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STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF TAMSULOSIN HYDROCHLORIDE AND DUTASTERIDE IN BULK AND ITS PHARMACEUTICAL DOSAGE FORMS

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Abstract:

A simple, effective, and rapid reverse-phase high-performance liquid chromatographic technique was developed for the simultaneous determination of TamsulosinHcl and Dutasteride in pure and its pharmaceutical dosage forms. The method was developed using an X-terra C8 column (4.6 250 mm, 5 m) with a mobile phase that consists of a 60:40 combination ratio of acetonitrile and potassium dihydrogen ortho-phosphate buffer (pH-4.5).The effluents were monitored at 238 nm at a flow rate of 1 ml/min. TamsulosinHcl and Dutasteride had retention times of 3.15 and 5.625 minutes, respectively. The results of the analysis were statistically validated in accordance with the ICH norms. TamsulosinHcl and Dutasteride had 99.43 percent and 100.59 percent recovery rates, respectively. A calibration curve for TamsulosinHcl was plotted, with a range of 8-12 µg/ml and 10-15 µg/ml for Dutasteride. Accuracy, precision, specificity, robustness, ruggedness, Limit of Detection, Limit of Quantification, and the system suitability parameters were validated and reported. Forced degradation studies under various conditions like acidic, basic, thermal, oxidation, and photolysis results were noted and their respective chromatograms of TamsulosinHCl and Dutasteride were observed, and reported. All of the results were acceptable, indicating that the approach was suitable for its intended usage in routine quality control and stability studies.

Keywords:

Tamsulosin Hydrochloride, Dutasteride, Reverse Phase HPLC, Simultaneous Determination, Forced Degradation.

Introduction:

Tamsulosin hydrochloride (TAM)is an antagonist of alpha 1A adrenoceptors in the prostate. It is chemically designated as (-)-(R)-5-[2-[[2-(o-Ethoxyphenoxy) ethyl] amino] propyl]-2- methoxy benzene sulfonamide, monohydrochloride).The empirical formula of TAM is $C_{20}H_{28}N_2O_5S$• HCl representing a molecular weight of 444.98.TAM is used in men to treat the symptoms of an enlarged prostate (benign prostatic hyperplasia or BPH) which include difficulty in urinating, hesitation, dribbling, weak stream, and incomplete bladder emptying, painful urination, and urinary frequency and urgency. TAM is in a class of medications called alpha blockers[1]. It works by relaxing the muscles in the prostate and bladder so that urine can flow easily.[2]Dutasteride (DUTA) is a synthetic 4-azasteroid compound that is a selective inhibitor of both type 1 and type 2 isoforms of steroid 5 alpha-reductase, an intracellular enzyme that converts testosterone to dihydrotestosterone (DHT). It is chemically designated as (5α, 17β)-N-{2, 5 bis (trifluoromethyl) phenyl}-3oxo-4-azaandrost-1-ene-17-carboxamide. The empirical formula of DUTA is $C_{27}H_{30}F_6N_2O_2$ representing a molecular weight of 528.5.DUTA is also used in the treatment of BPH and its related symptoms and may reduce the chance of developing acute urinary retention (sudden inability to urinate). DUTA is in a class of medications called 5-alpha reductase inhibitors[3]. It works by blocking the production of a natural substance that enlarges the prostate. Structures of TamsulosinHcl and Dutasteride are shown in figures 1 and 2 respectively.

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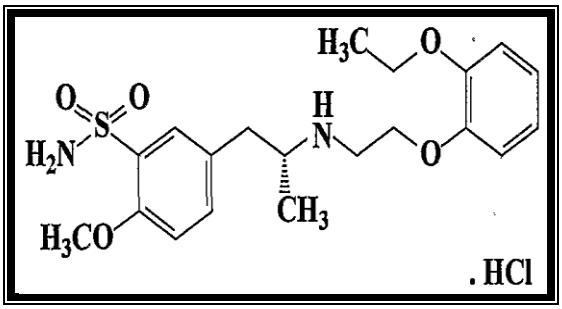

