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To Develop New RP HPLC Method for the Simultaneous Estimation of Tamsulosin Hydrochloride and Dutasteride in Pharmaceutical Dosage Form

Syed Hafeez Ahmed[1*]
Karunakranth D[2]
Sridhar Babu R[3]
Khasim SM[4]
Mohammed Arbaaz Khan[5]
Syed Ghouse[5]
Syed Aizaz Ehsan[5]
Neha Fatima[5]
Saquib Pasha[5]

[1]*Department of Pharmaceutical Analysis and Quality Assurance, Shadan College of Pharmacy, Hyderabad, India*
[2]*Department of Pharmaceutical Chemistry, Shadan College of Pharmacy, Hyderabad, India*
[3]*Department of Pharmaceutics, Shadan College of Pharmacy, Hyderabad, India*
[4]*Department of Pharmacology, Shadan College of Pharmacy, Hyderabad, India*
[5]*Department of Pharmacyy, Shadan College of Pharmacy, Hyderabad, India*

Abstract

A simple and selective LC method is described for the determination of Tamsulosin Hydrochloride and Dutasteride in pharmaceutical dosage form. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 40 volumes of 20mM Ammonium acetate buffer pH 3.5:30 volumes of Acetonitrile: 30 volumes of Methanol with detection of 223nm. Linearity was observed in the range 19.2-44.8 µg/ml for Tamsulosin Hydrochloride (r^2 = 0.996) and 24-56 µg/ml for Dutasteride (r^2 = 0.998) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing % RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: RP HPLC, Tamsulosin Hydrochloride, Dutasteride, Mobile phase

Introduction

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 [1]. 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.

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 [2,3]. 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 [4]. Approximately 70% of the alpha1-receptors in human prostate are of the alpha-1A subtype. Blockage of these receptors causes relaxation of

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***Corresponding author:** Syed Hafeez Ahmed, Department of Pharmaceutical Analysis and Quality Assurance, Shadan College of Pharmacy, Hyderabad, India. Tel: +91-7702543154; Email: syed.hafeez30(at)gmail.com

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smooth muscles in the bladder neck and prostate, and thus decreases urinary outflow resistance in men.

Dutasteride

Dutasteride belongs to a class of drugs called 5-alpha-reductase inhibitors, which block the action of the 5-alpha-reductase enzymes that convert testosterone into dihydrotestosterone (DHT). Finasteride also belongs to this group, but while Dutasteride inhibits both isoforms of 5-alpha reductase, finasteride inhibits only one. Even so, a clinical study done by GlaxoSmithKline, the EPICS trial, did not find Dutasteride to be more effective than finasteride in treating BPH [5].

Mechanism of action: Dutasteride inhibits the conversion of testosterone to 5 alpha-dihydrotestosterone (DHT), which is the androgen primarily responsible for the initial development and subsequent enlargement of the prostate gland. Testosterone is converted to DHT by the enzyme 5 alpha-reductase, which exists as 2 isoforms, type 1 and type 2 [6]. Dutasteride is a competitive and specific inhibitor of both type 1 and type 2, 5-alpha-reductase isoenzymes, with which it forms a stable enzyme complex. Dissociation from this complex has been evaluated under *in vitro* and *in vivo* conditions and is extremely slow. Dutasteride does not bind to the human androgen receptor [7,8].

Aim and Objective

Aim

To develop new RP HPLC method for the simultaneous estimation of Tamsulosin Hydrochloride and Dutasteride in pharmaceutical dosage form.

Objective

- Solubility determination of Tamsulosin Hydrochloride and Dutasteride in various solvents and buffers.
- Determine the absorption maxima of both the drugs in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development.
- Optimize the mobile phase and flow rates for proper resolution and retention times.
- Validate the developed method as per ICH guidelines.

Materials and Methods

Tables 1 and 2

Mobile phase

A mixture of 40 volumes of 20mM Ammonium acetate buffer pH 3.5:30 volumes of Acetonitrile: 30 volumes of Methanol. The mobile phase was sonicated for 10 min to remove gases.

