Validated RP-HPLC and TLC methods for simultaneous estimation of tamsulosin hydrochloride and finasteride in combined dosage forms

DIPTI B. PATEL*
NATUBHAI J. PATEL

*S. K. Patel College of Pharmaceutical
Education and Research, Department
of Pharmaceutical Chemistry, Ganpat
University, Kherva, Mehsana, Gujarat
India-382711*

Reversed-phase high-performance liquid chromatography (RP-HPLC) and thin-layer chromatography (TLC) methods have been developed and validated for simultaneous estimation of tamsulosin hydrochloride and finasteride in bulk drug and in combined dosage forms. RP-HPLC separation was achieved on a Phenomenex C_{18} column using methanol/0.02 mol L^{-1} ammonium acetate buffer/triethylamine (79.9 + 20 + 0.1, $V/V/V$) (pH 9.2) as mobile phase. TLC separation was achieved on an aluminium-backed layer of silica gel 60 F_{254} using toluene/ methanol/ triethylamine (9 + 1.5 + 1, $V/V/V$) as eluent. Quantification was achieved with photodiode array (PDA) detection at 235 nm over the concentration range 0.5–16 and 1–50 µg mL^{-1} with mean recovery of 99.8 ± 0.9 and 100.0 ± 0.8 % for tamsulosin hydrochloride and finasteride, respectively, by the RP-HPLC method. Quantification was achieved with UV detection at 270 nm over the concentration range 100–2000 ng per spot and 250–5000 ng per spot with mean recovery of 98.9 ± 0.9 and 99.6 ± 0.7 % for tamsulosin hydrochloride and finasteride, respectively, by the TLC method. Both methods are simple, precise, accurate and sensitive and are applicable to the simultaneous determination of tamsulosin hydrochloride and finasteride in bulk drug and in combined dosage forms.

Keywords: tamsulosin hydrochloride, finasteride, RP-HPLC, TLC

Tamsulosin hydrochloride (TAM), (–)-(*R*)-5-[2-[[2-(*o*-ethoxy phenoxy)ethyl]amino] propyl]-2-methoxy benzene sulfonamide, monohydrochloride, a selective antagonist of α_1-adrenoreceptor, exhibits selectivity for α_1-receptors in human prostate. TAM is used to reduce urinary obstruction and relieve the symptoms associated with symptomatic benign prostatic hyperplasia. Chemically, finasteride (FINA), is *N*-(1,1-dimethylethyl)- -3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide. It is a specific inhibitor of steroid type-

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* Correspondence; e-mail: diptibpatel_24980@yahoo.co.in

-II 5α-reductase, an intracellular enzyme that converts the androgen testosterone into 5α-dihydrotestosterone hormone responsible for prostate growth. Combination of both drugs is indicated for the treatment of symptomatic benign prostatic hyperplasia in men with an enlarged prostate (1-3).

Literature survey reveals various LC-MS (4-6) and HPLC (7) methods for determination of TAM in biological fluids and in pharmaceuticals. Recently, a LC-MS-MS method has been found for simultaneous estimation of tamsulosin hydrochloride and dutasteride in human plasma (8). A few LC-MS (9-11), HPLC (12, 13) and HPTLC (14) methods have been previously reported for the determination of FINA in biological fluids and in pharmaceuticals.

To the author's best knowledge, no method has been reported for simultaneous estimation of TAM and FINA in combined pharmaceutical dosage forms. Hence, it was thought to be of interest to develop RP-HPLC and TLC methods for simultaneous estimation of TAM and FINA in bulk as well as in combined dosage forms.

EXPERIMENTAL

Apparatus

A Shimadzu (Japan) RP-HPLC instrument (LC-2010HT) equipped with a photodiode array detector, autosampler with 20-μL loop, Phenomenex C_{18} column (250 mm × 4.6 mm id, 5 μm particle size) and Class-VP software were used. For TLC, a Linomat V autosprayer, Scanner-III, flat bottom and twin trough developing chambers and viewing cabinet with dual wavelength UV lamps (Camag, Switzerland) were used. TLC plates used were silica gel with a fluorescent indicator at 254 nm, layer thickness 0.2 mm, 10 × 10 cm, aluminium backing (E. Merck, Germany).

