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Journal of Pharmaceutical and Biomedical Analysis 46 (2008) 94–103
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Development of a validated RP-LC/ESI-MS–MS method for separation, identification and determination of related substances of tamsulosin in bulk drugs and formulations

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Received 21 May 2007; received in revised form 5 September 2007; accepted 5 September 2007
Available online 14 September 2007

Abstract

A reversed-phase high performance liquid chromatographic (RP-HPLC) method for evaluation of purity of tamsulosin in bulk drugs and pharmaceuticals was developed. The separation was accomplished on an Inertsil C_{18} column using 10 mM ammonium acetate: acetonitrile as a mobile phase in a gradient elution mode. A photodiode array detector set at 280 nm was used for detection. The impurities were identified by ESI-MS–MS. The detection limits were 0.06–0.11 μg/ml. The method was validated with respect to accuracy, precision, linearity, ruggedness and limits of detection and quantification. It finds application not only for monitoring the reactions during the process development but also on quality assurance of tamsulosin.

Keywords: Tamsulosin; Benign prostatic hyperplasia (BPH); RP-HPLC; ESI-MS–MS; Process related impurities; Bulk drugs and formulations

1. Introduction

Benign prostatic hyperplasia (BPH) is a common condition in ageing men. It affects severely the quality of life (QOL) of not only the patient but also the partner through sleep disturbance, disruption of social life and psychological burden [1]. Its impact on QOL is worse when compared with other diseased conditions. TamsulosinHCl[(−)-(*R*)-5-[2-[[2-(*o*-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide] is a new type of highly selective α_1-adrenergic receptor antagonist approved by the Food and Drug Administration (FDA), USA for treatment of BPH. Compared to other α-antagonists, tamsulosin has greater specificity for α_1 receptors in the human prostate [2,3] and does not affect receptors on blood vessels [4,5]. It is the most frequently prescribed medication for the treatment of lower urinary tract symptoms suggestive of BPH [6,7]. Determination of its quality is important for the benefit of the patients who are ultimately treated for BPH.

A thorough literature search has revealed that only a few analytical methods are available for determination of tamsulosin in bulk drugs and pharmaceuticals [8,9]. Liquid chromatography–mass spectrometry [10–13], LC with fluorescence detection were used to determine tamsulosin in human plasma [14–16]. Many chromatographic methods were reported for determination of enantiomeric purity of tamsulosin in pharmaceutical preparations [17–21]. However, the separation and determination of process related impurities in the finished products of tamsulosin were not addressed. Further to the best of our knowledge, no method for determination of the impurities either in bulk drugs or pharmaceuticals has been reported. Thus, there is a need for development of analytical methods, which will be useful to monitor the levels of impurities in the finished products of tamsulosin during process development. In the present study, a reverse phase high performance liquid chromatographic (RP-HPLC) method for separation and determination of tamsulosin and its pro-

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doi:10.1016/j.jpba.2007.09.009

cess related impurities was developed and validated. The impurities in bulk drugs and formulations were identified by LC-MS^n.

2. Experimental

2.1. Materials and reagents

All the reagents were of analytical reagent grade unless stated otherwise. Glass-distilled and de-ionized water (Nanopure, Barnsted, USA), HPLC-grade acetonitrile and ammonium acetate (S.D. Fine Chem., Mumbai, India) were used.

2.2. Apparatus

The HPLC system composed of two LC-10AT VP pumps, an SPD-10Avp diode array detector an SIL-10AD VP auto injector, a DGU-12A degasser and SCL-10 A VP system controller (all from Shimadzu, Kyota, Japan) was used. The chromatographic and the integrated data were recorded using HP-Vectra (Hewlett Packed, Waldron, Germany) computer system. The chromatographic columns used were:

1. Hypersil BDS C_{18} (250 mm × 4.6 mm) 5 μm (Thermo Electron Corporation, Runcorn, UK).
2. Inertsil ODS 3v (250 mm × 4.6 mm) 5 μm (G.L. Sciences, Tokyo, Japan).
3. XTerra C18 (250 mm × 4.6 mm) 5 μm (Waters, Milford, MA, USA).
4. Kromasil KR100-5C_{18} (250 mm × 4.6 mm) 5 μm (Eka Chemicals, Bohus, Sweden).
5. Symmetry C18 (250 mm × 4.6 mm) 5 μm (Waters, Milford, MA, USA).

2.3. Chromatographic conditions

The mobile phase was 10 mM ammonium acetate–acetonitrile in a gradient elution mode of a flow rate of 1.0 ml/min at 30 °C (Table 1). Before delivering into the system the solvent was filtered through 0.45 μm, PTFE filter and degassed using vacuum. The injection volume was 20 μl. Chromatograms were recorded at 280 nm using a SPD-M 10 Avp diode array detector.

Table 1
Optimum conditions for gradient elution of solvents A and B

Time (min)	Flow (ml/min)	Solvent A (%)	Solvent B (%)	Curve
0	1.0	95	5	Linear
10	1.0	80	20	Linear
25	1.0	40	60	Linear
30	1.0	20	80	Linear
35	1.0	0	100	Linear
36	1.0	95	5	Isocratic
45	1.0	95	5	

Solvent A: 10 mM ammonium acetate; solvent B: acetonitrile.

2.4. ESI-MS–MS conditions

The MS^n experiments were performed using a LCQ ion trap mass spectrometer (Thermo Finnigan, San Jose, CA), equipped with an ESI source. The data acquisition was under the control of xcalibur software. The typical source conditions were: spray voltage, 5 kV; capillary voltage, 15–20 V; heated capillary temperature, 200 °C; tube lens offset voltage, 20 V; sheath gas (N_2) flow rate, 30 units; helium was used as damping gas. For the ion trap mass analyzer, the automatic gain control (AGC) settings were 2×10^7 counts for a full-scan mass spectrum and 2×10^7 counts for a full product ion mass spectrum with a maximum ion injection time of 200 ms. The collision energies were used at 20–40%. The excitation time used was 30 ms. All the spectra were recorded under identical experimental conditions with an average of 25–30 scans.

