European Journal of Chemistry 5 (1) (2014) 181-185

European Journal of Chemistry

Journal homepage: www.eurjchem.com

Simultaneous quantitative analysis of tamsulosin and finasteride in pharmaceutical dosage form by U-HPLC Tandem mass spectrometry

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ARTICLE INFORMATION

DOI: 10.5155/eurjchem.5.1.181-185.942

Received: 04 October 2013
Received in revised form: 02 November 2013
Accepted: 02 November 2013
Online: 31 March 2014

KEYWORDS

Validation
Tamsulosin
Finasteride
Diphenhydramine
Pharmaceutical dosage form
Liquid chromatography tandem mass spectrometry

ABSTRACT

A sensitive, rapid, selective and accurate liquid chromatography coupled to quadrupole tandem mass spectrometry (LC-MS/MS) method was developed for simultaneous identification and quantification of tamsulosin and finasteride in bulk and in their combined dosage form. Chromatography was performed on a Hypersil gold 50 mm × 2 mm (1.9 µm) column, using acetonitrile:ammonium acetate (90:10, *v:v*) pH = 3.5 as the mobile phase. Protonated ions formed by a turbo ion-spray in positive mode were used to detect the analytes as well as the internal standard (IS). MS/MS detection was carried out by monitoring the fragmentation of 408.74 → 227.29 (*m/z*), 373.11 → 304.96 (*m/z*) and 255.75 → 166.15 (*m/z*) for tamsulosin, finasteride and diphenhydramine (IS), respectively, on a triple quadrupole mass spectrometer. The linearity was obtained over the concentration range of 1.6-40.0 ng/mL for tamsulosin and 20.0-500.0 ng/mL for finasteride with a lower limit of detection of 0.5 ng/mL and 5.0 ng/mL for the two drugs, respectively. The proposed method was successfully applied to tamsulosin and finasteride determination in pharmaceutical dosage form. The results obtained were statistically analyzed and compared with those of reference ones; in addition, the method was validated according to USP 34 recommendations. The simplicity and sensitivity of this method allows its use in the quality control of the cited drugs and can be extended for routine analysis of the drugs in their pharmaceutical preparations.

1. Introduction

Tamsulosin (TAM) is a sulfamoylphenethylamine derivative (Figure 1) commonly used to treat signs and symptoms of benign prostatic hyperplasia (BPH) [1,2]. TAM is a selective, potent and competitive α_1-adrenoceptor antagonist [3,4]. The USP has described a potentiometric method for the analysis of TAM [5], besides; various analytical techniques have been reported for the determination of TAM in bulk, pharmaceutical formulations and biological samples. These techniques include HPLC [6-9], stability indicating HPLC [10], stability indicating HPTLC [11], LC-MS/MS [12-14], potentiometry [15], voltametry [16], capillary electrophoresis [17], spectrofluorimetry [18], UV [19] and visible spectrophotometry [20].

Finasteride (FIN), *N*-(1,1-dimethylethyl)-3-oxo-(5α,17β)-4-azaandrost-1-ene-17-carboxamide (Figure 1), is a synthetic antiandrogen which acts by inhibiting type II 5-α reductase, the enzyme that converts testosterone to dihydrotestosterone (DHT). It is used as a treatment in benign prostatic hyperplasia in low doses, and prostate cancer in higher doses. It is also used for treatment of male-pattern baldness in men at a dose of 1 mg daily [21]. In addition to the HPLC method described by the USP [5], several methods for determination of FIN in bulk, pharmaceutical formulations and biological samples have been developed. These methods include HPLC [22], polarography [23] and LC-MS [24,25].

There are few published methods for the high throughput determination of TAM and FIN in bulk and combined dosage forms previously reported using HPLC and TLC methods [26,27].

The lack of LC-MS/MS methods for the simultaneous analysis of both TAM and FIN has motivated us to develop a simple, sensitive and validated LC-MS/MS method for their determination. For best detection up to nano-gram level of TAM and FIN; the chromatographic conditions and the mass spectrometric parameters were thoroughly studied and adjusted. The method was subsequently used to determine the concentration of the drugs in laboratory prepared mixtures as well as in combined dosage forms. Our experimental results were statistically analyzed and compared with those of reference ones.

2. Experimental

2.1. Materials and reagents

Tamsulosin HCl was kindly supplied from Osmopharm S. A., Bedano, Switzerland, while, finasteride and diphenhydramine

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
http://dx.doi.org/10.5155/eurjchem.5.1.181-185.942

Table 1. Tandem mass spectrometric parameters of tamsulosin, finasteride and diphenhydramine.

