

The aim of the present work was to develop an accurate, specific, reproducible, and stability indicating method for the determination of Tamsulosin HCl in the presence of its degradation products and related impurities as per ICH guideline.

MATERIAL AND METHODS:

Reagents and Materials

Bulk sample of Tamsulosin HCl was obtained from Sun Pharm lab, India and tablets (Label Claim: 10 mg per tablet, Product Name: Veltam and Manufacturer: Intas (Arron) was procured from the market. Triple distilled water (T.D.W) was used throughout the work. Methanol and Acetonitrile (HPLC grade) were procured from Merck chemicals

Chromatographic condition

A Shimadzu HPLC separation module LC 10AT VP series pumps equipped with SPD 10AVP UV-Visible detector was used. Data acquisition was performed by Shimadzu Class-VP version 6.12 SPI software. Analysis was carried at 275nm with a Lichocart / Lichrosphere 100 C-18 (0.4cm X 25 cm, packed with 5 μ) at ambient temperature (25°C). The mobile phase was of a mixture of Acetonitrile: T.D.W. in the ratio 40: 60v/v. The flow rate was 0.8 ml / min and the retention time of Tamsulosin HCl and Celecoxib were found to be 1.608 and 2.767min respectively. The mobile phase was degassed and filtered through 0.45 μ m membrane filter before pumping into the HPLC system.

Preparation of standard stock solutions:

Stock solution of Tamsulosin HCl (1000 μ g/ml) was prepared by dissolving 25 mg of Tamsulosin HCl in 25 ml of volumetric flask containing 10 ml of mobile phase. The solution was sonicated for about 20 minutes and then made up to volume with mobile phase. Working standard solutions of Tamsulosin HCl was prepared by suitable dilution of the stock solution with appropriate mobile phase. Similarly stock solution of internal standard was prepared by dissolving 25 mg of Celecoxib in 10 ml of mobile phase, sonicated for 20min. then made up to the volume with mobile phase. Working standard solutions of Tamsulosin HCl were prepared by taking suitable aliquots of drug solution from the standard stock solution 1000 μ g/ml, spiked with internal standard solution 0.1ml (From 1000 μ g/ml stock of Celecoxib) and the volume was made upto 10 ml with mobile phase.

Preparation of sample solution

Twenty five tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 25 mg of Tamsulosin HCl was extracted with mobile phase in a 25ml volumetric flask using ultra sonicator. This solution was filtered through 0.45 μ m filter paper. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined. An aliquot of the internal standard was added to the sample solution prior to the dilution. All determinations were carried out in triplicate.

Procedure for calibration curve:

The contents of the mobile phase were filtered before use through 0.45 μ m filter paper, and pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. Then, 20 μ l of each of standard and sample solutions were injected into the HPLC system for six times to get the chromatograms. The retention time, average peak areas and peak area ratios of drug to internal standard were recorded. Taking conc. plotted a graph on X-axis and peak area ratios on Y-axis. The linearity range was found to be in between 1-200 μ g/ml for Tamsulosin HCl.

Method validation

Precision:

The precision of each method was ascertained separately from the peak area ratios obtained by actual determination of eight replicates of a fixed amount of drug and internal standard. The percent relative standard deviations were calculated for Tamsulosin HCl and presented in the table 3.7. The precision of the assay was also determined in terms of intra day variation in the peak areas for a set of drug solutions and was calculated in terms of % RSD.

Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, 120%) of bulk samples of BUP along with internal standard within the linearity ranges were taken and added to the pre-analyzed formulation of concentration 10 μ g/ml. From that percentage recovery values were calculated.

