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RP-HPLC METHOD FOR THE ESTIMATION OF TAMSULOSIN AND SOLIFENACIN IN BULK AND ITS DOSAGE FORMS

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Keywords:

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ABSTRACT: A liquid chromatographic method has been developed and validated for the determination of the clinical trial combination of Solifenacin succinate and Tamsulosin hydrochloride. Effective chromatographic separation was achieved on an eclipse XDB-C18 (4.6 mm X 150 mm, 5µm) column using isocratic reverse phase technique. The mobile phase employed was ACN: 20Mm sodium phosphate buffer (0.2% Triethylamine) (30:70), the pH was adjusted to 3.0 by ortho phosphoric acid. The flow rate was maintained at 1.0 mL/min and elutewasmonitoredat225nm. A linear response was observed over the concentration range 15-75 µg/mL (R^2=0.999) of Solifenacin and the concentration range 1-5 µg/mL (R^2=0.999) of Tamsulosin.The limit of quantitation (LOQ) and limit of detection (LOD) for Solifenacin were 0.04 and 0.14 µg/mL, respectively and for Tamsulosin were 0.05 and 0.1 µg/mL, respectively. The method was successfully validated in accordance to ICH guideline Q2. The RSD for intra-day and for inter-day precision were found to be lesser than 1.5% for Solifenacin. The RSD for intra-day and for inter-day precision were found to be lesser than 1% for Tamsulosin. The percentage recovery was found to be greater than 98.0 %.The method was found to be accurate, precise, linear, specific, sensitive, rugged, robust and stability indicating.

INTRODUCTION: Benign prostatic hyperplasia is commonly occurring condition in elderly men and this is often associated with lower urinary tract symptoms.

Clinical trial study shows that Tamsulosin, Solifenacin are safe and effectively responsible for cure diseases Tamsulosin selectively act on a A1 receptor on prostate where Solifenacin is a urinary antispasmodic i.e., Antimuscarinic agent.

QUICK RESPONSE CODE

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Several analytical method UV, HPLC were developed for individual drug[1-10] but there is no simultaneous estimation and stability indicating assay method available for Tamsulosin and Solifenacin.

Drug profile(s):

(a) Tamsulosin: Tamsulosin is a selective antagonist at alpha-1A and alpha-1B-adrenoceptors in the prostate, prostatic capsule, prostatic urethra, and bladder neck.

At least three discrete alpha1-adrenoceptor subtypes have been identified: alpha-1A, alpha-1B and alpha-1D; their distribution differs between human organs and tissue.

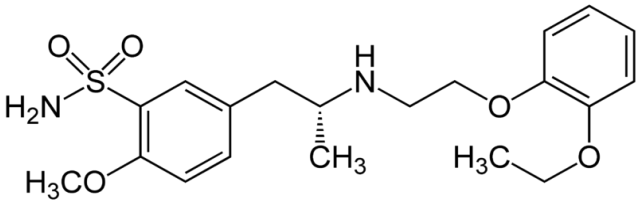

FIGURE: 1 STRUCTURE OF TAMSULOSIN

Chemical name: 5-[(2R)-2-{[2-(2-ethoxyphenoxy) ethyl] amino} propyl]-2-methoxybenzene-1-sulfonamide

Chemical formula: $C_{20}H_{28}N_2O_5S$

Molecular weight: 408.512gms/mol.

PKa: 9.28

Solubility: Sparingly soluble in water and Methanol, slightly soluble in glacial acetic acid and Ethanol, and practically insoluble in ether.

Description: white crystalline powder**.**

Category: Antimuscarinic

Mechanism of Action: Tamsulosin is a selective antagonist at alpha-1A and alpha-1B-adrenoceptors in the prostate, prostatic capsule, prostatic urethra, and bladder neck. At least three discrete alpha1-adrenoceptor subtypes have been identified: alpha-1A, alpha-1B and alpha-1D; their distribution differs between human organs and tissue. Approximately 70% of the alpha1-receptors in human prostate are of the alpha-1A subtype. Blockage of these receptors causes relaxation of smooth muscles in the bladder neck and prostate, and thus decreases urinary outflow resistance in men.

(b) Solifenacin: Solifenacin (rINN), marketed as Solifenacin succinate under the trade name Vesicare, is a urinary antispasmodic of the anticholinergic class. It is used in the treatment of overactive bladder with urge incontinence.

