



DEVELOPMENT AND VALIDATION OF RP - HPLC METHOD FOR THE DETERMINATION OF TAMSULOSIN HYDROCHLORIDE

**MANDAVA V. BASAVESWARA RAO^{*}, B. C. K. REDDY,
M. SUBBA RAO^a and B. SREEDHAR^b**

Department of Chemistry, G.I.T.A.M University, VISAKHAPATNAM - 530 045 (A.P.) INDIA

^a Inorganic and Physical Chemistry Division, Indian Institute of Chemical Technology,
HYDERABAD – 500 607 (A.P.) INDIA

^b Department of Chemistry, A.N.U. P.G. Centre, NUZIVEEDU, Krishna D.T. (A.P.) INDIA

ABSTRACT

A simple and precise isocratic reverse phase high performance liquid chromatographic method has been developed for the determination of tamsulosin hydrochloride pellets 0.2%. Good resolution was achieved with inertsil ODS 3V (5 microns, 15 x 4.6 mm) column, in an isocratic mode, using acetonitrile:buffer (30 : 70) as mobile phase. The flow rate is 2 mL/minute and elution was monitored at 220 nm. The factors involved in the method development are discussed. This method was validated as per International Conference on Harmonization (ICH) guidelines. The method is linear in the range 75 to 150 ppm. pH was adjusted to 3.0 with perchloric acid.

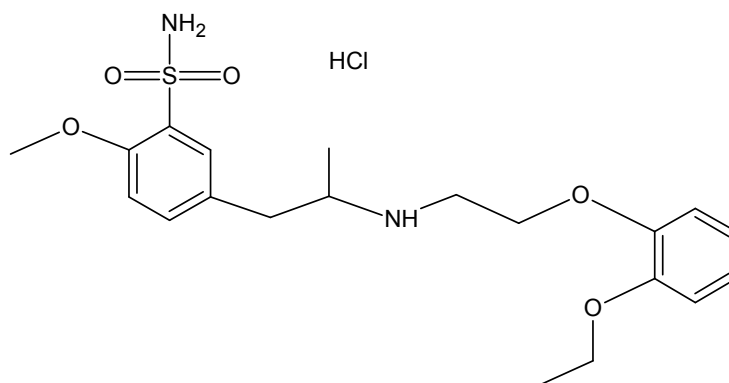
Key words: Development, Validation, Tamsulosin hydrochloride, Perchloric acid, Acetonitrile, Propyl Parahydroxybenzoate.

INTRODUCTION

Regulatory issues related to the presence of impurities have arisen with a greater frequency due to enhanced technological capability in identifying impurities and focus on their potential impact on human health¹. As per the guidelines from the European Medicines Agency on the limits of toxic impurities, threshold of toxicological concern (TTC) value of 1.5 µg/day intake of toxic impurity is considered to be associated with an acceptable risk for the most of the pharmaceuticals. The concentration limit of the permitted toxic impurity in ppm is the ratio of TTC in microgram per day and dose in gram per day².

^{*} Author for correspondence; E-mail : vbrmandava@yahoo.com

Tamsulosin hydrochloride is used to treat the signs and symptoms of benign enlargement of the prostate. Chemically, tamsulosin hydrochloride^{5,6} is known as 5-[(2R)-2-[[2 - (2 - ethoxy phenoxy)ethyl] amino] propyl } - 2 - methoxybenzenesulfonamide hydrochloride [molecular formula C₂₀H₂₈N₂O₅.HCl, Molecular Weight 444.97]. The methodology followed by us is not reported in any of the pharmacopeia. A survey of literature reveals that HPLC methods⁷⁻⁹ are reported for the determination of tamsulosin hydrochloride in bio Pharmacokinetics of the α -1A-adrenoreceptor antagonist, Chiral separation of tamsulosin by capillary electrophoresis and comparison of vascular α -Adrenoreceptor antagonism of tamsulosin in oral controlled absorption system (OCAS) and modified release (MR) formulations.



Tamsulosin hydrochloride

However, there is no HPLC method reported for its estimation in commercial dosage form. This method is validated as per the ICH Guidelines in terms of limits of detection (LOD), limit of quantification (LOQ), linearity, precision, accuracy, specificity and robustness. Hence, a reverse phase HPLC method for the determination of tamsulosin hydrochloride in pharmaceutical solid dosage forms is described.

EXPERIMENTAL

Instrument

High Performance Liquid Chromatograph, Shimadzu 2010. (Rheodyne injector with 50 μ l loop) with microprocessor attachment was used for chromatography.