Ruggedness and robustness of the method

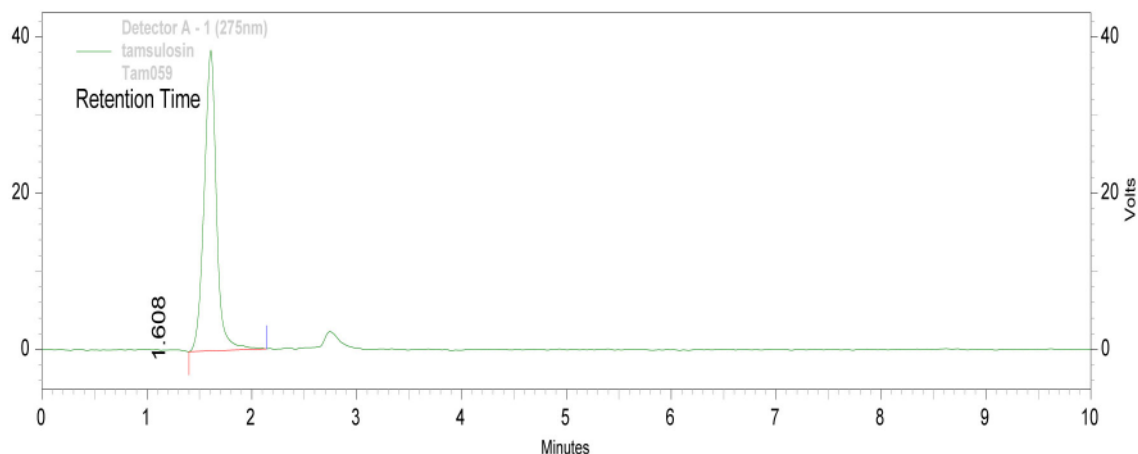
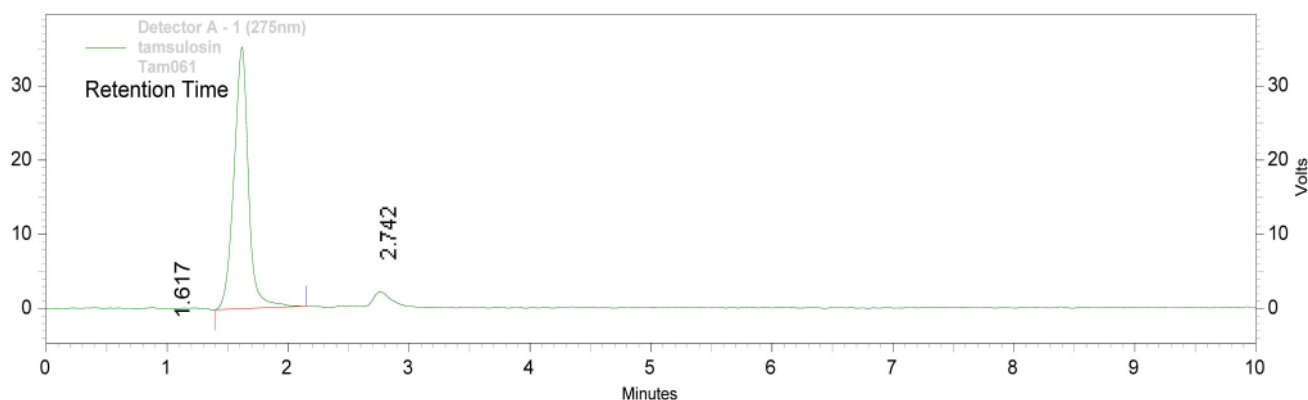
Method robustness and ruggedness was determined by analyzing same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, flow rate, instrument and analyst. The robustness and ruggedness of the method was established as the % deviation from mean assay value obtain from precision study is less than $\pm 2.0\%$. LOD and LOQ were established by slope method as mentioned below.

$$\text{LOD} = \frac{3.3 \times \text{standard deviation of } y - \text{intercept}}{\text{Slope of the calibration curve}}$$

$$\text{LOQ} = \frac{10 \times \text{standard deviation of } y - \text{intercept}}{\text{Slope of the calibration curve}}$$

Table 1: Summary of Validation Parameters.