2.5. Analytical procedures

Solutions (1000 μg/ml) of tamsulosin and its process impurities were prepared by dissolving known amounts of the components in methanol. The solutions were adequately diluted with the mobile phase to study accuracy, precision, linearity, limit of detection and limit of quantification. The specification concentration of tamsulosin was taken as 500 μg/ml.

3. Results and discussion

The chemical structures of tamsulosin(V) and its process related impurities (I, III, IV, VI, VII–IX) including the starting material (II) are shown in Fig. 1. The starting materials and intermediates could be present as potential impurities in the finished products of tamsulosin. The present study was aimed at developing a chromatographic system capable of separation and quantitative determination of tamsulosin and its impurities.

3.1. Method development

All the impurities and V were subjected to separation on different commercial C_{18} columns. Initially, buffers such as 0.01 M KH_2PO_4 (pH adjusted to 3.0 with H_3PO_4) and 0.01 M CH_3COONH_4 (pH adjusted to 4.0 with CH_3COOH) with organic modifiers, viz; methanol and acetonitrile were tried. Since the compounds I and III were close in structure with similar polarities so their separation became critical. Different compositions of 0.01 M CH_3COONH_4 and methanol were tried without adjusting the pH. However, the separation of compounds II and III was poor and peaks exhibited tailing on all the columns. Later, acetonitrile was tried instead of methanol. The peak shapes and resolutions were improved. To minimize the tailing and to improve peak shapes, acetonitrile was chosen as an organic modifier with ammonium acetate buffer. A gradient elution mode was selected since the impurities possessed varying polarities.

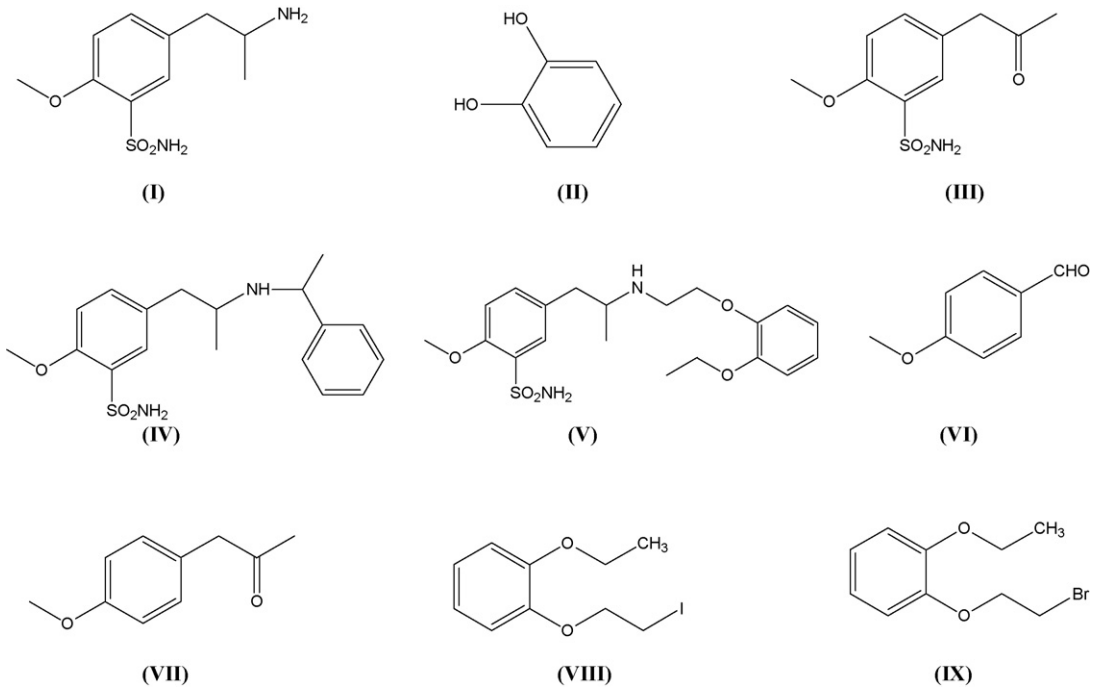

Fig. 1. Chemical structures of tamsulosin(V) and its process impurities.

3.2. Optimization of the chromatographic conditions

The HPLC conditions were optimized by studying the effect of concentration of acetate buffer, column selectivity, buffer pH and column temperature on retention of analytes.

3.2.1. Column selectivity

Five different C_{18} columns as described in Section 2.2 were tried. The selectivity and resolution of all compounds on the columns is described in Table 2. It could be seen from Table 2 that on Inertsil C_{18}, all compounds were well separated and the tailing was minimum. So it was chosen for further development.

3.2.2. Effect of organic modifier

The separation of impurities I–III became critical as they eluted very close to each other. With methanol, broad peaks were observed and separation was poor. When acetonitrile was used as an organic modifier, peaks became sharp and resolution was improved for I–III ($Rs > 2.0$). The initial concentration of organic modifier was kept at 5% in the gradient elution mode.

3.2.3. Effect of buffer concentration

The effect of concentration of ammonium acetate on separation was studied by varying its concentration from 10 to 50 mM on Inertsil C_{18} column maintained at 30 °C. The concentration of ammonium acetate had no effect on the retention of the test compounds, and there was not much effect on resolution (Fig. 2a) Tailing was increased (Fig. 2b) with an increase in concentration of ammonium acetate. So with 10 mM of ammonium acetate, sharp symmetrical peaks with good resolution were obtained. The desired symmetry and resolution were obtained with 10 mM of ammonium acetate, it was used for further optimization of other variables.