Determination of working wavelength (λ Max)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of Tamsulosin Hydrochloride

25 mg of Tamsulosin Hydrochloride was weighed and transferred in to 250ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg/ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of Dutasteride

25mg of **Dutasteride** was weighed in to 250ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg/ml of solution by diluting 1ml to 10ml with methanol.

Were soluble it was used as solvent for **λ max** determination by UV-Visible Spectroscopy.

Assay

Preparation of samples for assay

Preparation of mixed standard solution: Standard stock solutions of Tamsulosin Hydrochlorideand Dutasteride (microgram/ml) were prepared by dissolving 40 mg of Tamsulosin Hydrochloride and 32 mg of Dutasteride dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100 ml with mobile phase. Further dilutions are prepared in 5 replicates of 40 µg/ml of Tamsulosin Hydrochlorideand 32 µg/ml of Dutasteride was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Sample preparation: 10 tablets (each tablet contains 0.5 mg of Tamsulosin Hydrochloride and 0.4 mg of Dutasteride) were weighed and taken into a mortar uniformly mixed. Test stock solutions of Tamsulosin Hydrochloride (40 µg/ml) and Dutasteride (32 µg/ml) were prepared by dissolving weight equivalent to 40 mg of Tamsulosin Hydrochloride and 32 mg of Dutasteride and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 40 µg/ml of Tamsulosin Hydrochloride and 32 µg/ml of Dutasteride was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Table 1: Reagents used.

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Dihydrogen Phosphate	AR Grade
Acetonitrile	HPLC Grade
Dipotassium hydrogen phosphate	AR Grade
Acetonitrile	HPLC Grade

Table 2: Drugs used.

QUINAPRIL AND TOLCAPONE drugs	Gift Samples obtained from Chandra labs, Hyd.
PFIZA (QUINAPRIL- 10mg & TOLCAPONE- 12.5**) Tablet dosage form**	Obtained from local pharmacy

Table 3: Assay results.

	QUINAPRIL		TOLCAPONE	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	1136.114	1120.050	2576.974	2541.448
Injection-2	1112.446	1121.051	2535.582	2551.500
Injection-3	1115.176	1123.043	2549.337	2545.160
Injection-4	1116.202	1118.821	2538.795	2551.600
Injection-5	1124.282	1112.446	2544.742	2535.582
Average Area	1120.844	1118.942	2549.086	2545.058
Standard deviation	3.615683		6.83985	
%RSD	0.323134		0.26875	
Assay(%purity)	99.83032		99.84198	

Calculation

The amount of TOLCAPONE and QUINAPRILpresent in the formulation by using the formula given below, and results shown in table 3.

$$\%\,\text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AT = Peak area of sample preparation,

AS = Average Peak area of standard preparation,

WS = Weight of drug in mg,

DS & DT = Dilution of standard and sample preparation,

WT = Weight of Sample in Assay preparation,

P = Percentage purity of working standard,

LC = Label Claim of drug.

Observation: The amount of Tamsulosine Hydrochloride and Dutasteride present in the taken dosage form was found to be 98.93 % and 99.16% respectively.

Method validation

Validation: Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity and potency of the drug substances and drug products.

Validation parameters

a) Specificity / Selectivity

b) Accuracy

c) Precision

d) Linearity & Range

e) Limit of Detection

f) Limit of Quantitation

g) Robustness

h) Ruggedness

i) System Suitability

Results and Discussion

Wavelength optimization by UV– spectroscopy

Figure 1

Method development and optimization of RP-HPLC method

Table 4

Method validation

System suitability: Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

Tables 5 and 6

Linearity

Tables 7,8 and 9

Figures 2 and 3

Observation: The correlation coefficient for linear curve obtained between concentration vs. Area for standard

Table 4: Optimized chromatographic conditions

Mobile phase	Methanol:Water
Ph	-
Column	Inertsil ODS 3V column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25°C)
Sample temperature	Room temperature(20-25°C)
Wavelength	220
Injection volume	20 µl
Run time	6 min
Retention time	About 2.707 min for Quinapril and 3.953 min for Tolcapone.