Chemicals and reagents

All chemicals and solvents were of analytical grade. Reference standard tamsulosin hydrochloride was procured from M/S. Suven Life Sciences, Acetonitrile (HPLC Grade)

and perchloric acid (AR Grade) were purchased from E-Merck. Inertsil ODS 3V (5 microns, 15 cm x 4.6 mm) and acetonitrile were used as stationary and mobile phases, respectively.

Chromatographic conditions

Analysis was carried on Shimadzu 2010. For convenience, the elution was monitored at 220 nm. Separation was achieved by using Inertsil ODS 3V column (15 cm x 4.6 mm), 5 µl with mobile phase containing a 70 : 30 acetonitrile : buffer . The flow rate of the mobile phase was kept at 1.0 mL/min. Acetonitrile was used as diluent. 15 µL of sample solution was injected each time.

Sample preparation

Perchloric acid solution : 30.5 mL perchloric acid was added to 95 mL water and 10.5 g of sodium hydroxide was added to the mixture. It was made up to 1000 mL with water and homogenized. 100 mL perchloric acid solution was added to 565 mL of water and homogenized. pH was adjusted to 2.0 with 1N sodium hydroxide solution. The solution was made up with water to 700 mL and then 300 mL acetonitrile was added and filtered under vacuum through a 0.45 µm nylon filter.

Internal standard preparation

40 mg Propyl p-hydroxybenzoate was taken as secondary standard into a 100 mL volumetric flask. It was dissolved in 70 mL acetonitrile and was made up to volume with water. The solution was homogenized. 50.0 mL of the solution was measured into a 100 mL volumetric flask and made up to volume with solvent.

Standard preparation

25 mg of tamsulosin hydrochloride was weighed into a 50 mL volumetric flask. It was dissolved in about 30 mL solvent by shaking and made up to volume with solvent and homogenized. 2.0 mL into 50 mL solution was transferred volumetric flask and made up to volume with mobile phase and homogenized. Part of the solution was transferred into brown coloured auto sampler vials. 10.0 mL of this solution was pipetted into a vessel, 2.0 mL of internal standard solution was added, homogenized and then injected.

Sample solution

Thoroughly mixed 500 mg of granules were weighed and transferred (corresponding to 1.0 mg tamsulosin hydrochloride) into a 50 mL volumetric flask. 20 mL

0.05N sodium hydroxide was added to it sonicated at 50°C for 30 minutes. 10 mL acetonitrile was added and shaken for 5 minutes. 5.5 mL 0.2N hydrochloride was added and shaken for 5 minutes. Then 10 ml of solvent was added and shaken well for 5 minutes. pH of the prepared solution is maintained at about 2.5 to 3.5. Fresh 0.05N sodium hydroxide and 0.2 N hydrochloric acid solutions were prepared to use them if necessary to correct the pH. The solution made up to the mark with mobile phase. The sample solution was homogenized and centrifuged it at 3000 rpm for 15 minutes. The supernatant was filtered into a brown-coloured auto sampler vial with 0.45 µm nylon filter. 10.0 mL of this solution was pipetted into a vessel, 2.0 mL internal standard solution was added, homogenized and then injected.

The results are tabulated as follows:

Table 1

Semi - formulation	S. No.	Label claim %	Amount estimate%	% label claim	% deviation	SD	RSD
Pellets	1		0.203	101.5	(+) 1.5		
	2		0.203	101.5	(+) 1.5		
	3		0.200	100.0	-		
		0.2				0.0017	0.86
	4		0.200	100.0	-		
	5		0.200	100.0	-		
	6		0.199	99.5	(-) 0.5		

Calibration

50 µL of the above working standard solutions were injected at a time interval of 15 minutes. Evaluation was performed with UV detector at 220 nm. The retention time is found to be around 6.618 minutes for tamsulosin and for internal standard 12.313 minutes. Peak areas were recorded and the calibration graph was obtained by plotting peak areas versus concentration.

Assay

50 µL of standard and sample solutions were injected into an injector of liquid chromatograph. The amount of tamsulosin hydrochloride was calculated by comparing the

peak ratio, with that of the standard (Fig. 1).