Figure 1: Tamsulosin Hydrochloride

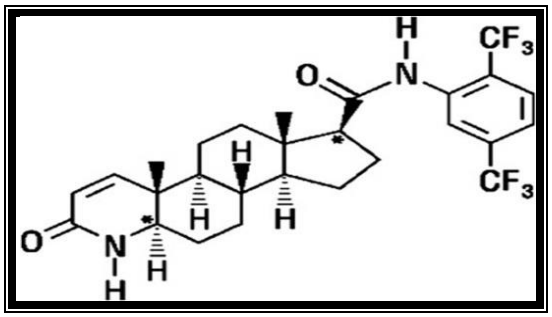

Figure 2: Dutasteride

The literature review reveals that methods including HPLC[4], RP-HPLC[5,6], HPTLC[7,8], TLC[9,10], LC/MS[11], UV[12,13,14], spectrofluorimetry[15,16,17], and spectrophotometry[18,19] have been reported for the estimation of TamsulosinHcl and Dutasteride individually as API and in Pharmaceutical dosage form. But no method has been reported so far in literature for simultaneous determinationof TamsulosinHcl and Dutasteride in bulk and Pharmaceutical dosage form by RP-HPLC method. Further, stability indicating RP-HPLC method for the simultaneous estimation of TamsulosinHcl and Dutasteride has not yet been cited in literature. The present work resolves the need of a method for simultaneous estimation of TamsulosinHcl and Dutasteride in tablet dosage form by RP-HPLC method. The method has been successfully used for quality-control analysis and for other analytical purposes for the combination.

Materials and method:

Instrumentation:

HPLC system (Shimadzu prominence) equipped with UV-detector was used for determination. The Method was developed on Waters 2695 separations module and X-terra C_8 column 4.6 × 250 mm, 5 µm.ER-180A analytical Balance, Sartorius-M500P microbalance, thermo scientific pH meter, Whatman filter paper, and Sartorius sonicator were used at different stages of method development. Glasswares (Borosil) were properly washed and rinsed before starting each day's work.

Reagents:

Most of the chemicals used in this method were of HPLC grade while the rest of them were of AR grade. The data acquisition was performed by Empower Software. Standards of TamsulosinHcl and Dutasteride were procured from PHARMATRAIN Kukatpally, Hyderabad, A.P, (India) which claimed to contain 0.4mg and 0.5mg was used in the analysis.

Preparation of mobile phase:

7 gm of potassium dihydrogen orthophosphate was dissolved in sufficient water to produce 1000 ml. The pH was adjusted to 4.5 using ortho phosphoric acid. The mobile phase was prepared by mixing phosphate buffer of pH 4.5 and acetonitrile in the ratio of 60:40. It was degassed and sonicated for 20 min and filtered prior to use.

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Preparation of Standard:

10mg of TamsulosinHCl and 12.5 mg of Dutasteridestandards were weighed accurately and transferred into a 100ml volumetric flask. About 10 ml of diluent was added and sonicated to dissolve. The volume was then made up to the mark with the same solvent. 1 ml of the stock solution was further diluted in a 10 ml volumetric flask with the same solvent. The chromatograms of standard TamsulosinHCl and Dutasteride are shown in figure 3.

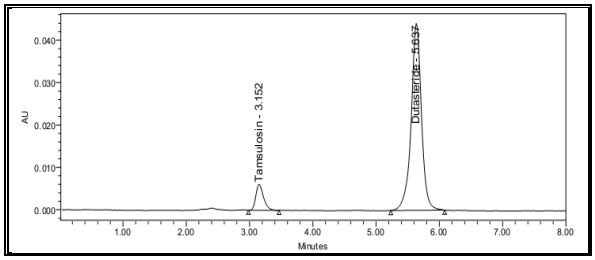

Figure 3: Chromatogram of standards

Preparation of sample:

10mg of TamsulosinHcl and 12.5 mg of Dutasteride samples were weighed accurately and transferred into a 100ml volumetric flask. About 10 ml of diluent was added and sonicated to dissolve. The volume was then made up to the mark with the same solvent. 1 ml of the stock solution was further diluted in a 10 ml volumetric flask with the same solvent. The chromatograms of sample TamsulosinHCl and Dutasteride are shown in figure 4.