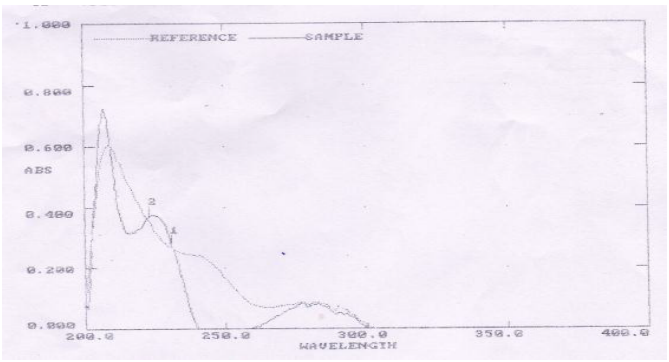

Figure 1: Wavelength optimization by UV– spectroscopy.

Table 5: Results for system suitability of Quinapril.

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.700	1136.114	2877	1.441
2	2.700	1112.446	2966	1.343
3	2.697	1115.176	2961	1.455
4	2.707	1116.202	2976	1.485
5	2.703	1124.282	2971	1.485
Mean	2.7014	1120.844	-	-
SD	0.003782	9.607	-	-
%RSD	0.139984	0.8574	-	-

Table 6: Results for system suitability of Tolcapone.

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	3.947	2576.974	2476	1.500
2	3.937	2535.582	2554	1.477
3	3.933	2549.337	2550	1.512
4	3.953	2538.795	2576	1.477
5	3.947	2544.742	2567	1.512
Mean	3.9434	2549.086	-	-
SD	0.008173	16.46919	-	-
%RSD	0.207261	0.646082	-	-

Table 7: Linearity preparations.

Preparations	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)	
			QUINAPRIL	TOLCAPONE
Preparation 1	0.75	10	2.5	5
Preparation 2	1.125	10	3.75	7.5
Preparation 3	1.5	10	5	10
Preparation 4	1.875	10	6.25	12.5
Preparation 5	2.25	10	7.5	1.5

Table 8: Linearity of Quinapril.

S.No.	Conc.(µg/ml)	Area
1	2.5	495.227
2	3.75	745.541
3	5	1015.117
4	6.25	1290.46
5	7.5	1470.799

Table 9: Linearity of Tolcapone.

S.No.	Conc.(µg/ml)	Area
1	5	1152.124
2	7.5	1807.304
3	10	2315.072
4	12.5	2929.514
5	15	3454.098

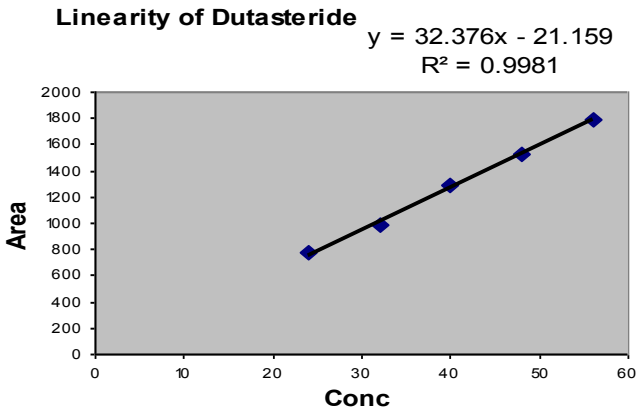

Figure 3: Linearity graph of Dutasteride.