3.2.4. Effect of pH

The pH had no effect on retention of compounds VIII and IX. While for I–VII, the retentions were increased as the pH of the buffer increased from 4.0 to 6.5. At pH 4.0 compound IV eluted very close to III (Fig. 3a). With increase in pH,

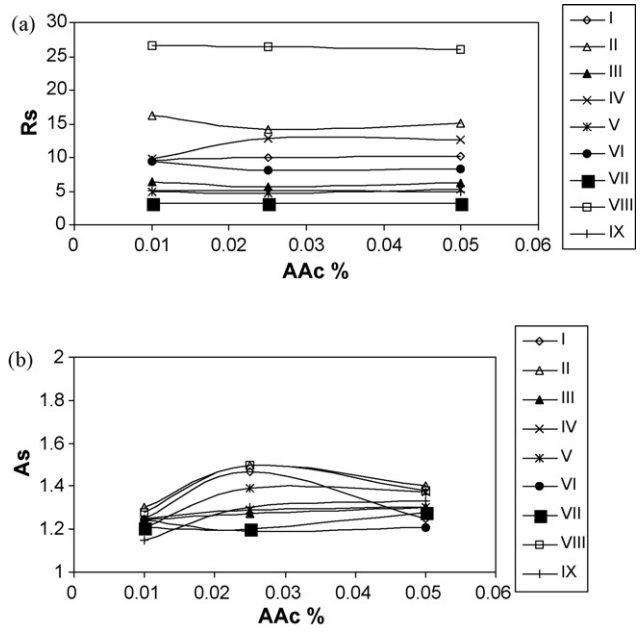

Fig. 2. Effect of concentration of ammonium acetate (AAc) on (a) resolutions (Rs) and (b) tailing factors (As).

Table 2
Selectivity of different C_{18} columns

	Compound								
	I	II	III	IV	V	VI	VII	VIII	IX
Hypersil BDS (250 mm × 4.6 mm) 5 μm									
k'	2.19	3.68	4.68	7.07	7.49	7.75	7.95	10.97	11.75
α	1.680	1.271	1.510	1.059	1.035	1.025	1.379	1.071	–
Rs	–	10.81	7.38	24.94	4.38	2.31	1.91	31.15	7.08
As	1.53	1.65	1.22	1.33	1.19	1.37	1.25	1.17	1.16
Inertsil ODS (250 mm × 4.6 mm) 5 μm									
k'	2.85	5.53	6.48	7.74	8.30	9.53	9.88	13.45	14.46
α	1.940	1.171	1.194	1.072	1.148	1.036	1.361	1.075	–
Rs	–	19.52	7.57	10.94	4.37	10.53	3.42	30.92	7.65
As	1.23	1.35	1.06	1.34	1.05	1.11	1.06	1.10	1.01
Kromasil C_{18} (250 mm × 4.6 mm) 5 μm									
k'	2.93	5.50	6.47	8.25	8.98	9.79	10.24	14.12	15.22
α	1.877	1.176	1.275	1.088	1.090	1.045	1.378	1.077	–
Rs	–	14.55	5.52	13.67	5.74	5.67	3.23	25.46	6.36
As	1.64	1.96	1.53	1.34	1.51	1.47	1.48	1.13	1.31
XTerra C_{18} (250 mm × 4.6 mm) 5 μm									
k'	1.46	3.12	3.98	6.55	7.20	7.33	7.53	10.53	11.14
α	2.136	1.275	1.645	1.099	1.018	1.027	1.398	1.057	–
Rs	–	12.24	6.86	27.03	7.20	1.47	2.14	33.81	6.82
As	1.04	2.51	1.01	1.09	1.01	1.34	1.09	1.03	1.04
Symmetry C_{18} (250 mm × 4.6 mm) 5 μm									
k'	2.40	4.94	5.98	7.88	8.72	9.21	9.69	13.34	14.36
α	2.058	1.210	1.317	1.106	1.056	1.052	1.376	1.076	–
Rs	–	18.45	9.26	20.26	7.46	4.50	5.54	38.10	9.06
As	1.60	2.60	1.00	1.82	2.18	1.20	0.99	1.00	1.08

k': retention factor; α: selectivity; Rs: resolution; As: tailing factor.

the resolutions were increased (Fig. 3b) for I, IV, V, VII, IX and tailing was reduced (Table 3) for most of the compounds. At pH 6.5, symmetrical peaks with good resolutions were obtained.

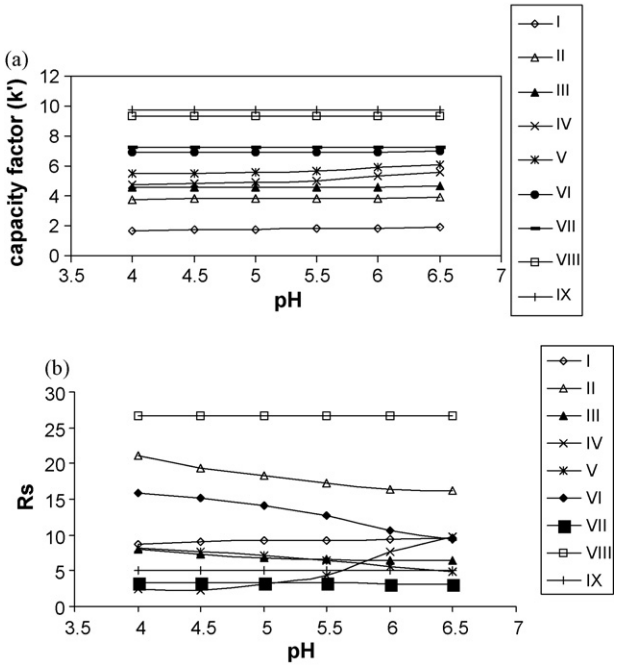

Fig. 3. Effect of pH on (a) retention (t_R) and (b) resolution (Rs).

3.2.5. Effect of temperature

The column was maintained at different temperatures ranging from 25 to 45 °C in a thermostated oven. Retentions were decreased slightly with increasing temperature and resolutions were decreased for compounds (I–III, V and VII) and slightly increased for (IV, VI, VIII and IX) (Fig. 4a). Tailing was minimum at 30 °C for almost all the compounds (Fig. 4b).