Reagents and materials

Tamsulosin hydrochloride and finasteride pure powder were gifts from Intaas Pharmaceutical Limited, India. Methanol (RP-HPLC grade) and toluene, methanol, ammonium acetate and triethylamine were purchased from s.d. Fine Chem. Ltd, India. Water for RP-HPLC was prepared by triple glass distillation and filtered through a nylon 0.45-μm membrane filter (Gelman Laboratory, India). Tablets of two different brands (brand A: Veltam F (0.4 mg TAM + 5 mg FINA), Intaas Pharmaceuticals Ltd, India, and brand B: Urimax F (0.4 mg TAM + 5 mg FINA), Sun Pharmaceutical Ltd, India) were purchased from the local pharmacy.

Chromatographic conditions

RP-HPLC method. – A Phenomenex C_{18} (250 mm × 4.6 mm id, 5 μm particle size) column was used at 27 °C. The mobile phase consisted of methanol/0.02 mol L^{-1} ammonium acetate buffer/triethylamine (79.9 + 20 + 0.1, *V/V/V*) (pH 9.2) and was pumped at a flow rate of 1 mL min^{-1}. The mobile phase was filtered through a nylon 0.45-μm mem-

brane filter and degassed before use. Elution was monitored at 235 nm and the injection volume was 20 μL.

TLC method. – Solutions of both analytes were applied to silica gel 60 F$_{254}$ TLC plates by means of a Linomat V automatic spotter equipped with a 100-μL syringe. The plate was developed in a twin trough chamber previously saturated for 30 min with the mobile phase, toluene/methanol/triethylamine (9 + 1.5 + 1, *V/V/V*) to 8.5 cm. Spots on the air-dried plate were scanned with a Scanner III at 270 nm using the deuterium source.

Preparation of TAM and FINA solutions

RP-HPLC method. – Accurately weighed pure powder of each TAM and FINA were dissolved in methanol to obtain a standard solution of both (100 μg mL^{-1}). Further TAM (20 μg mL^{-1}) and FINA (50 μg mL^{-1}) solutions were prepared with methanol.

TLC method. – Accurately weighed powder of each TAM and FINA were dissolved in methanol to obtain a standard solution of TAM and FINA of 1000 μg mL^{-1}. From this solution, working standard solutions of TAM of 100 μg mL^{-1} and FINA of 250 μg mL^{-1} were prepared.

Preparation of sample solution

Thirty tablets of each brand were weighed and powdered. A quantity of tablet powder equivalent to 1 mg of TAM and 12.5 mg of FINA was transferred to a 100-mL volumetric flask and 80 mL methanol was added. The solution was sonicated for 15 min, and the final volume was diluted to the mark with methanol and the solution was then filtered through a nylon 0.20-μm membrane filter to get solution of TAM (10 μg mL^{-1}) and FINA (125 mg mL^{-1}). This solution is used for TLC method. From this solution a ten fold dilution was obtained with methanol: of TAM (1 μg mL^{-1}) and FINA (12.5 μg mL^{-1}) for RP-HPLC method.

Validation

Both RP-HPLC and TLC methods were validated as per ICH guidelines (15).

Calibration curve for RP-HPLC method. – Calibration curves were plotted over the concentration range 0.5–16 μg mL^{-1} for TAM and 1–50 μg mL^{-1} for FINA. Accurately measured aliquots of working standard solutions of TAM and FINA were diluted with methanol to obtain final concentrations of TAM (0.5, 1, 2, 4, 8, 12 and 16 μg mL^{-1}) and FINA (1, 5, 10, 20, 30, 40 and 50 μg mL^{-1}). Calibration curves were constructed by plotting peak areas *vs.* TAM and FINA concentrations, and regression equations were calculated.

Calibration curve for TLC method. – Calibration curves were plotted over a concentration range of 100–2000 ng per spot and 250–5000 ng per spot for TAM and FINA, respectively. Standard solution of TAM and FINA (1, 2, 4, 8, 12, 16 and 20 μL) were applied to the plate. Spots were developed under the chromatographic conditions as described

above. Calibration curves were constructed by plotting peak areas *vs.* concentrations with the help of Win-CATS software.