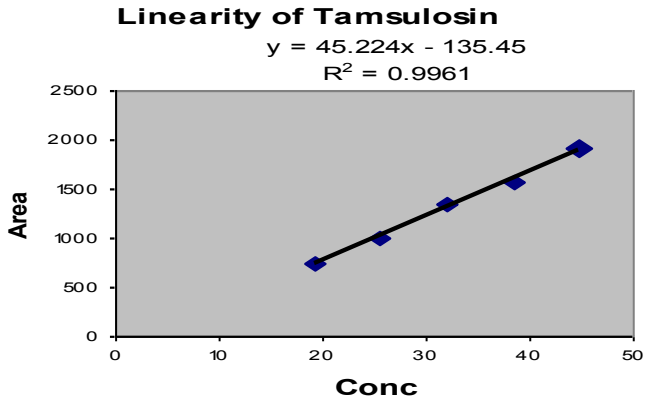

Figure 2: Linearity graph of Tamsulosin Hydrochloride.

preparations of Tamsulosin Hydrochloride and Dutasteride is 0.9961 and 0.9981.

Recovery

Tables 10 and 11

Observation: The percentage mean recovery of Tamsulosin Hydrochloride and Dutasteride is 100.23% and 99.47% respectively.

Precision

Observation: Test results for Tamsulosin Hydrochloride and Dutasteride are showing that the % RSD of Assay results are within limits.

Table 10: Recovery results for Quinapril.

Recovery level	Accuracy Quinapril				Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	%Recovery	
50%	2.5	1147.472	1142.193	101.985	101.54
	2.5	1147.472			
	2.5	1131.636			
100%	5	1282.181	1287.862	103.48	
	5	1290.460			
	5	1290.945			
150%	7.5	1391.221	1388.523	99.18	
	7.5	1373.610			
	7.5	1400.738			

Table 11: Recovery results for Tolcapone.

Recovery level	Accuracy Tolcapone				Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	%Recovery	
50%	5	2581.774	2573.486	102.06	102.81
	5	2581.774			
	5	2556.911			
100%	10	2933.859	2948.693	105.45	
	10	2936.438			
	10	2975.781			
150%	15	3186.091	3175.224	100.94	
	15	3146.856			
	15	3192.726			

Limit of detection

$$LOD = \frac{3.3\sigma}{S}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

LOD of Tamsulosin Hydrochloride = 0.74 µg/ml

LOD of Dutasteride = 1.29 µg/ml

Observation: The LOD for this method was found to be 0.74 µg/ml & area 33.46 for Tamsulosin Hydrochloride and 1.29 µg/ml & area 41.79 for Dutasteride.

Limit of quantification

$$LOQ = \frac{10\sigma}{S}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

LOQ of Tamsulosin Hydrochloride = 2.24µg/ml

LOQ of Dutasteride =3.91 µg/ml

Observation: The LOQ for this method was found to be 2.24 µg/ml & area 101.40 for Tamsulosin Hydrochloride and 3.91 µg/ml & area 126.64 for Dutasteride.

Robustness

Observation: From the observation it was found that the system suitability parameters were within limit at all variable conditions.

RUGGEDNESS

Observation: From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged.

Table 12: Method precision results for Quinapril and Tolcapone.

Quinapril		
S.No.	Rt	Area
1	2.660	1109.066
2	2.667	1110.202
3	2.680	1113.271
4	2.683	1112.450
5	2.680	1108.599
6	2.690	1109.570
Avg	2.676667	1110.526
stdev	0.011057	0.1564
%RSD	0.412278	**0.3421**

Tolcapone		
S.No.	Rt	Area
1	3.890	2518.891
2	3.900	2515.559
3	3.917	2514.373
4	3.903	2512.866
5	3.913	2517.609
6	3.923	2519.468
avg	3.907667	2516.461
stdev	0.012193	0.4321
%RSD	0.311401	**0.2653**

Discussion

A simple and selective LC method is described for the determination of Tamsulosin Hydrochloride and Dutasteride tablet dosage forms. Chromatographic separation was achieved on a C_{18} column using mobile phase consisting of a mixture of Phosphate buffer (KH_2PO_4) pH: 3.5:30 Acetonitrile: Methanol (40:30:30v/v), with detection of 223 nm. Linearity was observed in the range 19.2-44.8 µg/ml for Tamsulosin Hydrochloride (r^2 = 0.9961) and 24-56µg/ml for Dutasteride (r^2 = 0.9981) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing % RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Conclusion

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation Tamsulosin Hydrochloride and Dutasteride was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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