Finally the separation was carried out on Inertsil C_{18} column maintained at 30 °C in a gradient elution (Table 1) of 10 mM ammonium acetate (pH 6.5) as a buffer and acetonitrile as an organic modifier with PDA detector set at 280 nm. A typical chromatogram showing the separation of 10% (w/w) of each of the related substances spiked to V at the specified relative concentration of 500 μg/ml is shown in Fig. 5a. It is clear from Fig. 5a that all the compounds were eluted and separated with

Table 3
Effect of pH on peak tailing

pH	Tailing factors (As)								
	I	II	III	IV	V	VI	VII	VIII	IX
6.5	1.23	1.35	1.06	1.34	1.20	1.11	1.06	1.10	1.01
6.0	1.47	1.46	1.21	1.58	1.67	1.26	1.27	1.50	1.30
5.5	1.35	1.71	1.29	1.52	1.74	1.27	1.29	1.33	1.18
5.0	1.27	1.79	1.25	1.63	1.63	1.25	1.23	1.27	1.16
4.5	1.15	1.51	1.24	1.65	1.53	1.24	1.21	1.24	1.33
4.0	1.22	1.24	1.18	1.35	1.37	1.17	1.22	1.46	1.43

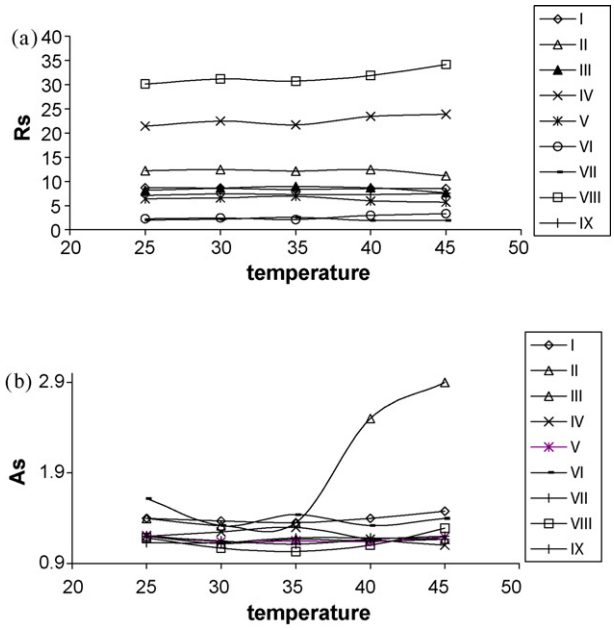

Fig. 4. Effect of temperature on (a) resolutions (Rs) and (b) tailing factors (As).

good peak shapes and resolution. The developed method was validated in terms of accuracy, precision and linearity as per ICH guidelines.

4. Validation

4.1. Specificity

Specificity is the ability of the method to measure the analyte response in presence of all potential impurities. For specificity determination, the known impurities were added to V and the detector response to each analyte in the mixture was compared with that of V. The assay of V for three determinations was 99.91% with 0.025% R.S.D. In presence of impurities (0.1%, w/w) it was found to be 99.86% with 0.04% R.S.D. These results suggest that the assay did not change in presence of the impurities. The specificity was checked by stressing tamsulosin under UV light at 254 nm, 60 °C temperature for 24 h and extreme conditions such as 0.1N HCl, 0.1N NaOH and 3% H_2O_2. Under thermal, acid, alkaline conditions and in the presence of peroxide, no change in the assay was observed, but in UV light, degraded products were formed and well separated from tamsulosin under the present conditions. It could be seen from Fig. 5b that the peaks corresponding to excipients in formulations did not interfere with the peaks of interest. Thus, the method was found to be applicable for quantitative determination of tamsulosin not only in bulk drugs but also pharmaceutical formulations.

4.2. System suitability

The system suitability was checked by making five replicate injections of V (500 μg/ml) spiked with 0.1% (w/w) of all the impurities. The system was deemed to be suitable for use if the tailing factors and resolutions for V and its impurities were not >1.20 and 2, respectively. Synthetic mixtures and samples collected during process development were analyzed under identical conditions. The quantities of impurities and assay of V were determined from the respective peak areas. The data is recorded in Table 4.

4.3. Accuracy

The recoveries of I–IV and VI–IX were determined by spiking each impurity at five different levels ranging from 0.5 to

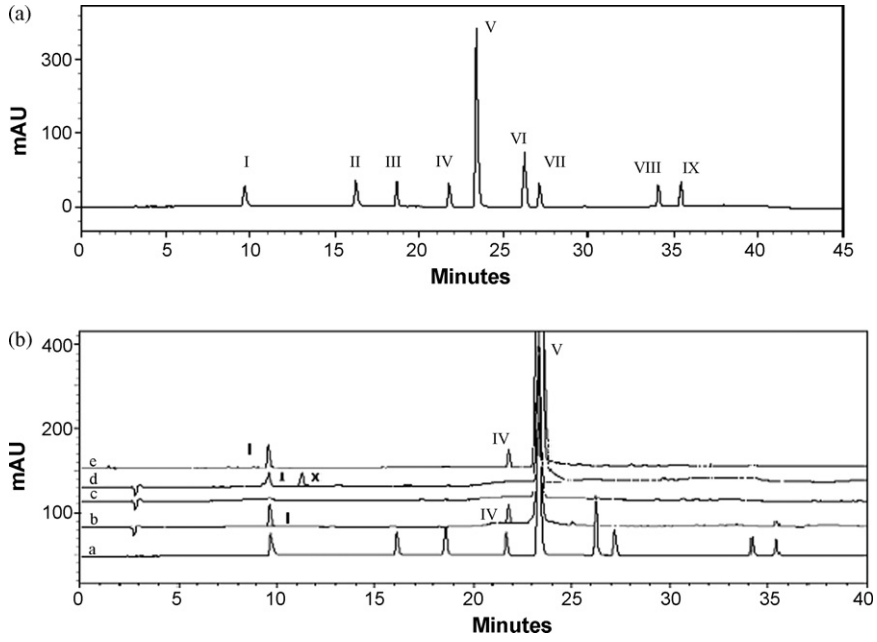

Fig. 5. (a) Typical chromatograms of tamsulosin(V) spiked with impurities and (b) different batches of bulk drugs and formulations.