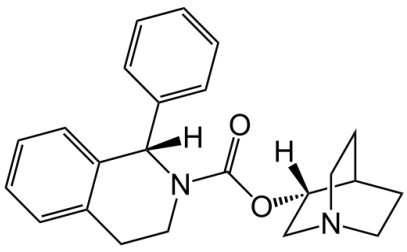

FIGURE: 2 STRUCTURE OF SOLIFENACIN

Chemical name: butanedioic acid (3R)-1-azabicyclo[2.2.2]octan-3-yl (1S)-1-phenyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate

Chemical formula: $C_{27}H_{32}N_2O_6$

Molecular weight: 480.5528gms/mol.

PKa: 8.88

Solubility: freely soluble at room temperature in water, Glacial acetic acid, dimethyl sulfoxide and methanol

Description: Pale- yellowish-White crystal or crystalline powder

Category: Alpha-1-Adrenergic blocker.

Mechanism of Action: Solifenacin is a competitive muscarinic acetylcholine receptor antagonist. The binding of acetylcholine to these receptors, particularly the M3 receptor subtype, plays a critical role in the contraction of smooth muscle. By preventing the binding of acetylcholine to these receptors, Solifenacin reduces smooth muscle tone in the bladder, allowing the bladder to retain larger volumes of urine and reducing the number of incontinence episodes.

MATERIALS AND METHOD:

Reagents & Chemicals: The Active pharmaceutical ingredient of Solifenacin succinate and Tamsulosin were obtained as gift sample from Hetero Drugs and Ogene system (I) Pvt Ltd. All solvent and reagent used were of HPLC and spectroscopic grade. HPLC grade, Acetonitrile, Orthophosphoric acid, Triethylamine was obtained from Merck Pvt.ltd. The synthetic mixture was prepared by using marketed formulation containing tablet of Tamsulosin; brand name Urimax; B -No 9865742; mfg by Cipla; mfg date AUG 2010; Exp JUL 2012, and API of Solifenacin. This was simulating dosage form. Millipore water obtained from (Milli Q) was used in all experiments.

Instrumentation: The chromatographic separation performed using JASCO-2080 model equipped with UV-2075 detector (JASCO). JASCO-BROWIN software was used for monitored and integrate the output single at wavelength 225nm.

Sample injection was done with a Rheodye 7725 injection valve via a 20ul loop. Digisum Electronic analytical balance (model DI 707) was used for weighing. Drug separation achieved at room temperature with Agilent eclipse XDB-C18 (4.6mm ×150mm) with pore size 5µm was used for method development.

Preparation of 20mM Sodium phosphate buffer: Solution was prepared by adding 0.27 g of mono sodium phosphate and 0.0001 g of disodium phosphate hepta hydrate to 100 mL of water.

Preparation of Mobile phase: Mix 30:70%v/v Acetonitrile and 20mM Sodium phosphate buffer pH 3 and sonicated for 5mins to degas the mixture.

Preparation of standard and sample solution: The standard solution of Tamsulosin and Solifenacin were prepared at a concentration of 4µg/mL and 60µg/mL respectively. Accurately about 4 mg Tamsulosin and 60 mg of Solifenacin were weighed transfer into 100 mL volumetric flask to this add 25 mL of methanol mixed well to dissolve and make up the volume with methanol

Pipette out 1 mL above solution into 10 ml volumetric flask diluted with mobile phase up to the mark.

Preparation of Pharmaceutical Dosage Form Solutions: Sample concentration was prepared as per new formulation vesomni. Weigh synthetic mixture equivalent to 4 mg Tamsulosin and 60 mg of Solifenacin were transfer into 100 mL volumetric flask to this add 25 mL of methanol mixed well to dissolve and make up the volume with methanol and sonicate for 15 min. Solution was centrifuge for 15 min at 5000 rpm; supernatant was collected and filtered through a 0.22µm membrane filter. Pipette out 1 mL above solution into 10 ml volumetric flask diluted with mobile phase up to the mark and injected in to HPLC system for analysis.

Optimization of the chromatographic conditions: The initial literature search indicated that many HPLC methods are available for individual drugs. Based on literature search, attempts were made to develop a simple method which has less retention time and higher selectivity, top priority was given for complete separation of Solifenacin and Tamsulosin. Solifenacin and Tamsulosin are hydrophilic almost soluble in aqueous solution and freely soluble in methanol. Several mobile phases were tested until good resolution obtained between two drugs in preliminary experiments, both drugs Solifenacin and Tamsulosin were subjected to separation by reverse phase HPLC using sodium phosphate 20mM buffer and methanol as organic modifiers. These drugs were able to be separated on the chromatogram but peak shape was not good for Solifenacin and tailing was seen more than 1.5.