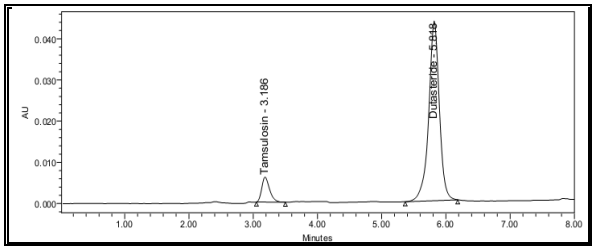

Figure 4: Chromatogram of sample

Results and discussion:

A series of trials for optimization of chromatographic conditions were run with variations in pH, flow rate, and composition of the mobile phase. This resulted in the selection of Phosphate buffer of pH 4.5: acetonitrile (in the ratio of 60:40) as mobile phase. A flow rate of 1 ml/min, a wavelength of 259 nm, and a run time of 10 min were used. Using such conditions as shown in Table 1, the peaks were separated more prominently. Thus, proposed chromatographic conditions were found to be appropriate for the quantitative determination of the drugs.

Table 1: Chromatographic conditions

Parameters	**Description**
Mobile Phase	Phosphate buffer (pH=4.5): ACN (60:40)
Column	X-terra C_8, 4.6 × 250 mm, 5 µm
Wave length	259 nm
Flow rate	1 ml/min
Run time	10 min
Injection volume	20 µl

Validation of the method:

The developed method has been validated for the assay of TamsulosinHcl and Dutasteride as per ICH guidelines by using the following parameters.

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Specificity and Selectivity:

The chromatogram obtained after injecting the synthetic mixture into theHPLC system is shown in figure 5. No peaks were found at the retention time of TamsulosinHcl and Dutasteride. The retention times were found to be 3.151 min and 5.626 min respectively. Specificity studies indicated that the excipients did not interfere with the analysis.

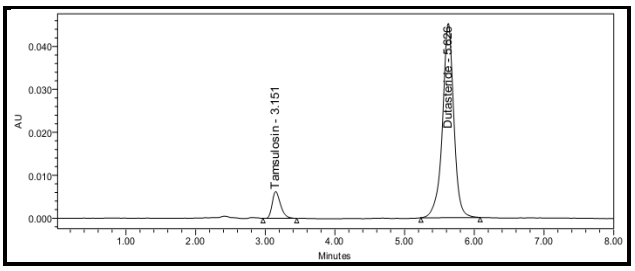

Figure 5:Chromatogram of synthetic mixture for specificity studies

Linearity:

Linearity was studied by preparing standard solutions of TamsulosinHcl and Dutasteride at different concentration levels. The calibration curves were plotted between different concentrations of the drugs and their corresponding mean peak areas. The responses were found to be linear in the range of 8 – 12 µg/ml and 10 -15 µg/ml for TAM and DUTA respectively. The calibration curves are shown in figures 6 and 7 and the data is shown in Table 2.

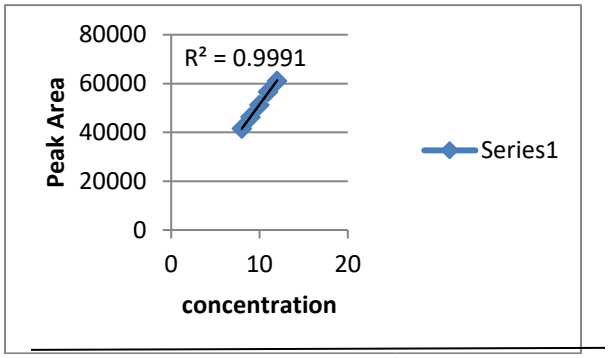

Figure 6: Calibration curve of TamsulosinHcl

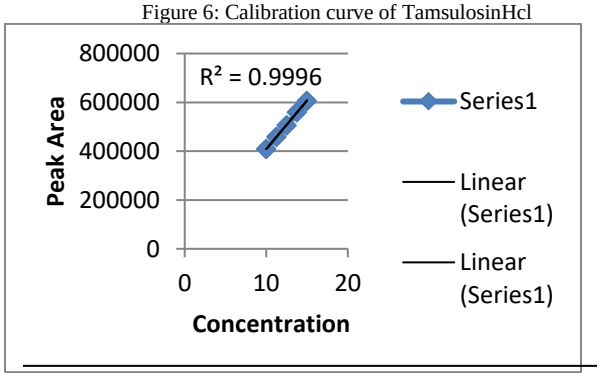

Figure 7: Calibration curve of Dutasteride

Table 2: Calibration curve data

TamsulosinHcl		**Dutasteride**	
Conc. (µg/ml)	**Peak area**	**Conc. (µg/ml)**	**Peak area**
8	41606	10	408900
9	46273	11.25	458835