Parameters	Values
LOD	0.36
LOQ	0.74
Accuracy (%)	98.88-100.03
Precision(%RSD)	
Intraday(n=3)	0.098
Interday(n=3)	0.125
Ruggedness(%assay)	
Analyst 1	99.34
Analyst 2	99.15
Robustness(%RSD)	Less than 2%

Figure 2: A Typical Chromatogram of Tamsulosin HCl in pure drug (20 g/ml) at λ_{\max} : 275nm.**Figure 3:** A Typical Chromatogram of Tamsulosin HCl in formulation (20 g/ml) at λ_{\max} :275nm.**Analysis of formulations:**

For analysis of commercial formulations, 25 tablets containing Tamsulosin HCl were taken and powdered. The powder equivalent to 10 mg of Tamsulosin HCl was taken in a 100 ml volumetric flask, containing 70 ml of mobile phase and sonicated for 30 minutes. The volume was made up to 100 ml with mobile phase and filtered to get a solution of concentrations 100 $\mu\text{g}/\text{ml}$. This was further diluted with to get a concentration within the linearity range. The amount of drug present in pharmaceutical formulation was calculated through peak area ratio of drug to that of internal standard by using the standard calibration curve (concentration in $\mu\text{g}/\text{ml}$ was taken on X-axis and peak area ratio on Y-axis).

SPECIFICITY (FORCED DEGRADATION) STUDIES:

The specificity of the method was demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative and photolytic degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient.

1. Acid Degradation:

About 10mg of Tamsulosin HCl pure drug was accurately weighed and transferred to 10ml volumetric flask and one ml of 0.1N HCl was added and kept aside for one hr and made up to volume with mobile phase. Then from this 10mcg/ml solution was prepared and injected in HPLC system to obtain chromatograms.

2. Alkaline Degradation:

About 10mg of Tamsulosin HCl pure drug was accurately weighed and transferred to 10ml volumetric flask and one ml of 0.1N NaOH was added and kept aside for one hr and made up to volume with mobile phase. Then from this 10mcg/ml solution was prepared and injected in HPLC system to obtain chromatograms.

3. Oxidative degradation:

About 10mg of Tamsulosin HCl pure drug was accurately weighed and transferred to 10ml volumetric flask and one ml of 3%w/v of hydrogen peroxide was added and kept aside for two hrs and made up to volume with mobile phase. Then from this 10mcg/ml solution was prepared and injected in HPLC system to obtain chromatograms.

4. Photolysis:

About 10mg of Tamsulosin HCl pure drug was accurately weighed and transferred to 10ml volumetric flask and made up to volume with mobile phase and kept aside for 8hr under direct sunlight. Then from this 10mcg/ml solution was prepared and injected in HPLC system to obtain chromatograms.

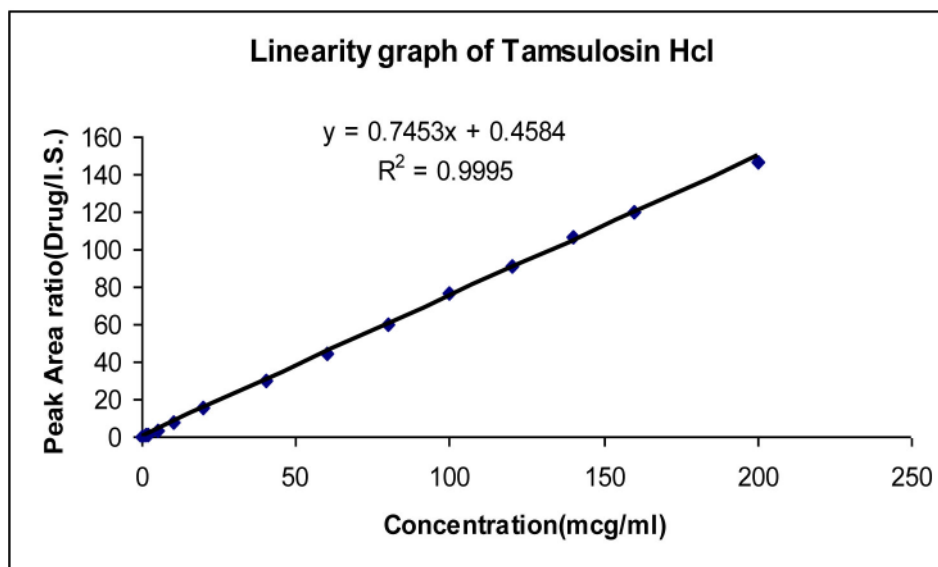
RESULTS & DISCUSSIONS:

A reversed-phase chromatographic technique was developed to quantify Tamsulosin HCl and it shows maximum absorbance (λ_{\max}) in acetonitrile at 275nm. In HPLC 275nm is selected, peaks are seen at this wave lengths using T.D.W. and acetonitrile (60:40) as mobile phase with retention time 1.608. The flow rate was increased from 0.8ml/minute to

Table 2: Estimation of amount present in tablet dosage form.