The effect of pH (6.5, 4.5 3.5 and 30) and mobile phase composition was also checked. It improved peak shape at pH 3.0 slight extent but the number of theoretical plates is very less. Acetonitrile was found to be better than methanol in terms of resolution and peak shapes. Initially try with mixture of methanol Acetonitrile it shows good peak shape with theoretical plate but Tamsulosin get merge with solvent peak. Resolution was also not good. Then only acetonitrile was used.

The chromatogram obtained was improved. For reducing tailing Triethylamine was used at the conc.0.1% but no improvement in tailing factor then 0.2% was used. Finally methods was developed with 30:70 (ACN: sodium phosphate buffer 20mM (0.2% TEA)) pH 3, the chromatogram obtained was better than the previous one in all aspects with good peak shape, tailing factor, resolution and theoretical plate as per USP requirement.

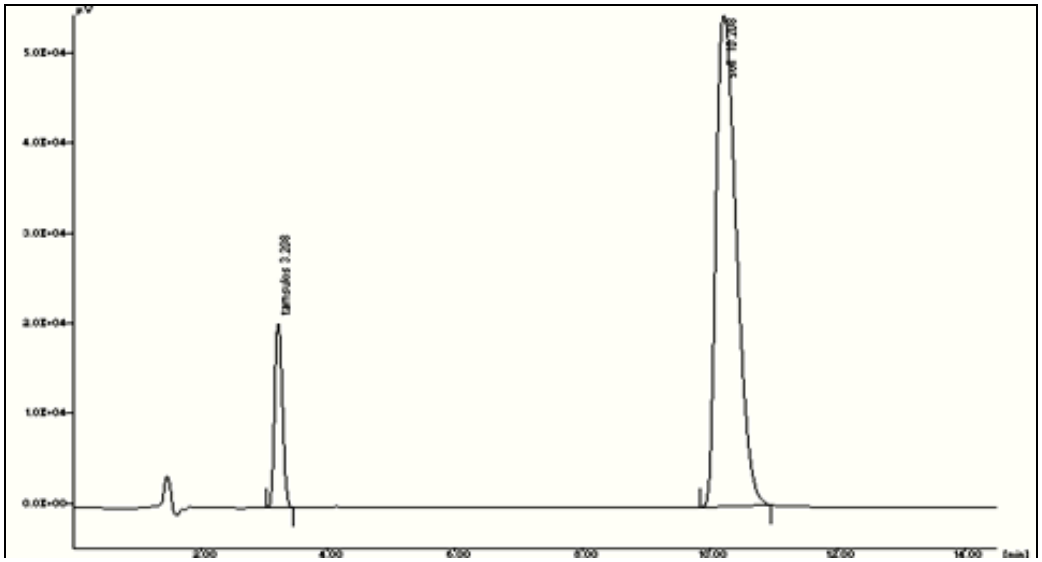

FIGURE: 3 CHROMATOGRAPHIC SEPARATION OF SOLIFENACIN AND TAMSULOSIN IN BULK

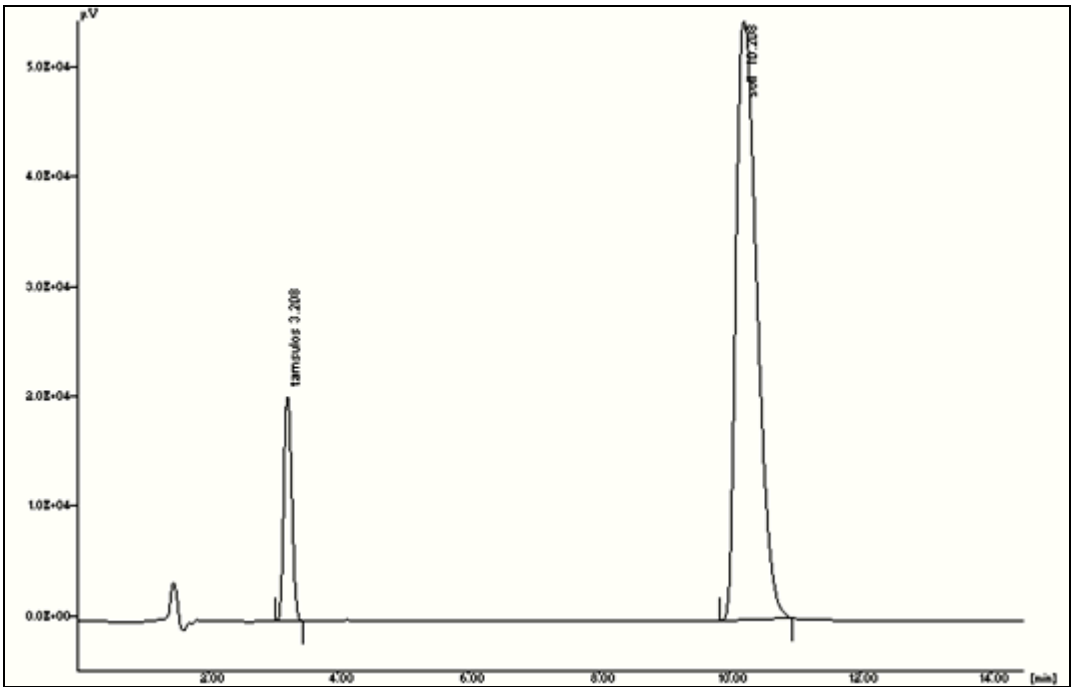

FIGURE: 4 CHROMATOGRAPHIC SEPARATION OF SOLIFENACIN AND TAMSULOSIN IN SYNTHETIC MIXTURE.

Validation: The method was successfully validated as per ICH guideline Q2 (R1): Validation of analytical procedures: text and methodology, International Conference on Harmonization, Food and Drug Administration, USA, November 2005. The method was validated and parameters were linearity, range, accuracy, precision, LOQ, LOD.