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10	51279	12.5	506011
11	56717	13.75	559755
12	61104	15	606556
R^2	0.999	R^2	0.999

Accuracy:

Accuracy was performed in triplicate for various concentrations of TamsulosinHcl and Dutasteride equivalent to 50, 100, and 150% of the standard amount was injected into the HPLC system as per the test procedure.Data of recovery studies are shown in Tables 3 and 4. The limit for a mean of % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Table 3: Accuracy Results of TamsulosinHcl

%Concentration(at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	26426	5.0	4.98	99.6%	100.26%
50%	25711	5.0	5.01	100.2 %	
50%	26071	5.0	5.05	101.0%	
100%	51958	10.0	9.98	99.80%	100.16%
100%	51608	10.0	10.01	100.1%	
100%	51480	10.0	10.06	100.6%	
150%	79162	15.0	14.99	99.93%	100.15%
150%	79258	15.0	14.97	99.93%	
150%	77620	15.0	15.01	100.06%	

Table 4: Accuracy Results of Dutasteride

%Concentration(at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	250581	6.25	6.24	99.84%	100.32%
50%	252846	6.25	6.28	100.48%	
50%	256906	6.25	6.29	100.64%	
100%	506045	12.5	12.4	99.2%	100.53%
100%	507718	12.5	12.6	100.8%	
100%	504814	12.5	12.7	101.6%	
150%	747696	18.75	18.72	100.06%	100.17%
150%	746674	18.75	18.77	100.2%	
150%	755641	18.75	18.79	100.26%	

Precision:

Precision was determined by replicate injections of mixed standard solution. The R.S.D of Area is present within the Acceptance criteria of 2 %. The reliability and sensitivity of the method could be seen from recovery studies. There is no interference due to excipients. The proposed method is simple, accurate, and rapid. The precision results are shown in Table 5.

Table 5: Precision Results of TamsulosinHcl and Dutasteride

Tamsulosin		Dutasteride	
Injection	**Area**	**Injection**	**Area**
Injection-1	51057	**Injection-1**	502914
Injection-2	51851	**Injection-2**	511238
Injection-3	52882	**Injection-3**	507029
Injection-4	52319	**Injection-4**	508057
Injection-5	52619	**Injection-5**	507187
Injection-6	53231	**Injection-6**	513631
Average	52326.5	**Average**	508342.7
Standard Deviation	781.0305	**Standard Deviation**	3713.832
% RSD	1.49	**% RSD**	0.73

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Robustness:

Robustness was done by small deliberate changes in the chromatographic conditions of TamsulosinHcl and Dutasteride. The factors selected were flow rate and variation in the organic phase. The results remained unaffected by small variations in these parameters. The results are shown in Tables 6 & 7.

Table 6: Robustness results of Tamsulosin and Dutasteride (Change in flow rate)

	System suitability results for TamsulosinHcl		System suitability results for Dutasteride	
Flow rate (ml/min)	**USP Plate count**	**USP Tailing**	**USP Plate count**	**USP Tailing**
0.8	3320	1.3	6499	0.94
1.0	3438	1.19	6407	0.94
1.2	3310	1.34	6433	0.93

Table 7: Robustness results of Tamsulosin and Dutasteride (Change in Organic phase composition)

	System suitability results for TamsulosinHcl		System suitability results for Dutasteride	
Organic phase	**USP Plate count**	**USP Tailing**	**USP Plate count**	**USP Tailing**
10 % less	3273	1.31	6411	0.92
Actual	3438	1.19	6407	0.94
10 % more	3302	1.33	6507	0.94

Ruggedness:

To evaluate the Ruggedness of the method, Precision is performed on a different day by using different columns on the same dimensions. The standard solution was injected six times andmeasured the area for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits. The ruggedness results are shown in Table 8.

Table 8: Ruggedness Results of Tamsulosin and Dutaseride

Tamsulosin		**Dutasteride**	
Injection	**Area**	**Injection**	**Area**
Injection-1	53255	**Injection-1**	502914
Injection-2	53128	**Injection-2**	511238
Injection-3	53934	**Injection-3**	507029
Injection-4	53442	**Injection-4**	508057
Injection-5	53416	**Injection-5**	507187
Injection-6	53626	**Injection-6**	513631
Average	53466.83	**Average**	508342.7
Standard Deviation	285.1178	**Standard Deviation**	3713.832
% RSD	0.53	**% RSD**	0.73

Detection Limit and Quantitation limit:

The limit of detection of Tamsulosin and Dutasteride were calculated and found to be 3.02 µg/ml and 2.96 µg/ml and the limit of quantification of Tamsulosin and Dutasteride were calculated and found to be 9.96 µg/ml and 9.98 µg/ml respectively.