Table 4
System suitability data

Sample	t_R (±S.D.)[a] (min)	RRT	Rs	As	RRF
I	9.68 ± 0.05	0.415	–	1.23	1.091
II	16.20 ± 0.03	0.694	16.23	1.35	1.023
III	18.59 ± 0.02	0.797	6.38	1.06	1.181
IV	21.69 ± 0.06	0.930	9.84	1.34	0.891
V	23.33 ± 0.09	1.00	4.87	1.20	1.000
VI	26.26 ± 0.01	1.25	9.49	1.11	1.251
VII	27.17 ± 0.09	1.164	3.19	1.06	1.117
VIII	34.18 ± 0.07	1.465	26.69	1.10	0.913
IX	35.44 ± 0.05	1.519	5.14	1.01	0.935

t_R: retention time; RRT: relative retention time; Rs: resolution; As: tailing factor; RRF: relative response factor.
[a] Average of five determinations.

5.0 μg/ml to V at the specified level (500 μg/ml). The recovery range and R.S.D. for all impurities were found to be 95.10–103.36% and 0.36–3.92%, respectively (Table 5). Similarly, the accuracy in the assay of V was checked at five concentration levels, i.e. 100, 200, 300, 400, 500 μg/ml each in triplicate for 3 days and the percentage recoveries are recorded in Table 5. The R.S.D. values were below 1.36%.

4.4. Precision

The precision of the method was tested by six ($n = 6$) injections of tamsulosin(V) spiked with 0.1% (w/w) of each impurity and the R.S.D. of retention time (t_R) and peak areas were determined. The R.S.D. ranged from 0.31 to 0.89%. The precision in determination of the assay was studied by repeatability, intermediate precision and reproducibility (ruggedness). Repeatability is the intra-day variation in assay obtained at different concentration levels of V expressed in terms of R.S.D. calculated for each day. The R.S.D. values were found to be below 1.0%, indicating a good repeatability (Table 6). The intermediate precision is the inter-day variation at the same concentration level determined on successive days. The inter-day variations calculated for three concentration levels from the above data of 3 days are expressed in terms of %R.S.D. values. At each concentration level, the %R.S.D. values were below 1.0%, indicating a good intermediate precision. The ruggedness of the method is defined as the degree of reproducibility obtained by analysis of the same sample under a variety of conditions at different labs, with different analysts, instruments and lots of reagents. The same samples of three concentrations were analyzed in triplicate on 2 days by another instrument (LC-20A Module HPLC, Shimadzu system containing two pumps and PDA detector) by a different analyst with different lots of reagents and columns. The data obtained were within 2% R.S.D. The precision of the method for determining the impurities was calculated at five concentration levels (0.5, 0.7, 1.0, 1.5 and 2 μg/ml) for 5 days. The intra- and inter-day precisions were in the range of 0.1–2.05% and 0.75–3.96% (%R.S.D.), respectively (Table 7).

Table 6
Inter- and intra-day assay of tamsulosin

Intra-day			
Day 0			
Mean of concentration (mg/ml, $n = 3$)	0.0997	0.2011	0.3002
S.D.	0.0003	0.0010	0.0007
R.S.D. (%)	0.30	0.51	0.23
Day 1			
Mean of concentration (mg/ml, $n = 3$)	0.0995	0.2006	0.2998
S.D.	0.0002	0.0012	0.0012
R.S.D. (%)	0.21	0.60	0.42
Day 2			
Mean of concentration (mg/ml, $n = 3$)	0.0997	0.2000	0.2994
S.D.	0.0003	0.0010	0.0006
R.S.D. (%)	0.26	0.5	0.21
Inter-day			
Mean of concentration (mg/ml, $n = 3$)	0.0994	0.2004	0.3004
S.D.	0.0003	0.0013	0.0022
R.S.D. (%)	0.31	0.66	0.74

4.5. Linearity

The linearity of detector response to different concentrations of impurities was studied in the range from 0.5 to 5.0 μg/ml at five different levels. Similarly, the linearity of V was also studied by preparing standard solutions at 10 different levels ranging from 50 to 500 μg/ml. The data were subjected to sta-

Table 5
Recovery data

Sample	Recovery[a] R.S.D. (%)				
Amount added (μg/ml)	0.5	0.7	1.0	1.5	2.0
I	100.5 ± 0.95	98.92 ± 1.66	98.08 ± 1.23	97.59 ± 1.79	98.12 ± 2.15
II	97.25 ± 1.83	98.52 ± 1.05	99.81 ± 0.62	98.60 ± 1.25	98.20 ± 0.55
III	97.96 ± 0.73	101.11 ± 0.73	95.70 ± 0.50	99.44 ± 0.79	97.85 ± 1.05
IV	100.5 ± 0.95	99.40 ± 0.61	98.08 ± 1.23	100.10 ± 0.45	95.10 ± 0.47
VI	98.65 ± 1.30	97.62 ± 2.08	97.62 ± 0.29	103.36 ± 1.15	101.02 ± 0.62
VII	99.48 ± 3.92	100.39 ± 0.73	102.54 ± 2.35	98.80 ± 1.12	99.54 ± 0.36
VIII	98.12 ± 2.15	97.93 ± 0.63	96.95 ± 0.63	99.07 ± 1.03	96.15 ± 0.89
IX	98.06 ± 2.25	97.94 ± 1.68	99.08 ± 0.63	100.40 ± 3.55	99.07 ± 1.03
Amount added (μg/ml)	100	200	300	400	500
V	98.92 ± 1.15	98.65 ± 0.58	98.78 ± 1.36	99.29 ± 0.84	98.92 ± 0.53

[a] Average of three determinations.