Formulation	Labeled amount (mg)	Observed amount*(mg)	% Recovery by proposed method	%RSD
VELTAM INTAS (Arron)	0.4	0.377	97.74±0.236	0.239

*Each value is average of five determinations ± standard deviation.

Figure 4: Linearity graph of Tamsulosin HCl.**Table 3:** Forced Degradation Readings.

Stress Conditions	Auc	Retention Time	Drug Recovered (%)	Drug Decomposed (%)
Standard Drug	92007	1.683	100	0
Acidic Hydrolysis	58776	1.617	63.88	36.12
Alkaline Hydrolysis	61149	1.608	66.46	33.53
Oxidative degradation	78240	1.658	85.03	14.92
Photolytic degradation	82240	1.658	89.38	10.61

1ml/ minute. Merging of peaks was seen at flow rate 1ml/min. At flow rate 0.8ml/minute peaks were well resolved. It was our intention to use low flow rates as the high flow rates more than 1 decrease the life time of both the column and the pump. It is desirable to have retention times less than 10 minutes as this allows multiple analyses to be carried out in a reduced time. Celecoxib was taken as the internal standard and the retention time was found to be 2.767. In case of symmetrical peaks, peak heights are taken into consideration. In the present study we had chosen peak area ratio of drug/ internal standard. The present method was developed by taking peak area ratio of drug/ internal standard and validated. The marketed formulation is analyzed and the percentage recovery values of pure drug from the preanalyzed solution of formulation were in between 97.74% (Table 2). It was found that the drug obeys linearity

within the concentration range of 1-200µg/ml and a straight line passing through the origin was observed (Figure 4). The LOQ was found to be 0.74µg/ml and the LOD was found to be 0.36µg/ml. The capacity factor was more than 2

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (273 and 277 nm), percentage of acetonitrile in the mobile phase (38 and 42%) and flow rate (0.7 and 0.9 mL min⁻¹). Robustness of the method was studied using six replicates at a concentration level of 20 µg mL⁻¹ of Tamsulosin HCL. The % RSD value of assay determined for the same sample under original conditions and robustness conditions was less than 2.0% indicating that the developed method was robust method was found to be simple, precise, accurate and robust for determination Tamsulosin

Figure 5a: Typical Chromatogram of Tamsulosin HCL (Degraded When 0.1N HCL Added).

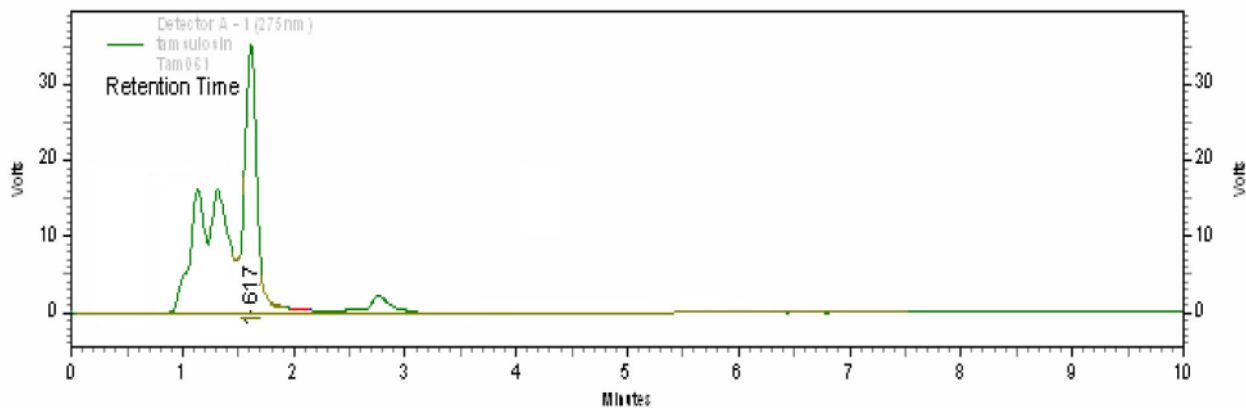


Figure 5b: Typical Chromatogram of Tamsulosin HCL (Degraded When 0.1N NaOH Added).