Accuracy. – The accuracy of the methods was determined by calculating TAM and FINA recoveries by the standard addition method. Known amounts of standard solutions of TAM (0.50, 1.00 and 1.50 μg mL^{-1}) and FINA (6.25, 12.50 and 18.75 mg mL^{-1}) were added to prequantified sample solutions of tablet dosage forms for the RP-HPLC method. TAM (50, 100 and 150 ng per spot) and FINA (625, 1250 and 1875 ng per spot) were added to prequantified sample solutions of tablet dosage forms for the TLC method. The amounts of TAM and FINA were estimated by applying these values to the regression equation of the calibration curve.

Instrument precision. – The precision of the instrument was checked by repeatedly injecting ($n = 6$) standard solutions of TAM (6 μg mL^{-1}) and FINA (20 μg mL^{-1}) for the RP-HPLC method and by repeated spotting of the same solution and repeated scanning of the same spot ($n = 6$) of TAM (800 ng per spot) and FINA (2000 ng per spot) without changing the position of plate for the TLC method. Repeatability is reported in terms of relative standard deviation (RSD).

Method precision. – The intra-day and inter-day precision of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days for 3 different concentrations (4, 8 and 12 mg mL^{-1}) of TAM and FINA (10, 20 and 30 mg mL^{-1}) for the RP-HPLC method, and 800, 1200 and 1600 ng of TAM per spot and 1000, 2000 and 3000 ng of FINA per spot for the TLC method.

Limit of detection and quantification. – The limit of detection (*LOD*) of the both drugs was found by the trial and error method (visual) by injecting progressively low concentrations of standard solutions. The lowest concentration of the range at which the analyte can be quantified with acceptable accuracy and precision was selected as the limit of quantification (*LOQ*).

RESULTS AND DISCUSSION

Method development

RP-HPLC method. – To optimize the RP-HPLC parameters, several mobile phase compositions were assayed. A satisfactory separation and good peak symmetry for TAM and FINA were obtained with a mobile phase consisting of methanol/0.02 mol L^{-1} ammonium acetate buffer/triethylamine (79.9 + 20 + 0.1, *V/V/V*). Clear baseline was achieved with photodiode array (PDA) detection at 235 nm (Fig. 1).

TLC method. – Several mobile phases were tested to accomplish good separation of TAM and FINA. Using the mobile phase toluene/methanol/triethylamine (9 + 1.5 + 1, *V/V/V*) and silica gel 60 F$_{254}$ aluminum-backed plates as a stationary phase, better separation was attained for TAM and FINA with R_f values of 0.36 and 0.65, respectively (Fig. 3). A wavelength of 270 nm was used for quantification of both drugs.
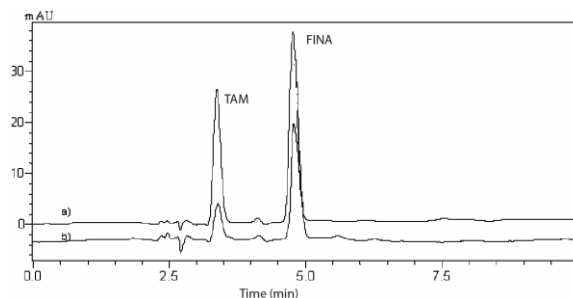
Fig. 1. RP-HPLC chromatograms of: a) standard solution with 10 µg mL^{-1} TAM + 50 µg mL^{-1} FINA, b) formulation with 2 µg mL^{-1} TAM + 25 µg mL^{-1} FINA, at 235 nm.

Validation of the methods

Linear correlation was obtained between the peak area and concentration of the drugs. In the RP-HPLC method, linearity range was 0.5–16 µg mL^{-1} and 1–50 µg mL^{-1} for TAM and FINA, respectively. In the TLC method, linearity was in the range of 100–2000 ng per spot and 250–5000 ng per spot for TAM and FINA, respectively. Linearity of the calibration curves was validated by the high value of correlation coefficients of the regression (Table I).