Parameter	Value		
Turbo ion spray temperature (°C)	400		
Capillary temperature (°C)	270		
Sheath gas (psi)	20		
Auxillary gas (psi)	2		
Ion spray voltage (V)	3600		
Capillary offset	35		
	Tamsulosin	**Finasteride**	**Diphenhydramine (IS)**
Collision energy (V)	23	27	25
SRM transition (*m/z*)	408.74 / 227.29	373.11 / 304.96	255.75 / 166.15

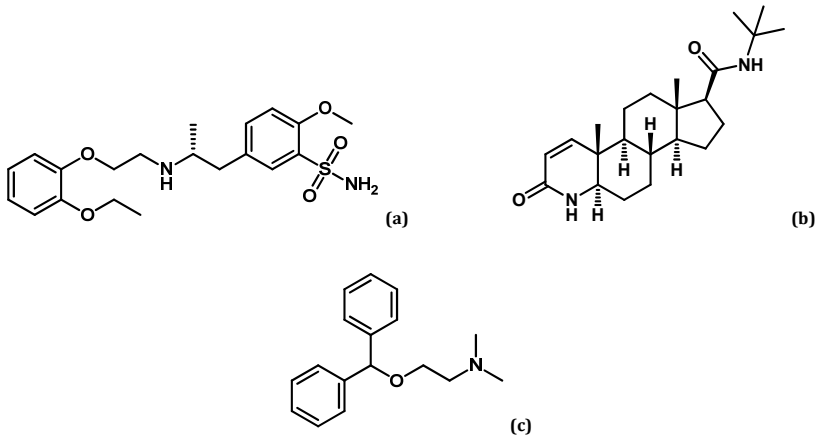

Figure 1. Chemical structures of tamsulosin (a), finasteride (b) and diphenhydramine as IS (c).

(IS) were purchased from Sigma Pharmaceutical Industries, Steinheim, Germany. Their purities were certified and analyzed by reference methods and were found to be 99.54% for TAM [26] and 99.86% for FIN [5]. They were used as provided. Urimax F tablets (0.4 mg TAM + 5 mg FIN) Cipla Pharmaceutical Ltd., Mumbai, India. All solvents and materials were of HPLC grade. Methanol was purchased from Fischer Scientific UK Ltd, Loughborough, UK. Acetonitrile and Ammonium acetate were purchased from Merck, Darmstadt, Germany. Deionized water (Purelab flex, ELGA) was used.

2.2. Instrumentation

The analysis was performed using a TSQ Quantum Access MAX triple stage quadrupole mass spectrometer, Thermoscientific, New York, USA, equipped with an electrospray ionization (ESI) source. Xcalibur software version 2.2 was used to control the LC-MS/MS system, collect and analyse the data. The HPLC system consisted of an Accela U-HPLC with Accela 1250 quaternary pump and Accela open autosampler, New York, USA operated at 15 °C.

2.3. Mass spectrometric conditions

The positive-ion mass spectrometric detection method utilised electrospray ionization and single reaction monitoring (SRM) mode. The optimized parameters are summarized in Table 1.

2.4. Chromatographic conditions

Chromatographic separation was performed on Hypersil-Gold column (C18-bonded ultrapure silica based column) 50 mm × 2.0 mm (1.9 µm) from Thermoscientific, New York, USA. Elution was performed at room temperature using the mobile phase 10 mM ammonium acetate:acetonitrile (10:90, *v*:*v*) (pH = 3.5). The LC system was operated isocratically at 250 µL/min. The injection volume was 10 µL. The total run time for each sample was 3 min.

2.5. Standard solutions

Stock solutions (0.1 mg/mL) of TAM, FIN and IS were separately prepared in 100 mL volumetric flasks in methanol while the working standard solutions were prepared by further dilution of the corresponding stock solutions with methanol. All stock solutions were kept at -20 °C until use, whilst the working solutions were kept at 4 °C and discarded within 30 days.

2.6. Procedures

2.6.1. Calibration curves

Six standard solutions of each drug were prepared in concentration ranges 1.6-40.0 ng/mL for TAM and 20.0-500.0 ng/mL for FIN with the addition of 40 ng/mL of IS on every standard solution. A 10 µL aliquot of each solution was injected onto the LC-MS system. Two calibration curves were established separately for each drug. The calibration curve was constructed by plotting of the peak area ratios of each analyte to IS obtained against the corresponding concentrations.

2.6.2. Laboratory prepared mixtures

Binary laboratory prepared mixtures of TAM and FIN were prepared by mixing their working solutions in different ratios, then 40.0 ng/mL of IS was added. A 10 µL aliquot of each solution was injected onto the LC-MS system, and the procedure was continued as stated under calibration curves.