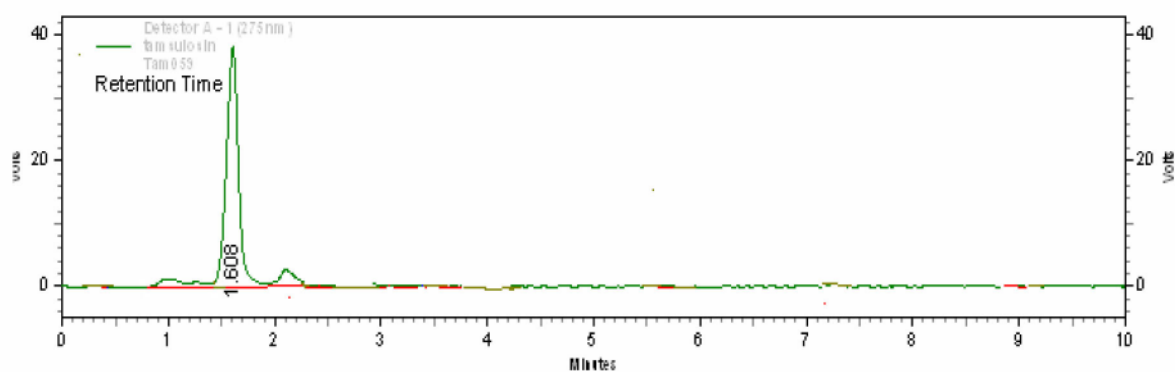


Figure 5c: Typical Chromatogram of Tamsulosin HCL (Oxidative Degradation).

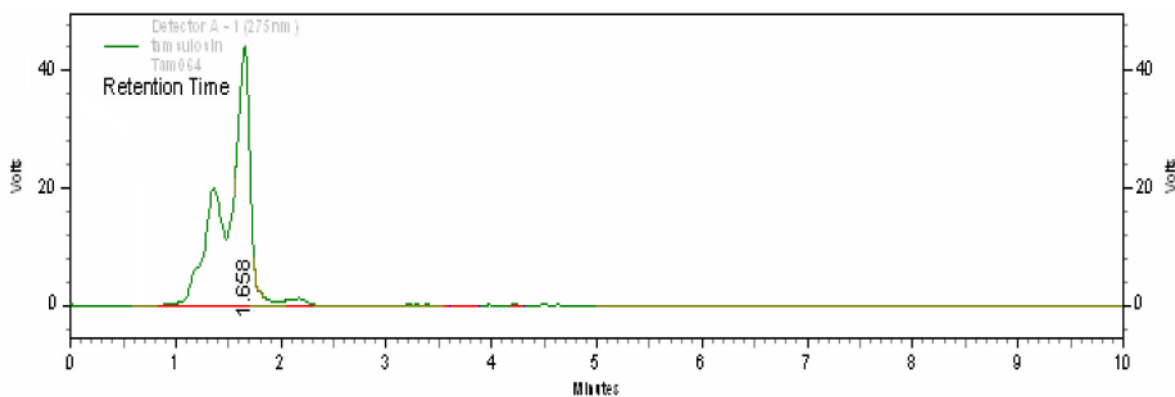
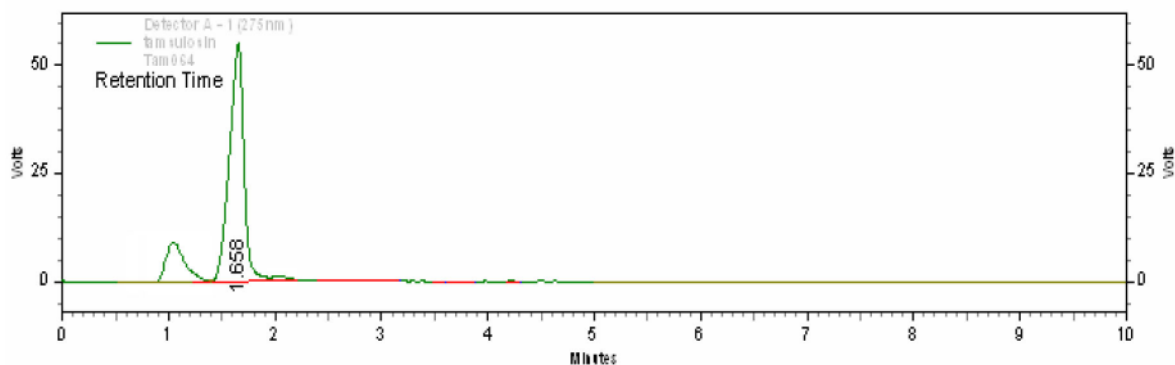