The accuracy of the method was studied by the standard addition method. The mean recoveries with standard deviation were 99.9 ± 0.9 and 100.0 ± 0.8 for TAM and FINA, respectively, by RP-HPLC, and 98.9 ± 0.9 and 99.6 ± 0.7 for TAM and FINA, respectively, by the TLC method. The results revealed no interference of excipients during the analysis by both methods are accurate (Figs. 1b and 2b and Table II).

Both the drugs were well resolved from each other by either HPLC or TLC.

The RSD values of instrument precision for both TAM and FINA were found to be 0.4 % using RP-HPLC and 0.5–0.6 %, using TLC (Table II). The RSD values of inter-day precision for TAM and FINA by RP-HPLC method were 0.8–1.6 % and intra-day preci-

Table I. Regression analysis of the calibration curves for TAM and FINA for RP-HPLC and TLC methods

Parameter	RP-HPLC		TLC	
	TAM	FINA	TAM	FINA
Concentration range	0.5–16 µg mL^{-1}	1–50 µg mL^{-1}	100–2000 ng per spot	250–5000 ng per spot
Slope[a]	28074 ± 324	82150 ± 23	1.518 ± 0.22	0.743 ± 0.086
Intercept[a]	7508 ± 77	8714 ± 561	239.34 ± 19.74	143.58 ± 32.91
Coefficient of determination (R^2)	0.9986	0.9981	0.9975	0.9978

[a] Mean ± SD, $n = 5$.

Table II. Validation parameters for the proposed RP-HPLC and TLC methods

Parameter	RP-HPLC method		TLC method	
	TAM	FINA	TAM	FINA
LOD	0.2 µg mL^{-1}	0.5 µg mL^{-1}	80 ng per spot	200 ng per spot
LOQ	0.5 µg mL^{-1}	1.0 µg mL^{-1}	100 ng per spot	250 ng per spot
Recovery (accuracy)(%)[a]	99.9 ± 0.9	100.0 ± 0.8	98.9 ± 0.9	99.6 ± 0.7
Instrument precision (RSD, %)[b]	0.4 %	0.4 %	0.5 %	0.6 %
Method precision (RSD, %)[b]				
Intra-day precision (RSD, %)[b]	0.5–1.0 %	0.7–0.9 %	0.8–1.0 %	1.0 %
Inter-day precision (RSD, %)[b]	0.7–1.1 %	0.9–1.4 %	1.0–1.3 %	1.1–1.6 %

[a] Mean ± SD, *n* = 6.
[b] *n* = 3.

sion 0.7–1.0 %. For TLC method inter-day precision RSD values for TAM and FINA were 1.0–1.6 %, and intraday precision were 0.8–1.1 %. Low RSD values indicate that the proposed methods are precise (Table II).

LOD for TAM and FINA was found to be 0.2 and 0.5 µg mL^{-1} for RP-HPLC and 80 and 200 ng per spot for TLC, respectively. *LOQ* for TAM and FINA was found to be 0.5 and 1.0 µg mL^{-1}, respectively, for RP-HPLC, and 100 ng per spot and 250 ng per spot respectively for the TLC method. These data show that both methods are sensitive for TAM and FINA determination (Table II).

System suitability parameters including retention time, theoretical plate number, tailing factor, asymmetry factor, capacity factor and resolution were investigated and are listed in Table III.

Table III. System suitability parameters for TAM and FINA for the RP-HPLC method

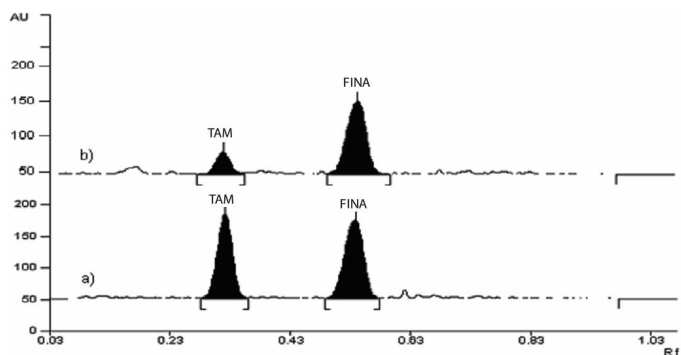| Parameters	TAM[a]	RSD (%)	FINA[a]	RSD (%)
Retention time (min)	3.271	0.5	4.735	0.5
Tailing factor	1.195	1.0	1.171	1.0
Asymmetry factor	1.139	1.0	1.268	1.0
Capacity factor	4.289	0.9	6.543	0.7
Number of theoretical plates (*N*)	2800.66	1.1	4918.52	1.0
Chromatographic resolution (*R*$_s$)	2.875	1.3	5.246	0.5

[a] Mean value, *n* = 5.