2.6.3. Dosage form

For analysis of the pharmaceutical dosage form, 10 tablets were pulverized well; an accurate amount of the powdered

tablets equivalent to 12.5 mg of FIN and 1.0 mg of TAM, was weighed and transferred into 100 mL volumetric flask and dissolved in methanol, then the procedure was continued as stated under calibration curves.

3. Results and discussion

For optimum detection up to nano-gram level of TAM, FIN and the IS it was necessary to adjust both the chromatographic conditions and the mass spectrometric parameters. Precursor ions and product ions were optimized by infusing 1.00 µg/mL neat solutions into mass spectrometer in about a 100-500 *m/z* range, in positive polarity mode using electrospray ionization technique. Best intensity for precursor ions and product ions was found in the positive mode for both drugs as they have the ability to accept protons. The protonated molecular ions $[M + H]^+$ of TAM, FIN and IS, observed on the full scan mass spectra, were 408.74, 373.11 and 255.75 *m/z*, respectively.

Moreover, the collision energy in Q2 produced significant fragments. The MS/MS transition 408.74 → 227.29, 373.11 → 304.96 and 255.75 → 166.15 for TAM, FIN and IS, respectively, were selected since these products ions represented the most abundant ions by applying sufficient collision activated dissociation gas and collision energy (Figure 2). Optimization of capillary temperature and sheath gas flow is important as they play a great role in minimizing ion suppression and altering the sensitivity. Adjustment of capillary temperature at 270 °C and sheath gas at 20 psi, augmented the intensity of the analytes. Minor changes in ion spray voltage did not have a marked effect on the signal intensity and was maintained at 3600 V.

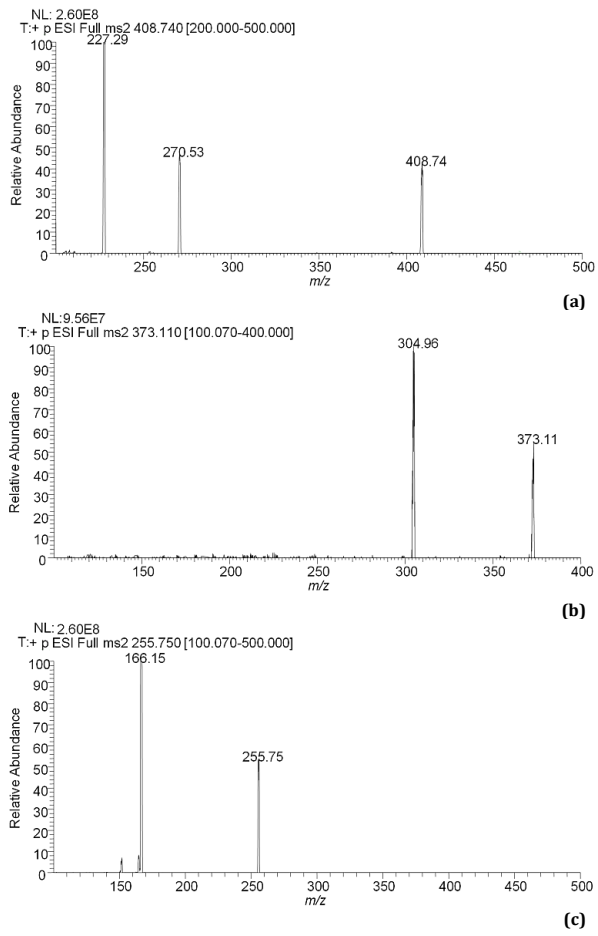

Figure 2. Product ion spectra of $[M + H]^+$ of TAM (a), FIN (b) and IS (c).

To obtain the best chromatographic separation with the desired response it was observed that, mobile phase as well as selection of column is an important criterion. Chromatographic analysis of the drugs and IS was initiated under isocratic conditions with the aim to develop a simple separation process with a short run time. Separation was tried using various combination of acetonitrile and buffer solution with varying contents of each component on different columns like Hypersil-Gold (C18-bonded ultrapure silica based column) and Bio Basic-8 to identify the optimal conditions that produce the best sensitivity, efficiency and peak shape. The use of buffer solution helped in achieving good response for MS detection operating in the positive mode. Thus, a mobile phase consisting of 10 mM ammonium acetate buffer pH adjusted to 3.5 with acetic acid:acetonitrile (10:90, *v:v*) was found suitable as the drugs were protonated and well separated by this phase (Figure 3). High content of acetonitrile (90%) in the mobile phase helped in eluting the drugs along with their IS within 3 min at a flow rate of 250 µL/min. Hypersil-Gold (50.0 mm × 2.0 mm, 1.9 µm particle size) column gave good peak shape and response even at LOQ levels for both drugs.