Linearity and range: The Linearity of detector response to different concentration of all the three drugs was studied with a series of working standard solutions prepared by diluting the stock solution with mobile phase. The Standard plots were constructed between concentration vs. peak area a linear response of peak area was observed over the concentration range of 15-90 µg/mL for Solifenacin and 1-6µg/mL for Tamsulosin. Ten microlitre of each sample was injected under above chromatographic conditions and peak area was measured. Keeping the values to the straight line equation of calibration curve, quantification was carried, the data of linearity curve was summarized in the table 2 and 3 for Solifenacin and Tamsulosin respectively, figure 5 & 6 for Solifenacin and Tamsulosin respectively. It was found that correlation coefficient (R^2) and regression analysis were within the limit which is summarized in the **table 1.**

TABLE: 1 LINEARITY DATA SHOWING EQUATION OF REGRESSION LINE AND COEFFICIENT OF DETERMINATION

Drug	Conc. Range	Equation	R^2
Solifenacin	15-90µg/ml	Y=43503.1x+1664.3	**0.999**
Tamsulosin	1-6 µg/ml	Y=58198x+3917.9	**0.999**

TABLE: 2 LINEARITY DATA FOR SOLIFENACIN (n=3)

S. No.	%Spike level	Concentration (µg/ml)	Peak area
1	25	15	**652042**
2	50	30	**1298921**
3	75	45	**1960012**
4	100	60	**2608569**
5	125	75	**3261112**
6	150	90	**3912845**