Forced degradation studies:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform stress degradation studies on Tamsulosin and Dutasteride using the proposed method.

Standard stock solution preparation

Accurately weigh and transfer about 10 mg Tamsulosin and Dutasteride of standard into a 100 ml dry volumetric flask add about 70 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

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Hydrolytic degradation under acidic condition:

2.4 ml of Tamsulosin, 3.0 ml of Dutasteride above stock solution, and 3 ml of 0.1N HClwere added in 10 ml of volumetric flask. The volumetric flask was kept at normal condition for 90 minutes and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.45 microns syringe filters and place in vials. The chromatogram of acid degradation is shown in figure 8.

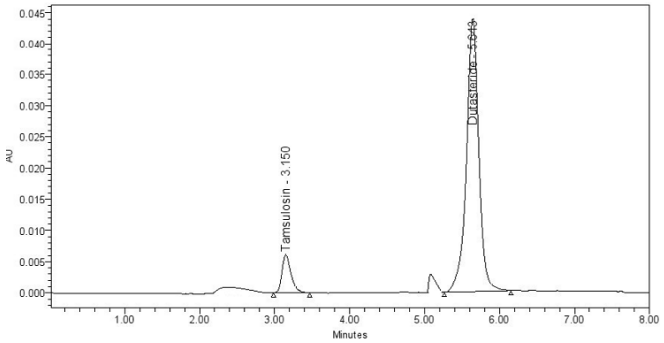

Figure 8: Chromatogram of Acid Degradation

Hydrolytic degradation under alkaline condition:

2.4 ml of Tamsulosin, 3.0 ml of Dutasteride above stock solution, and 3 ml of 0.1N NaOHwere added in 10 ml of volumetric flask. The volumetric flask was kept at normal condition for 90 minutes and then neutralized with 0.1 N HCL and make up to 10 ml with diluent. Filter the solution with 0.45 microns syringe filters and place in vials. The chromatogram of alkaline degradation is shown in figure 9.

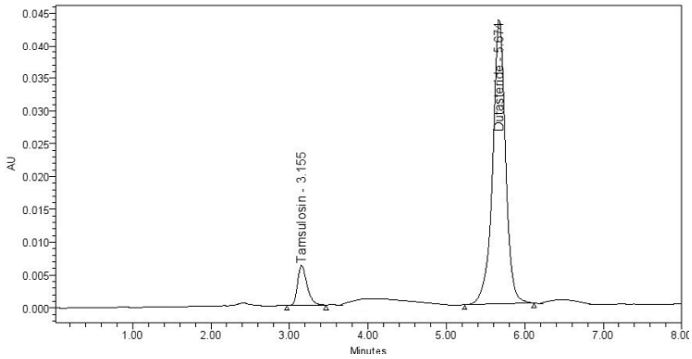

Figure 9: Chromatogram of Alkaline Degradation

Thermal induced degradation:

2.4 ml of Tamsulosin, 3.0 ml of Dutasteride above stock solution, and 3 ml of diluent were added in 10 ml of volumetric flask. The volumetric flask was kept at reflex condition for 60 minutes and make up to 10 ml with diluent. Filter the solution with 0.45 microns syringe filters and place in vials. The chromatogram of thermal degradation is shown in figure 10.

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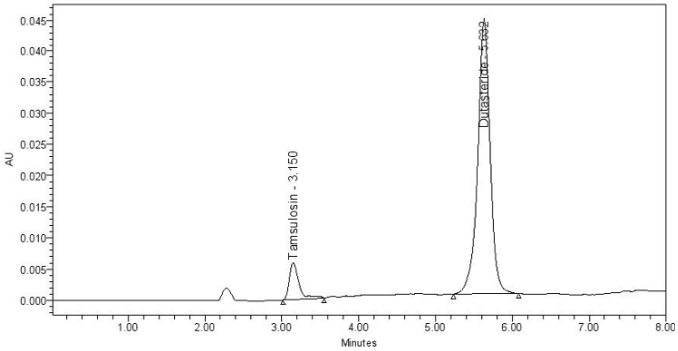

Figure 10: Chromatogram of Thermal Degradation

Oxidative degradation:

2.4 ml of Tamsulosin, 3.0 ml of Dutasterideabove stock solution, and 1 ml of 3 % w/v of hydrogen peroxide were added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials. The chromatogram of peroxide degradation is shown in figure 11.