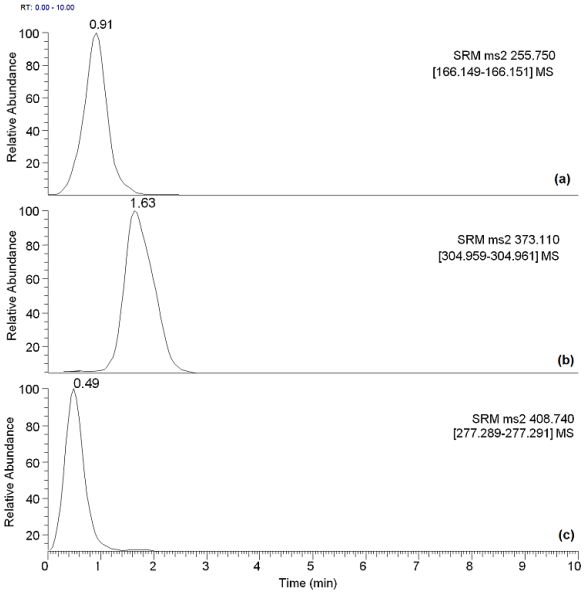

Figure 3. Representative SRM chromatograms of IS (40 ng/mL) (a), FIN (300 ng/mL) (b) and TAM (25 ng/mL) (c).

3.1. Method validation

3.1.1. Linearity and range

Under the optimum chromatographic and mass spectrometric conditions a linear relationship was established. The calibration graphs were found to be rectilinear within the concentration range of 1.6-40.0 ng/mL for TAM and 20.0-500.0 ng/mL for FIN. The linearity of standard curves (r^2) for all analytes were greater than 0.99. The calibration curve had a reliable reproducibility across the calibration range. The corresponding regression equations are cited in Table 2, where the slopes are consistent with the relative peak areas.

3.1.2. Accuracy and precision

The accuracy of the proposed method was evaluated by analyzing six levels of standard solutions of the studied drugs, each three times. The results obtained by the proposed method were favorably compared with those of reference ones [26] for TAM and [5] for FIN.

Table 2. Performance data for tamsulosin and finasteride by the proposed LC-MS/MS method *.

Parameter	Tamsulosin	Finasteride
Linearity range	1.60 - 40.00 ng/mL	20.00 - 500.00 ng/mL
Regression equation	7.21×10^{-3} C - 1.82×10^{-3}	1.85×10^{-4} C - 6.56×10^{-3}
Slope (b)	7.21×10^{-3}	1.85×10^{-4}
Intercept (a)	1.82×10^{-3}	6.56×10^{-3}
Correlation coefficient (r)	0.9998	0.9997
R^2	0.9997	0.9995
SE of slope	6.45×10^{-5}	2.12×10^{-6}
SE of intercept	1.42×10^{-3}	5.84×10^{-4}
$S_{y/x}$	2.11×10^{-3}	8.67×10^{-4}
LOD	0.50 ng/mL	5.00 ng/mL
LOQ	1.60 ng/mL	20.00 ng/mL

* C: Concentration, $S_{y/x}$: Standard deviation of residuals, LOD: Limit of detection, LOQ: Limit of quantification.

Table 3. Accuracy and precision data obtained by the proposed LC-MS/MS method and the reference ones for the analysis of tamsulosin and finasteride in pure form [a].

Item	Tamsulosin		Finasteride	
	Proposed	Reported [26] [c]	Proposed	Reported [5] [d]
Mean [b] ± SD	100.11±0.78	99.54±0.88	100.01±0.61	99.86±0.81
% RSD	0.78	0.88	0.69	0.81
% REr	0.32	0.44	0.28	0.40
n	6	4	6	4
Variance	0.61	0.77	0.47	0.65
t- test (2.31)	1.08		0.32	
F- test (5.409)	1.26		1.37	
Intraday precision [b]	99.83±0.77		99.75±0.63	
Inter-day precision [b]	99.16±0.83		99.06±1.01	

[a] SD: Standard deviation, %RSD: Percent relative standard deviation, %REr: Percent relative standard error, Values in parenthesis are the theoretical values of t and F at $p = 0.05$ [28].
[b] Average of three different determinations.
[c] The reported method for tamsulosin [26] is an HPLC method and was performed using C18 Column 250 × 4.6 mm (particle size of 5 µm). Mobile phase: acetonitrile: (0.05 M) KH_2PO_4 buffer (45:55) at flow rate 1.8 mL/min. The detection was monitored at 240 nm.
[d] The official method for finasteride [5] is an HPLC method and was performed using , C18 Column. Mobile phase, water:tetrahydrofuran (4:1, *v:v*). The detection was monitored at 215 nm.

