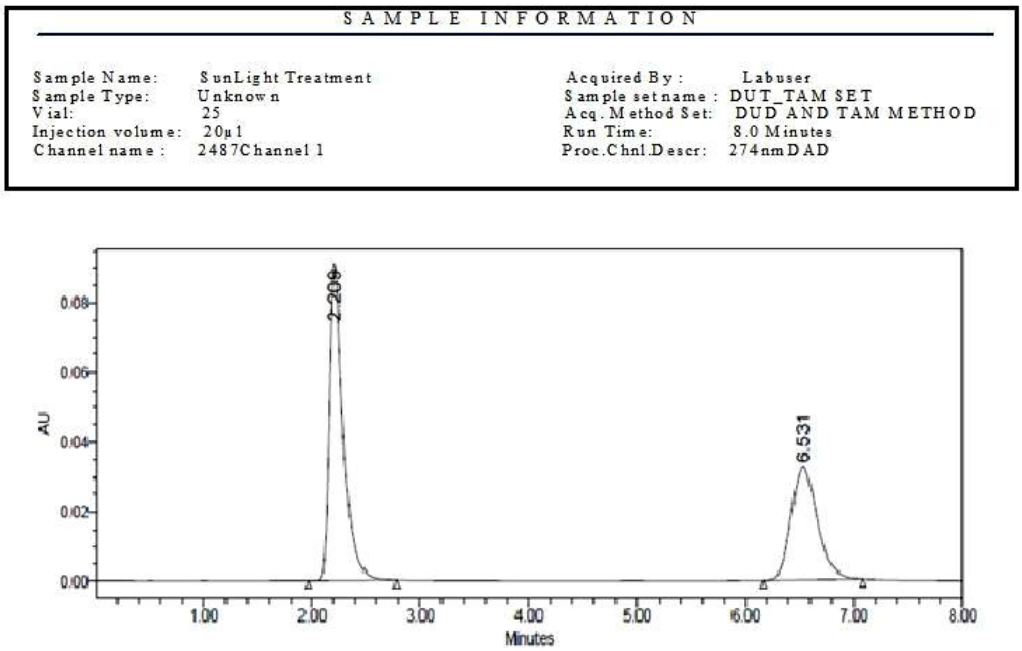


Fig 6: A Typical Chromatogram for Dutasteride and Tamsulosin - Photolytic stress



CONCLUSIONS

The isocratic RP-HPLC method developed for quantitative determination of Tamsulosin and Dutasteride simple, specific, sensitive, precise, reproducible and validated stability-indicating HPLC method for simultaneous estimation of Dutasteride and Tamsulosin in the presence of degradation products. The method was completely validated and satisfactory results were obtained for all the method validation data tested. A clear separation of the drugs and degradation products was achieved in the tablet with no interference from excipients. In almost all the cases, chromatographic pattern was similar .The method could be applied with success even to the analysis of marketed products, as no interference was observed due to excipients or other components present.

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