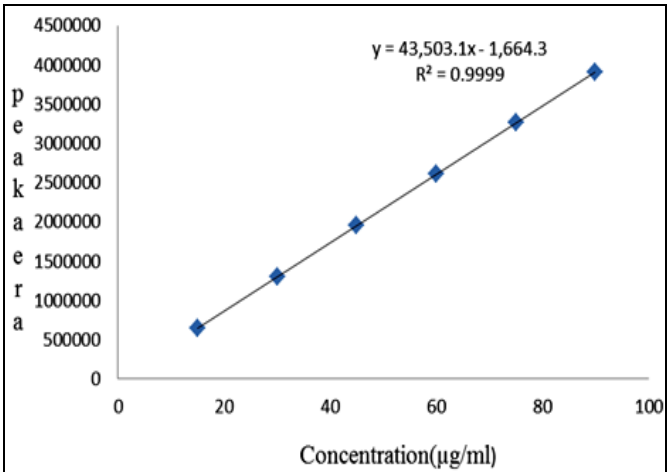

FIGURE: 5 CALIBRATION GRAPH OF SOLIFENACIN

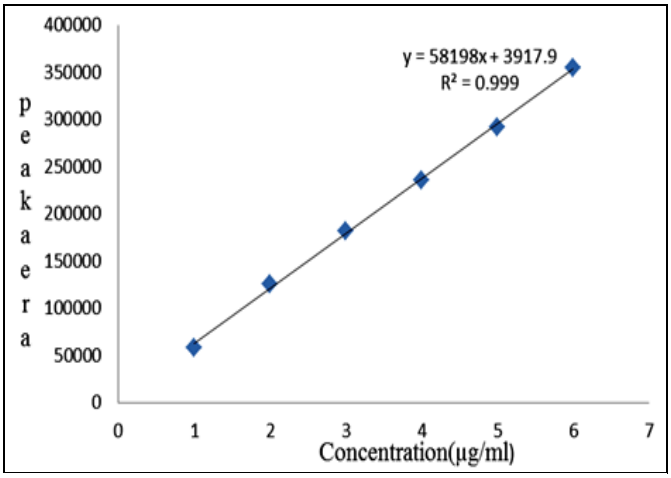

FIGURE: 6 CALIBRATION GRAPH OF TAMSULOSIN

TABLE: 3 LINEARITY DATA FOR TAMSULOSIN (N=3)

S. No.	%Spike level	Concentration (µg/ml)	Peak area
1	25	1	**58009**
2	50	2	**124843**
3	75	3	**181113**
4	100	4	**235626**
5	125	5	**291673**
6	150	6	**354391**

Limit of Detection (LOD) and Limit of Quantification (LOQ): A study to establish the Limit of detection and Limit of Quantification of Solifenacin and Tamsulosin. A series of solutions having Solifenacin and Tamsulosin were injected were established by identifying the concentration which gives signal to noise ratio about 3.

Limit of quantitation was established by identifying the concentration which gives signal to noise ratio about 10. The LOD and LOQ were estimated in **table 4**.

TABLE: 4 LOQ, LOD VALUES

Drugs	LOD µg/mL	LOQ µg/mL
Solifenacin	**0.04**	**0.14**
Tamsulosin	**0.05**	**0.1**

Precision: According to ICH guidelines repeatability should be assessed by using a minimum of nine determinations covering the specified range for the procedures (i.e., three concentrations and three replicates of each concentration) precision was studied to find out intra and inter day variations of the proposed method at three different levels (50, 100 and 150%

or 80, 100, 120%) 30, 60 & 90 µg/mL for Solifenacin and 1, 4 & 6µg/mL for Tamsulosin on the same and on three different days respectively. The results were interpreted by statistical analysis by calculating % RSD values and all the results were within the acceptance criteria of not more than 2 % and the results are tabulated in the table: 5, 6 for Solifenacin and 7, 8 for Tamsulosin. The % RSD values for intraday and inter day were <2%, indicating that the method was sufficiently precise.

TABLE 5: INTRADAY PRECISION STUDIES OF SOLIFENACIN

S. No.	Concentration (µg/mL)	% Assay	Statistical parameters
1	29.98	99.93	Mean=99.9
2	29.79	99.30	SD=0.55
3	30.12	100.40	%RSD=0.55
4	59.89	99.82	Mean=100.1
5	60.21	100.35	SD=0.27
6	60.04	100.07	%RSD=0.27
7	90.12	100.13	Mean=100.0
8	90.01	100.01	SD=0.09
9	89.97	99.97	%RSD=0.09

TABLE 6: INTER DAY PRECISION STUDIES OF SOLIFENACIN

S. No.	Concentration (µg/mL)	% Assay	Statistical parameters
1	29.96	99.87	Mean=100.0
2	29.97	99.90	SD=0.20
3	30.07	100.23	%RSD=0.20
4	59.98	99.97	Mean=100.0
5	60.01	100.02	SD=0.06
6	59.94	99.90	%RSD=0.06
7	90.06	100.07	Mean=100.0
8	89.92	99.91	SD=0.08
9	89.98	99.98	%RSD=0.08

TABLE 7: INTRADAY PRECISION STUDIES OF TAMSULOSIN

S. No.	Concentration (µg/mL)	% Assay	Statistical parameters
1	1.98	99.0	Mean=99.7
2	1.99	99.5	SD=0.76
3	2.01	100.5	%RSD=0.77
4	3.97	99.3	Mean=99.8
5	3.99	99.8	SD=0.63
6	4.02	100.5	%RSD=0.63
7	6.01	100.2	Mean=99.9
8	5.99	99.8	SD=0.25
9	5.98	99.7	%RSD=0.25