Table 7
Inter- and intra-day variations

Sample	Day	Concentration[a] (μg/ml)	R.S.D. (%)	Concentration[a] (μg/ml)	R.S.D. (%)	Concentration[a] (μg/ml)	R.S.D. (%)	Concentration[a] (μg/ml)	R.S.D. (%)	Concentration[a] (μg/ml)	R.S.D. (%)
Intra-day											
I	0	0.51	0.62	0.71	1.40	1.03	1.47	1.51	0.38	2.11	0.54
	1	0.52	1.27	0.71	0.80	1.02	0.98	1.52	0.65	2.12	0.27
	2	0.53	0.75	0.73	1.39	1.04	1.10	1.53	0.37	2.13	0.83
II	0	0.52	0.57	0.72	0.27	1.13	0.88	1.53	1.30	2.23	1.36
	1	0.53	0.65	0.73	0.75	1.14	1.33	1.51	0.76	2.22	0.68
	2	0.51	0.59	0.71	0.45	1.11	1.36	1.51	0.17	2.15	0.46
III	0	0.53	0.18	0.72	0.13	1.12	0.89	1.50	0.36	2.12	1.18
	1	0.51	0.40	0.71	0.42	1.11	0.90	1.51	0.59	2.13	0.46
	2	0.52	1.14	0.73	0.14	1.13	1.83	1.52	0.19	2.03	1.02
IV	0	0.51	0.38	0.71	0.49	1.13	0.88	1.51	0.06	2.02	1.02
	1	0.52	0.50	0.72	0.77	1.12	1.02	1.52	0.65	2.03	1.23
	2	0.53	0.10	0.70	0.56	1.02	0.98	1.53	0.39	2.12	1.18
V	0	0.52	0.58	0.72	0.34	1.12	0.89	1.51	0.66	2.04	1.29
	1	0.51	0.29	0.71	0.42	1.11	0.52	1.52	0.37	2.12	1.14
	2	0.50	0.61	0.70	0.96	1.03	0.97	1.52	0.06	2.04	1.29
VI	0	0.51	0.49	0.71	0.21	1.02	0.98	1.51	0.06	2.06	0.74
	1	0.52	0.29	0.72	0.73	1.11	1.36	1.50	0.21	2.11	0.47
	2	0.50	0.79	0.70	0.96	1.12	0.51	1.52	0.62	2.22	0.68
VII	0	0.51	0.40	0.70	0.28	1.05	0.95	1.51	0.20	2.04	0.74
	1	0.50	0.23	0.71	0.08	1.11	1.37	1.50	0.51	2.10	0.27
	2	0.52	0.11	0.72	0.65	1.22	2.05	1.52	0.26	2.22	0.68
VIII	0	0.51	1.86	0.70	1.50	1.02	2.02	1.52	0.38	2.03	1.13
	1	0.52	1.82	0.71	0.89	1.12	1.36	1.53	0.57	2.13	0.94
	2	0.53	1.03	0.72	0.70	1.21	1.25	1.50	0.25	2.19	0.52
IX	0	0.51	0.58	0.71	0.37	1.13	0.88	1.51	0.66	2.02	0.49
	1	0.50	1.08	0.71	1.10	1.12	1.78	1.52	0.65	2.11	0.27
	2	0.52	0.69	0.72	0.69	1.11	1.37	1.50	0.76	2.19	1.31
Inter-day[b]											
I		0.52	1.96	0.72	3.50	1.14	3.06	1.49	2.53	2.15	2.32
II		0.53	0.92	0.73	3.15	1.18	2.13	1.53	1.73	2.08	3.66
III		0.53	3.27	0.72	1.32	1.14	3.06	1.50	1.01	2.15	2.32
IV		0.51	2.90	0.72	3.54	1.13	3.33	1.52	2.06	2.19	1.84
V		0.53	1.82	0.72	3.11	1.13	1.02	1.50	3.65	2.19	1.58
VI		0.52	2.75	0.73	2.15	1.18	2.54	1.50	3.71	2.22	3.62
VII		0.52	3.00	0.72	1.37	1.10	3.96	1.53	1.38	2.20	2.09
VIII		0.52	2.55	0.72	0.99	1.23	2.04	1.53	0.75	2.16	2.89
IX		0.52	2.42	0.73	1.10	1.22	1.24	1.51	1.22	2.23	3.58

[a] Mean of five determinations.
[b] Mean of 5 days.

Table 8
Linearity data

Sample	Range[a] (μg/ml)	Regression equation	r^2	LOD (μg/ml)	LOQ (μg/ml)
I	0.5–5.0	$y = 19{,}913x + 365.26$	0.9998	0.09	0.30
II	0.5–5.0	$y = 8370.5x + 244.73$	0.9997	0.10	0.35
III	0.5–5.0	$y = 23{,}326x - 840.96$	0.9995	0.09	0.29
IV	0.5–5.0	$y = 18{,}028x - 202.19$	0.9994	0.10	0.35
V	50–500	$y = 11{,}481x - 32{,}353$	0.9999	0.09	0.30
VI	0.5–5.0	$y = 17{,}0510x - 765.6$	0.9997	0.06	0.21
VII	0.5–5.0	$y = 9602.6x + 80.205$	0.9997	0.11	0.37
VIII	0.5–5.0	$y = 11{,}434x - 92.562$	0.9989	0.10	0.36
IX	0.5–5.0	$y = 16{,}482x - 71.945$	0.9999	0.11	0.39

[a] Ten calibration levels for V and five levels for I–IV and VI–IX were used.