Table 4. Determination of tamsulosin and finasteride in laboratory prepared mixtures by the proposed LC-MS\MS method.

Concentration (ng/mL)		% Recovery *	
Tamsulosin	Finasteride	Tamsulosin	Finasteride
30.00	30.00	101.04	97.95
30.00	60.00	100.45	98.06
40.00	20.00	99.76	99.95
10.00	125.00	100.35	99.55
30.00	375.00	98.76	100.09
20.00	200.00	100.59	98.50
Mean ± SD		100.16±0.80	99.02±0.96
% RSD		0.80	0.97
% Rer		0.33	0.40
Variance		0.64	0.93

* Average of three different determinations.

Statistical analysis [28] obtained by the proposed and reported methods using student´s t-test and variance ratio F-test, showed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively, (Table 3).

In addition, the intraday precision was evaluated through replicate analysis of the standard solutions of the drugs. However, the interday precision was performed through replicate analysis of the standard solutions of the drugs on three successive days. The percentage recoveries as well as the percentage relative standard deviations were calculated as abridged in Table 3. The repeatability of the proposed method is good as indicated by small value of standard deviation (SD).

3.1.3. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to USP 34 recommendations, below which the calibration graph is nonlinear and was found to be 1.6 ng/mL for TAM and 20.0 ng/mL for FIN. The limit of detection (LOD) was determined by evaluating the lowest concentration of the analyte that can be readily detected and was found to be 0.5 ng/mL for TAM and 5.0 ng/mL for FIN.

3.1.4. Robustness of the method

The robustness of the method adopted was demonstrated by the consistency of the relative peak area values with the deliberately minor changes in the chromatographic and mass spectrometric parameters, e.g. the pH of the buffer (±0.5), the turbo ion spray temperature or capillary temperature (±5 °C), sheath gas flow (±5 psi) and collision energy (±5 V).

3.2. Application of the proposed method

The proposed method was successfully applied to the analysis of TAM and FIN in laboratory prepared mixtures containing both drugs in different ratios. The average percent recoveries were based on the average of three replicate determinations (Table 4).

To indicate the potential of LC-MS for quality control and routine analysis of TAM and FIN in pharmaceutical formulation; Urimax F tablets were analyzed.

Table 5. Application of standard addition technique for the determination of tamsulosin and finasteride in pharmaceutical formulation [a].

Item	Taken concentration (ng/mL)		Added concentration (ng/mL)		% Recovery [b]	
	Tamsulosin	Finasteride	Tamsulosin	Finasteride	Tamsulosin	Finasteride
	5.00	62.50	5.00	62.50	99.95	98.89
	5.00	62.50	10.00	125.00	99.99	98.54
	5.00	62.50	15.00	187.50	98.16	98.24
	5.00	62.50	20.00	250.00	99.00	97.98
	5.00	62.50	25.00	312.50	98.85	99.69
	5.00	62.50	30.00	375.00	98.05	97.00
Mean ± SD	98.76±0.78	98.12±0.89			99.00±0.84	98.39±0.90
% RSD	0.79	0.91			0.85	0.92
% Rer	0.32	0.37			0.35	0.37
Variance	0.61	0.79			0.70	0.82

[a] SD: Standard deviation, %RSD: Percent relative standard deviation, %REr: Percent relative standard error.
[b] Average of three different determinations.

The concentrations of the drugs were calculated referring to the corresponding regression equation. The coupling of LC with MS/MS detection in the SRM mode showed high specificity, because only the ions derived from the analytes of interest were monitored, thus, commonly used tablet excipients did not interfere in the analysis as indicated by the percentages found. The results obtained by applying the standard addition technique are abridged in Table 5.

4. Conclusion

A rapid and precise liquid chromatography with electrospray ionization tandem mass spectrometry method for the determination of tamsulosin and finasteride in bulk powders and in pharmaceutical formulation was developed and validated. The method offers several advantages such as unnecessary complete separation of analytes, non-tedious sample preparation. In addition, the short run time and the relatively low flow rate allows the analysis of a large number of samples with less mobile phase that proves to be cost effective. The data validation shows that the optimized LC-MS/MS possess specificity, sensitivity, linearity, precision and accuracy, thus, the proposed method can be used for the routine analysis of tamsulosin and finasteride in pharmaceutical dosage forms.

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