TABLE 8: INTER DAY PRECISION STUDIES OF TAMSULOSIN

S. No.	Concentration (µg/mL)	% Assay	Statistical parameters
1	1.97	98.5	Mean=99.0
2	1.99	99.5	SD=0.50
3	1.98	99.0	%RSD=0.51
4	3.97	99.3	Mean=99.6
5	3.98	99.5	SD=0.36
6	4.00	100.0	%RSD=0.36
7	6.00	100.0	Mean=99.7
8	5.97	99.5	SD=0.25
9	5.98	99.7	%RSD=0.25

Accuracy: The accuracy of the HPLC method was confirmed by recovery studies by spiking 50,100 & 150% of pure drugs to the pre analyzed samples and the samples after dilution injected into the system (n=3). The peak area of each drug was measured and the recovery values for paracetamol, Phenylephrine and caffeine were determined by using the formula. The statistical data was presented in the table 9 amd 10 for solifenacin and Tamsulosin respectively;

$$\% \text{ Recovery} = \frac{T-A}{S} \times 100$$

T= total amount of drug estimated; A= Amount contributed for formulation; S = Amount of pure drug added

TABLE 9: RECOVERY FOR SOLIFENACIN

S. No.	%Spike level	Amount added (µg/mL)	Amount found (µg/mL)	%Recovery	Statistical parameters
1	50	30.21	29.87	98.9	Mean=98.8
2	50	30.44	29.96	98.4	SD=0.32
3	50	30.23	29.94	99.0	%RSD=0.32
4	100	60.25	59.97	99.5	Mean=99.4
5	100	60.41	59.92	99.2	SD=0.19
6	100	60.27	59.96	99.5	%RSD=0.19
7	150	90.55	90.13	99.5	Mean=99.7
8	150	90.32	90.21	99.9	SD=0.17
9	150	90.47	90.18	99.7	%RSD=0.17

TABLE 10: RECOVERY FOR TAMSULOSIN

S. No.	%Spike level	Amount added (µg/mL)	Amount found (µg/mL)	%Recovery	Statistical parameters
1	50	2.05	2.03	99.0	Mean=99.0
2	50	2.08	2.05	98.6	SD=0.47
3	50	2.01	2.00	99.5	%RSD=0.48
4	100	3.91	3.90	99.7	Mean=99.4
5	100	3.96	3.92	99.0	SD=0.38
6	100	3.94	3.92	99.5	%RSD=0.39
7	150	5.98	5.93	99.2	Mean=99.6
8	150	6.02	6.01	99.8	SD=0.35
9	150	5.96	5.94	99.7	%RSD=0.35

System suitability parameters: According to USP System suitability tests are an integral part of chromatographic method validation. The tests were used to verify that the reproducibility of the chromatographic system is adequate for analysis. To ascertain its effectiveness system suitability tests were carried out on freshly prepared standard stock solution containing 60 µg/mL for Solifenacin and 4 µg/mL for Tamsulosin.

10µL of solution was injected into the optimized chromatographic system. For system suitability 6 replicates of working standard samples were injected and the parameters like Retention time (RT), Plate number(N), Peak area and peak asymmetry of sample were calculated these results are presented in **table 11**.

TABLE 11: SYSTEM SUITABILITY PARAMETER (N=6)

Drug	RT	TP	AF	Resolution
Solifenacin	10.95	5036	1.11	18.48
Tamsulosin	3.31	4568	1.02	0

Assay of synthetic mixture: The validated method was applied to determination of Solifenacin and Tamsulosin in commercial tablet. Weight and transfer 4 mg Tamsulosin and 60 mg of Solifenacin synthetic mixture were transfer into 100 mL volumetric flask to this add 25 mL of methanol mixed well to dissolve and make up the volume with methanol and sonicate for 15 min. Solution was centrifuge for 15 min at 5000 rpm; supernatant was collected and filtered through a 0.22µm membrane filter.Pipette out 1 mL above solution into 10 ml volumetric flask diluted with mobile phase. The result assay indicates that the method is selective for routine analysis. Result mention in **table 12.**

TABLE 12: ASSAY OF SOLIFENACIN AND TAMSULOSIN IN SYNTHETIC MIXTURE

S. No	% Assay	
	Solifenacin	**Tamsulosin**
1	99.78	99.89
2	99.39	100.35
Average	**99.6**	**100.1**
SD	**0.3**	**0.3**
% RSD	**0.3**	**0.3**

CONCLUSION: A simple, specific, accurate, precise, stability indicating reverse phase high performance liquid chromatography (RP-HPLC) method has been developed which can be used accurately quantitative estimation of Solifenacin and Tamsulosin for routine analysis of individual and combination of drugs. Method was validated as per ICH Q2 (R2) so it can be used by QC department.

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