Table 9
Results of analysis of impurities in bulk drugs and formulations

Sample	(%) Impurities (w/w) ± S.D.[a] (w/w)							
	I	II	III	IV	VI	VII	VIII	IX
Bulk drug-1	0.02 ± 0.001	–	–	0.02 ± 0.0011	–	–	–	0.04 ± 0.0021
Bulk drug-2	0.01 ± 0.001	–	–	0.04 ± 0.0012	–	–	0.01 ± 0.015	–
Bulk drug-3	0.03 ± 0.0012	–	–	0.01 ± 0.0025	–	–	–	–
Formulation-1	0.01 ± 0.0025	–	–	0.03 ± 0.0015	–	–	–	
Formulation-2	–	–	–	0.02 ± 0.0021	–	–	–	0.04 ± 0.001
Formulation-3	0.04 ± 0.0024	–	–	0.01 ± 0.001	–	–	–	–

[a] Average of three determinations.

tistical analysis using a linear-regression model. The regression equations and coefficients (r^2) are given in Table 8. According to ICH guidelines [22,23] the related substances and impurities above 0.1% in drug products should be quantified. The proposed linearity range was useful to quantify impurities at the level of 0.1% and above present in tamsulosin. Thus, the proposed method meets the requirement of ICH guidelines and the results indicated a good linearity.

4.6. Limits of detection and quantification

Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background by injecting a blank and calculating the signal-to-noise ratio for each compound by injecting a series of solutions until the S/N ratio 3 for LOD and 10 for LOQ. The results are given in Table 8. It could be seen from Table 8 that the lowest limits of quantitation (LLOQ) were in the range of 0.21–0.39 μg/ml much below the working range (0.5–5 μg/ml) indicating the suitability of the developed method for quantification of impurities at levels of 0.1% as per ICH guidelines.

4.7. Analysis of bulk drugs and formulations

High–low chromatographic techniques were employed for detecting trace level impurities in bulk drugs and capsule/tablet formulations of tamsulosin. Ten capsules/tablets were accurately weighed (equivalent to 2.5 g), ground to powder and an equivalent of 4 mg of active ingredient dissolved in methanol was taken into a 25 ml volumetric flask, ultra sonicated for about 10 min and made up to the mark with the mobile phase. The supernant liquid was collected. For determining the impurities, the same solution was used. The results are recorded in Table 9. The peaks were identified by injecting and comparing with the retention times of the individual compounds, absorption spectra of PDA detector and LC–ESI-MS^n fragmentation. Fig. 6 shows the UV spectra of tamsulosin and its impurities recorded using the PDA detector. The concentration of impurities relative to tamsulosin were in the range of 0.01–0.04 % (Table 9). The assay of tamsulosin was carried out by diluting the above solutions to 100 μg/ml with the mobile phase. The results are given in Table 10.

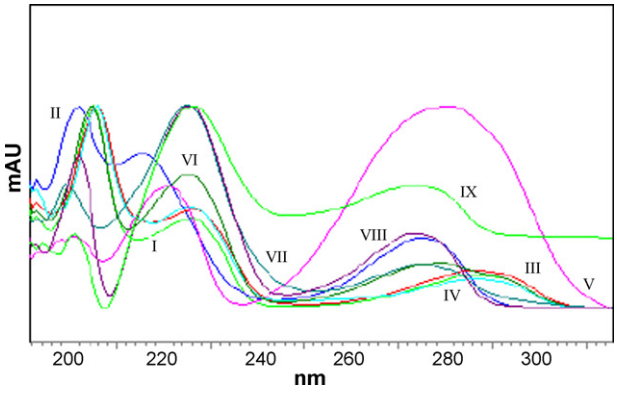

Fig. 6. Overlay UV spectra of tamsulosin(V) and its process impurities.

Different batches of tamsulosin(V) were analyzed and the results are recorded in Table 9. Three impurities having more than 0.1% area at retention times of 9.68, 11.2 and 21.69 min were detected. In order to characterize these impurities ESI-MS^n was used. The MS analysis carried out in positive ion mode using electro spray ionization technique. The impurity at 9.68 min had perfectly matched with the retention time and fragmentation pattern of (I) with *m/z* 245 (100%) and daughter ions *m/z* 228 and 200. This had supported the impurity as I. Later the impurity at 11.1 min did not match with any of the process intermediates studied in present investigation. It showed *m/z* 349 with stable daughter ions at 228 and 200. It was identified as compound (X). Another impurity at 21.69 perfectly matched with fragmentation pattern of (IV), which showed *m/z* 349 with daughter ions at 245, 228 and 200. In positive ion mode tamsulosin had

Table 10
Results of analysis of tamsulosin in bulk drugs and formulations

Sample	Concentration of tamsulosin (mg/ml)			
	Theoretical value	Found value[a]	Recovery (%)	R.S.D. (%)
Bulk drug-1	0.050	0.0501	100.2	0.52
Bulk drug-2	0.200	0.2018	100.9	0.81
Bulk drug-3	0.500	0.4980	99.6	0.34
Formulation-1	0.080	0.0802	100.25	0.33
Formulation-2	0.120	0.1205	100.44	0.42
Formulation-3	0.150	0.1497	99.82	0.10

[a] Average of three determinations.

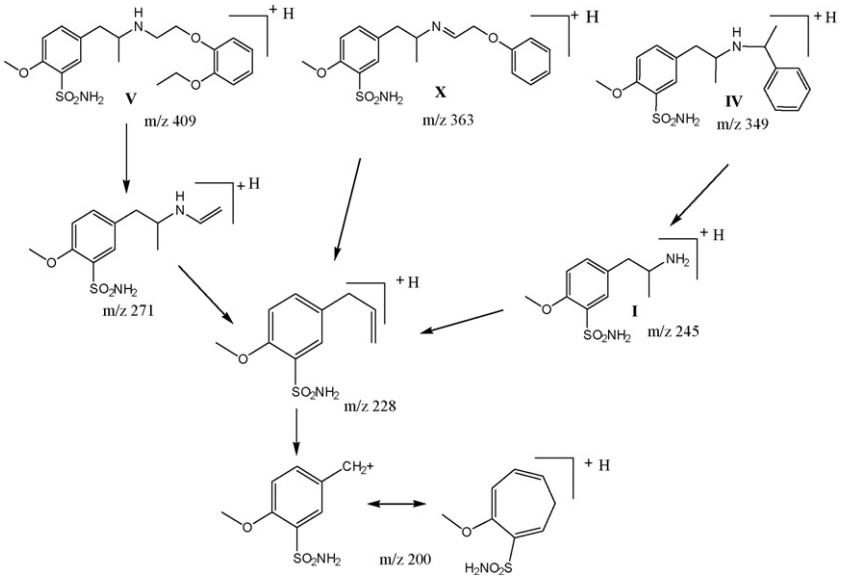

Fig. 7. Mass spectral fragmentation pattern of tamsulosin(V) and its impurities I, IV and X.