Fig. 2. TLC densitograms of: a) standard solution with 1600 ng TAM + 400 ng FINA per spot, b) formulation A with 300 ng TAM + 3750 ng FINA per spot at 270 nm.

Assay of the tablet dosage form

The proposed methods were applied to determine TAM and FINA in the combined tablet dosage form (brands A and B). Good compliance (99–101 %) with the label claim was found for both analytes by both HPLC and TLC (Table IV).

Table IV. Assay of the combined tablet dosage forms using RP-HPLC and TLC methods

Formulation		Label claim	RP-HPLC		TLC	
			Mass found (mg)	Assay (%)[a,b]	Mass found (mg)	Assay (%)[a,b]
A	TAM	0.40	0.40	98.9 ± 0.6	0.40	99.6 ± 1.2
	FINA	5.00	5.03	100.7 ± 1.1	4.99	99.8 ± 1.1
B	TAM	0.40	0.40	99.7 ± 0.8	0.402	100.6 ± 0.8
	FINA	5.00	4.97	99.2 ± 1.0	5.00	100.0 ± 1.0

[a] Percent of the label claim.
[b] Mean ± SD, n = 5.

CONCLUSIONS

RP-HPLC and TLC methods were developed for determination of TAM and FINA in pharmaceutical formulations. Both methods are simple, precise, accurate, specific and sensitive. Hence, both methods can be extended for routine simultaneous analysis of TAM and FINA in tablet formulations.

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S A Ž E T A K

Validirane RP-HPLC i TLC metode za simultano određivanje tamsulozin hidroklorida i finasterida u istom dozirnom pripravku

DIPTI B. PATEL i NATUBHAI J. PATEL

U radu su opisani razvoj i validacija inverzno fazne kromatografije visoke učinkovitosti (RP-HPLC) i tankoslojne kromatografije (TLC) za simultano određivanje tamsulozin hidroklorida i finasterida kao čistih supstancija i u kombiniranim tabletama. Za RP-HPLC odjeljivanje korištena je Phenomenex C_{18} kolona (250 mm, 4,6 mm, 5 µm) i metanol/0,02 mol L^{-1} pufer s amonijevim acetatom/trietilamin (79,9+20+0,1, *V/V/V*) (pH 9,2) kao pokretna faza, pri protoku 1 mL min^{-1}. TLC odjeljivanje rađeno je na silikagelu $60F_{254}$ na aluminijskoj podlozi, koristeći toluen/metanol/trietilamin (9+1,5+1, *V/V/V*) kao eluens. Za detekciju u RP-HPLC metodi korištena je fotodioda (PDA) pri 235 nm te je provedena kvantitacija u koncentracijskom području 0,5–16 µg mL^{-1} i 1–50 µg mL^{-1}, uz srednji analitički povrat od 99,8 ± 0,9 % za tamsulozin hidroklorid i 100,0 ± 0,8 % za finasterid. Za kvantitaciju u TLC metodi korištena je UV detekcija pri 270 nm u koncentracijskom području 100–2000 ng po točki za tamsulozin hidroklorid i 250–5000 ng po točki za finasterid, uz srednji analitički povrat od 98,9 ± 0,9, odnosno 99,6 ± 0,7 %. Obje metode su jednostavne, precizne, točne i osjetljive i mogu se primijeniti za simultano određivanje tamsulozin hidroklorida i finasterida kao čistih supstancija i u kombiniranim dozirnim oblicima.

Ključne riječi: tamsulozin hidroklorid, finasterid, RP-HPLC, TLC

S. K. Patel College of Pharmaceutical Education and Research, Department of Pharmaceutical Chemistry, Ganpat University, Kherva, Mehsana, Gujarat, India-382711