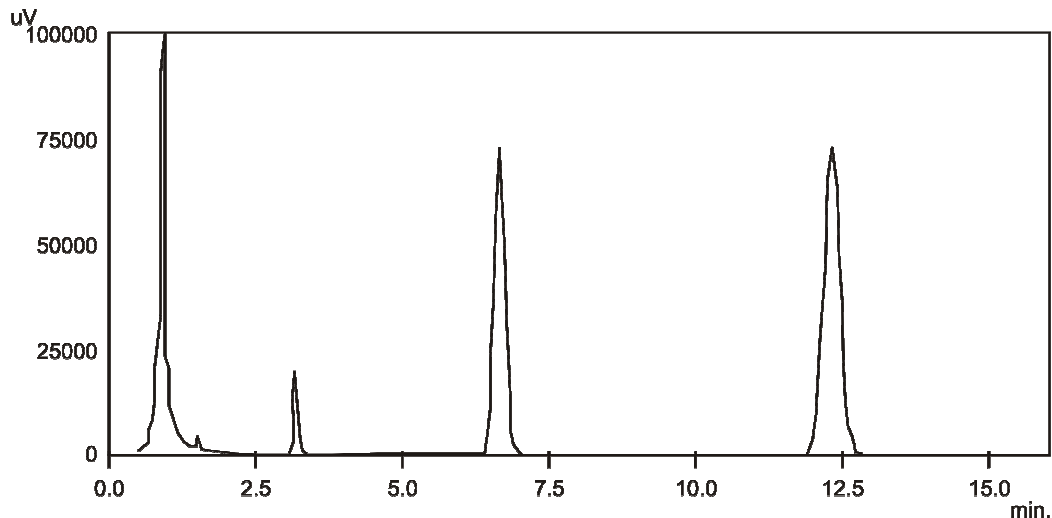


Fig. 1: Chromatogram of sample semi formulation containing tamsulosin hydrochloride

Recovery studies

To study the linearity, accuracy and precision of proposed method, recovery experiments were carried out. Known quantities of standard at two different levels were added to the preanalyzed sample and the recovery was estimated to be more than 99%.

RESULTS AND DISCUSSION

Method development

System suitability test is applied to a representative chromatogram to check various parameters such as efficiency, resolution and peak tailing. The results obtained are shown in Table 2, which are in concurrence with the USP requirements⁸.

Linearity

The linearity of tamsulosin hydrochloride is established by plotting a graph of peak area of standard solutions versus concentration. The linearity was found to be between 100-500 $\mu\text{g/mL}$.

Chromatography

The mobile phase of acetonitrile and buffer in the ratio of 30 : 70 is found to be ideal for analysis of tamsulosin hydrochloride. The concentration of tamsulosin hydrochloride was found to be within limits and the RSD values are reasonably low.

Table 2

Parameter	Tamsulosin HCl
Theoretical plate	5829
Tailing factor	1.099
RSD of 6 injections	0.0934218

The precision of the method is studied by making 5 injections of standard and very low RSD values indicate good precision. The reproducibility and reliability of the method has been tested by performing recovery studies, which showed good results.

CONCLUSION

The proposed method is very simple, rapid and involves efficient reproducible methodology. High percentage of recovery shows that the method is free from all the interferences of the excipients used in the semi formulations. Therefore, the method can be useful in routine quality control analysis.

REFERENCES

1. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human use; ICH Harmonised Tripartite Guidelines on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human use, S2(R1) (2008).
2. European Medicines Agency, Evaluation of Medicines for Human Use, Guidelines on the Limits of Genotoxic Impurities, CPMP / SWP / 5199 / 02, EMEA / CHMP / QWP / 251344 / (2007).
3. Indian Pharmacopoeia, The Indian Pharmacopoeia Commission, India, **2**, 714, (2007).
4. United States Pharmacopoeia, The United States Pharmacopoeial Convention, Rockville, M D, **31(2)** 1400 (2008).
5. British Pharmacopoeia, The Department of Health, Great Britain, **1**, 137 (2008).

6. European Pharmacopeia, Council of Europe, France Volume **6**, Issue **2**, 1173 (2008).
7. E. J. van Hoogdalem, H. Kamimura and S. Higuchi, *European J. Pharma. Sci.*, **4**, Supplement 1, 60 (1996).
8. Vítězslav Maier, Jana Horáková, Jan Petr, Eva Tesařová, Pavel Coufalb and Juraj Ševčík *Il Farmaco*, **60**, 834-839 (2005).
9. Michel, C. Korstanie, W. Krauwinkel, M. Shear, J. Davies and A. Quartel, *Euro. Urol. Supplements*, **4**, 45-52.
10. The United States Pharmacopeia, US Pharmacopeia Convention Inc., Washington D.C. Edition XXX.

Accepted : 23.08.2008