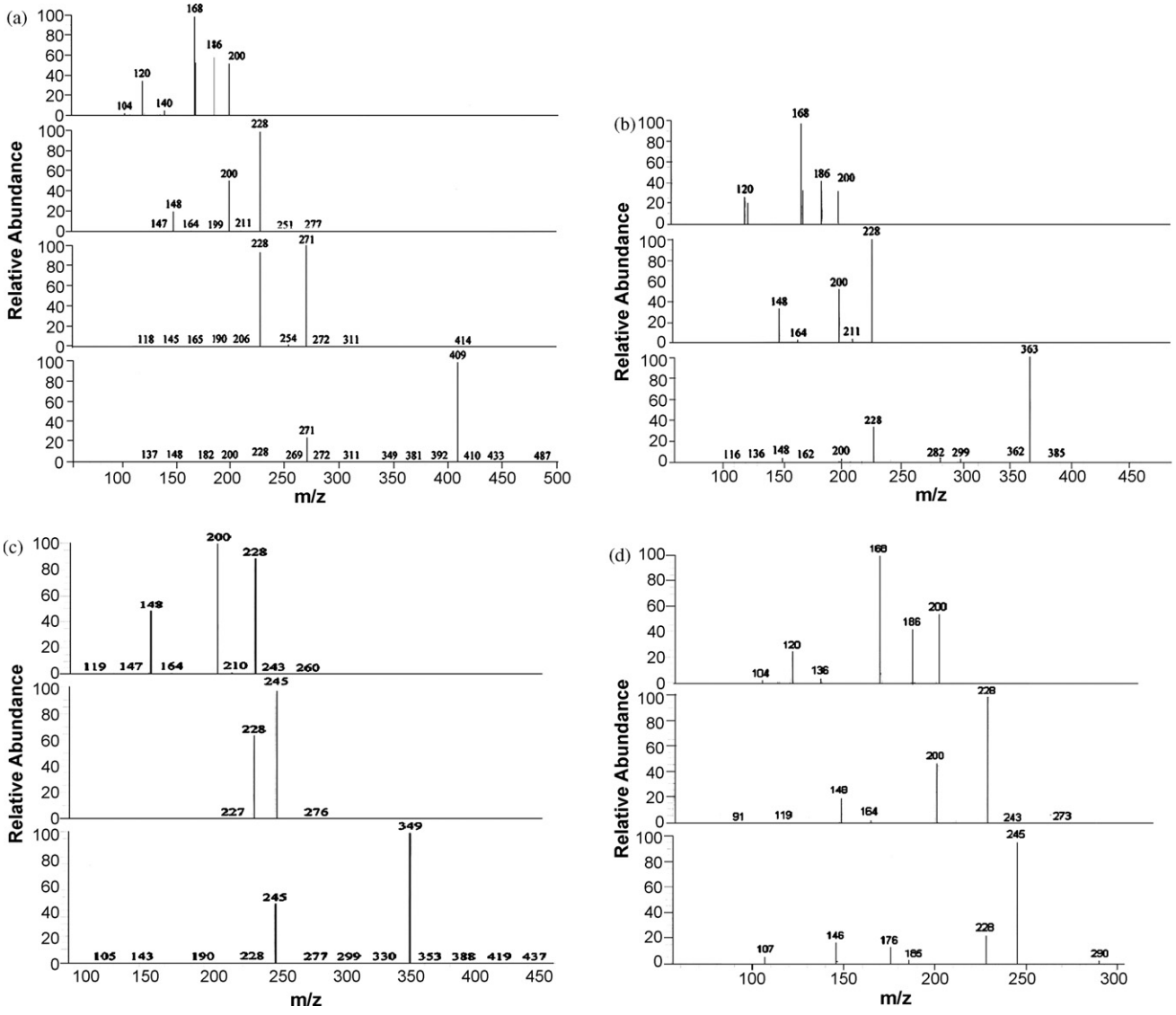

Fig. 8. ESI-MS–MS spectra of (a) tamsulosin, (b) impurity, X (c) impurity IV and (d) impurity I.

shown a protonated molecular ion at *m/z* 409. Its further ESI-MS^n fragmentation showed daughter ions 271, 228 and 200. The chemical structures and ESI-MS^n fragmentation and spectra of tamsulosin and its major impurities are shown in Figs. 7 and 8, respectively.

5. Conclusions

A simple and rapid gradient RP-HPLC method was developed and validated for determining the process related substances of tamsulosin in bulk drugs and pharmaceuticals. Attempts were made to separate tamsulosin from its process related impurities on different commercial C_{18} columns. The chromatographic conditions were optimized by studying the effects of temperature of the column and concentration and pH of ammonium acetate buffer. The developed method was found to be selective, sensitive, precise, linear, accurate and reproducible in determining the tamsulosin and its potential impurities, which may be present at trace level in the finished products. The method was found suitable for identification of major impurities of tamsulosin by ESI-MS^n. Thus, the method could be of use for process development as well as quality assurance of tamsulosin in bulk drugs as well as pharmaceutical formulations.

Acknowledgements

The authors wish to thank Dr. J.S. Yadav, Director and Dr. M. Vairamani, Head of the Analytical Chemistry Division, Indian Institute of Chemical Technology for encouragement and permission to communicate the manuscript for publication.

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