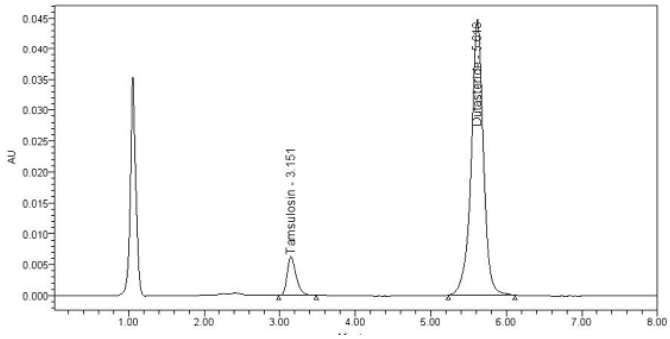

Fig 11: Chromatogram of Peroxide Degradation

Photolytic degradation:

2.4 ml of Tamsulosin and 3.0 ml of Dutasterideabove stock solution were taken in 10 ml of volumetric flask and the volume was made up to the mark with diluents. The volumetric flask was then exposed to light for 12 hr. Filter the solution with 0.45 microns syringe filters and place in vials. The chromatogram of photolytic degradation is shown in figure 12.

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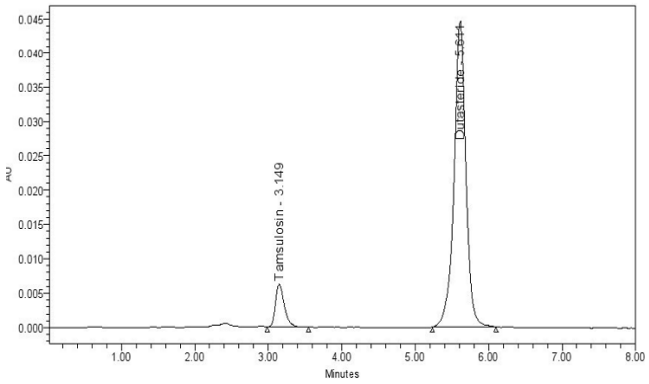

Fig 12: Chromatogram of Photolytic Degradation

The sample solutions were subjected to acidic, basic, peroxide, temperature, and light.In acidic, thedegradation for Tamsulosin and Dutasteride was found to be -5.05 % and -5.96%respectively. In basic, the degradation for Tamsulosin and Dutasteride was found to be -5.69 % and -11.1 % respectively. Degradation by peroxide for Tamsulosin and Dutasteride was found to be -12.02 % and -11.82 %.The solid sample was subjected to light for 7 days and then the degradation of Tamsulosin and Dutasteride was found to be -11.77% and -19.8 %. Thermal degradation for Tamsulosin and Dutasteride was found to be -6.17 % and -8.51 %. The degradation of Tamsulosin and Dutasteride results are reported in Tables 9 and 10 respectively.

Table 9: Degradation results for Tamsulosin

	Area	**% Assay**	**% Degradated**
Acid	48586	94.95	-5.05
Base	48256	94.31	-5.69
Thermal	48014	93.83	-6.17
Peroxide	45021	87.98	-12.02
Photolytic	45147	88.23	-11.77

Table 10: Degradation results for Dutasteride

	Area	**% Assay**	**% Degradated**
Acid	457856	91.31	-5.96
Base	445782	88.90	-11.1
Thermal	458762	91.49	-8.51
Peroxide	442172	88.18	-11.82
Photolytic	402147	80.20	-19.8

Conclusion:

A simple, rapid, isocratic, precise, and accurate stability indicating reversed phase high performance liquid chromatographic method has been achieved for the simultaneous determination of TamsulosinHcl and Dutasteride in the presence of degradation products. The method successfully passed all the criteria of validation.

A clear separation of the drugs and degradation products was achieved in the tablets with no interference from excipients. The method is suitably applicable for the analysis of TamsulosinHcl and Dutasteride in combined tablet dosage forms available in the market as well as in quality control laboratories.

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