Figure 5d: Typical Chromatogram of Tamsulosin HCL (Photolytic Degradation).



HCl in pure and its dosage forms and also reveals that the commonly used excipients and additives in the pharmaceutical formulations were not interfering in the proposed method.

On Acid treatment (1ml of 0.1NHCl) it was found there was increase in peak resolution, shown by increase in AUC. The retention time was changed from 1.608 to 1.617 and the additional peaks observed are due to degradation products. The percent drug remaining after acid treatment was found to be 63.88 which was calculated from standard graph.

On Alkaline treatment (1ml of 0.1NNaOH) it was found there was increase in peak resolution, shown by decrease in AUC. The retention time was changed from 1.608 to 1.608 and the additional peaks observed are due to degradation products. The percent drug remaining after alkaline treatment was found to be 66.46 which was calculated from standard graph.

The 2 hr exposure to 3 % v/v H₂O₂ also affects the peak resolution much and the degradants are few and well separated from the drug peak.

When exposed to direct sunlight for 8 hrs, the retention time increased to 1.658 and the drug increases to 89.38 % of the initial amount. Although the peaks of degradants are well separated from drug peak, it's better to keep the solutions in dark/in containers which cut off light.

CONCLUSION:

The proposed method was found to be simple, precise, accurate and rapid for determination Tamsulosin HCl from pure and its dosage forms & validated as per ICH guidelines. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Tamsulosin HCl in pure form and its dosage forms and can also be used for dissolution or similar studies.

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REFERENCES:

- Martindale, The Extra Pharmacopoeia, Council of Royal Pharmaceutical Society of Great Britain, 29th edition, 1989.
- S.Budavari, The Merck Index, Merck and Co. Inc., White House Station, NJ, USA, 14th edition, 1996.
- Kakizaki H, Ameda K, Kobayashi S, Tanaka H and Shibata T Tomohiko Koyanagi, Urodynamic effects of α 1-blocker tamsulosin on voiding dysfunction in patients with neurogenic bladder. *Int. J. Urol.* 2003; 10: 11576.
- Shrivastava A, Saxena P, Gupta VB, Spectrophotometric estimation of tamsulosin hydrochloride by acid-dye method. *Pharmaceutical methods.* 2011; 2(1): 53-59.
- Susmitha K, Radha K and Venkateshwarlu G. Extractive Spectrophotometric methods for determination of tamsulosin hydrochloride in pharmaceutical formulations using acidic tri phenylmethane dyes. *Asian journal of Research in Chemistry.* 2011; 4(7):1114-18.
- Nanda RK, Gaikwad J, Prakash A, Estimation of TamsulosinHCl and Tolterodine in its pharmaceutical dosage form by spectrophotometric method. *Int. J. Pharm. Tech. Res.* 2009; 1(3): 420-423.
- V.V. Pande, Simultaneous estimation of Tamsulosin hydrochloride and Dutasteride in combined dosage form by UV spectroscopy method. *Journal of Pharmacy Research.* 2009; 2(3): 507-509.
- Meiling Qi, Peng Wang, Rui-Hua Cong, Determination of the enantiomers of Tamsulosin hydrochloride and its synthetic intermediates by chiral liquid chromatography. *Chromatographia.* 2004; 59: 251-254.
- Patel DB, Patel NJ, Validated stability indicating HPTLC method for the determination of tamsulosin hydrochloride in pharmaceutical dosage forms. *Int J Chem Tech Res.* 2010; 2(1): 646-652.
- S. B. Bari, A. R. Bakhshi, P. S. Jain, and S. J. Surana, Development and Validation of Stability-Indicating HPTLC Determination of Tamsulosin in Bulk and Pharmaceutical Dosage Form. *Chromatography Research International.* 2011; Volume 2011: 893260.
- J. Macek, J. Klíma, P. Ptáček, Rapid determination of tamsulosin in human plasma by high-performance liquid chromatography using extraction with butyl acetate. *Journal of Chromatography B.* 2004; 809 (2):307-311.
- G. Chandorkar, V. B. Kotwal, N. S. Dhande, S. G. Gurav, V. V. Pande, and P. V. Yadav, A sensitive HPLC method for simultaneous estimation of tamsulosin hydrochloride and its impurity. *Pakistan Journal of Pharmaceutical Sciences.* 2008; 21(3), 307-310.
- Richa Kumari, P. P. Dash, V. K. Lal, A. Mishra and P. N. Murthy, RP – HPLC method for the estimation of Tamsulosin Hydrochloride in Tablet Dosage Form. *Indian Journal of Pharmaceutical Sciences.* 2010; 72(6): 785-787.
- Sudha Thomas, Jitendra Dhomane, A validated RP-HPLC method for the determination of impurities in tamsulosin hydrochloride. *Int JChem Res* 2011; 2(4): 29-33.
- Fan HR, Gu Y, Si DY, Liu CX, Determination of tamsulosin in dog plasma by a high sensitive liquid chromatography tandem mass spectrometric method. *Yao Xue Xue Bao.* 2007; 42(8): 872-76.
- L. Ding, M. Li, P. Tao, J. Yang, and Z. Zhang, Quantitation of tamsulosin in human plasma by liquid chromatography-electrospray ionization mass spectrometry. *Journal of Chromatography B.* 2002; 767(1): 75-81.
- N. V. S. Ramakrishna, K. N. Vishwottam, S. Manoj, M. Koteswara, S. Wishu, and D. P. Varma, Rapid, simple and highly sensitive LC-ESI-MS/MS method for the quantification of tamsulosin in human plasma. *Biomedical Chromatography.* 2005; 19(10): 709-719.
- Ksycinska H, Rudzki PJ, Sarosiek A, LC-MS Determination of Tamsulosin in human plasma and its application to pharmacokinetic study. *Acta Pol. Pharm* 2006; 63(5):417-419.
- Keski-Rahkonen P, Parssinen O, Leppanen E, Mauriala T, Lehtonen M, Aurola S, Determination of tamsulosin in human aqueous humor and serum by liquid chromatography-electro spray ionization tandem mass spectrometry. *J Pharm Biomed Anal* 2007; 43(2): 606-12.
- Matsushima H, Takanuki KI, Kamimura H, Watanabe Tand Higuchi S, Highly sensitive method for the determination of tamsulosin hydrochloride in human plasma dialysate, plasma and urine by high performance liquid chromatography-electro spray tandem mass spectrometry. *Drug Metab. Dispos.* 2004; 26(3): 240-245.
- Nageswra Rao R, Kumar Talluri MV, Narasa Raju A, Shinde DD, Ramanjaneyulu GS, Development of a validated RP-LC/ESI-MS-MS method for separation, identification and determination of related substances of tamsulosin in bulk drug and formulations. *J Pharm Biomed Anal.* 2008; 46(1): 94-103.
- ICH Q2A Guideline, Validation of Analytical Procedures: Definition and Terminology (CPMP III/ 5626/94), ICH, Geneva, Switzerland, 1995.
- ICH—Guidelines Q2B, Validation of Analytical Procedures: Methodology (CPMP/ICH/281/95), ICH, Geneva, Switzerland, 1996.
- ICH-Guidelines Q1A- Q1E, Stability Testing of New Drug Substances and Products, 2003.