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

Tamsulosin Hydrochloride is a selective antagonist at alpha-1A and alpha-1B-adreno receptors in the prostate, prostatic capsule, prostatic urethra, and bladder neck.

4.1 Drug Profile: \cite{124-26}

Synonyms: Tamsulonim

Brand Names: Alfatam, Contiflo, Dynapes, Veltam

Molecular Structure:

![Figure 4.1: Structure of Tamsulosin Hydrochloride (TSH)](image)

Chemical Name: \((R)-5-(2-\{2-(2-ethoxyphenoxy)ethyl\}amino)propyl-2-methoxybenzene-1-sulfonamide\)

Molecular Formula: \(C_{20}H_{28}N_2O_5\cdot S\cdot HCl\)

Molecular Weight: 445 gm/mol

Category: Uroselective Alpha1a-Adrenergic Receptor Antagonist.

Solubility: Soluble in water, methanol, and acetonitrile.

Physical state: White to almost white

Half-life: 5-7 hours

Absolute bioavailability: 100% (oral)

Melting point: 226-228 °C (HCl salt)

Mechanism of action: Tamsulosin Hydrochloride is a selective antagonist at alpha-1A and alpha-1B-adreno receptors in the prostate, prostatic capsule, prostatic urethra, and bladder neck. Approximately 70% of the alpha1-receptors in human prostate are of the alpha-1A subtype. Blockage of these receptors causes relaxation of smooth muscles in the bladder neck and prostate, and thus decreases urinary outflow resistance in men.
**Therapeutic uses**: Tamsulosin Hydrochloride (TSH) is an uroselective alpha1A-adrenergic receptor antagonist which is used in benign prostatic hyperplasia (BPH also known as enlarged prostate), but is sometimes used for the passage of kidney stones by the same mechanism of smooth muscle relaxation via alpha antagonism. Tamsulosin Hydrochloride is an alpha-blocker that works by relaxing the muscles in the bladder neck and prostate. Relaxing these muscles leads to relief of symptoms of BPH such as the feeling of needing to urinate frequently or urgently, weak stream, difficulty in beginning the flow of urine, and the need to urinate during the middle of the night. This medication should not be used to treat high blood pressure.

**Dose**: The recommended dose is 0.4 or 0.8 mg once daily about 30 minutes after the same meal time each day. When taken on an empty stomach, more of the medication is absorbed. This could cause a greater effect and potentially a drop in blood pressure.

**Pharmacodynamics**: Tamsulosin Hydrochloride, a sulfamoylphenethylamine-derivative alpha-adrenoceptor blocker with enhanced specificity for the alpha-adrenoceptors of the prostate, is commonly used to treat benign prostatic hyperplasia (BPH). The drug is commercially available in a racemic mixture of 2 isomers, and is pharmacologically related to doxazocin, prazosin, and terazosin. However, unlike these drugs, Tamsulosin Hydrochloride has a higher affinity for the alpha-1A-adrenergic receptors, which are located in vascular smooth muscle. Studies show that Tamsulosin Hydrochloride has about 12 times greater affinity for alpha-1 adrenergic receptors in the prostate than those in the aorta, which may result in a reduced incidence of adverse cardiovascular effects.

**Pharmacokinetics**:

**Absorption**: Absorbs rapidly and completely following oral administration. Peak plasma half-life is 5-7 hrs.

**Distribution**: Protein binding is >98% (e.g. to albumin and α1-acidglycoprotein)

**Metabolism**: Tamsulosin Hydrochloride is extensively metabolized by cytochrome P450 enzymes in the liver; however, the pharmacokinetic profile of the metabolites in humans has not been established.

**Elimination**: Tamsulosin Hydrochloride is extensively metabolized by cytochrome P450 enzymes in the liver and less than 10% of the dose is excreted in urine unchanged. The metabolites of Tamsulosin Hydrochloride undergo extensive conjugation to glucuronide or sulfate prior to renal excretion.
Adverse Reactions\textsuperscript{59}: Patients taking Tamsulosin Hydrochloride are prone to a complication known as floppy iris syndrome. Tamsulosin Hydrochloride can cause a drop in blood pressure, rarely resulting in dizziness, headache, nasal congestion and palpitation.

Drug Interactions: Indomethacin and other NSAIDS attenuate the anti-hypersensitive action of Tamsulosin Hydrochloride. Cimetidine inhibits Tamsulosin Hydrochloride metabolism. Propranolol attenuates Tamsulosin Hydrochloride action against hypertension.

Storage: Store the formulations in a tightly closed container at 25\textdegree C; excursions permitted between 15-30\textdegree C, protect from light and excessive humidity.
4.2 LITERATURE REVIEW

Raghu K et al.,60 described two visible spectrophotometric methods for the assay of Tamsulosin Hydrochloride in pure and solid dosage forms. The first method $M_1$ is based on the formation of yellowish brown coloured species by the drug with Folin reagent and exhibits absorption maxima at 440 nm. Second method ($M_2$) is based on the formation of purple red coloured species with sodium nitroprusside-acetaldehyde reagent exhibiting maximum absorption at 560 nm. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges (16-48) µg/ml for method $M_1$ and (8-24) µg/ml for method $M_2$ respectively.

Bari S et al.,61 developed two UV-Spectrophotometry and First order derivative methods for estimation of Tamsulosin Hydrochloride in bulk and tablets. Methanol: water (2:8) was used as a solvent. In UV spectrophotometric method absorbance of samples are recorded at 280 nm. In first order derivative method the amplitude of trough was recorded at 298 nm. Tamsulosin Hydrochloride follows linearity in the concentration range of 10-90 µg/ml.

Hemanth K et al.,62 developed and validated a UV spectrophotometric method with good sensitivity for the simultaneous determination of Finasteride and Tamsulosin Hydrochloride in standard solutions and tablets. In methanol, the $\lambda_{\text{max}}$ of Finasteride and Tamsulosin Hydrochloride was found to be 219 and 224nm respectively. Using an Elico UV-Visible spectrophotometer (model SL – 159) with matched quartz cells, in this proposed method both these drugs obeyed linearity individually and in mixture with the concentration range of 12.5-62.5 µg/ml for Finasteride and 1-5 µg/ml for Tamsulosin Hydrochloride with a correlation coefficient of 0.9981 and 0.9989. The relative standard deviation was found to be 0.5974 and 0.4096.

Nilam A et al.,63 developed three UV methods for the estimation of Tamsulosin Hydrochloride in pure drug and tablet dosage form. Method A is absorbance maxima at 279.0nm, Method B is the first order derivative spectra showed a sharp peak at 298nm, method C is the area under curve, were the wavelength range selected at 298-263nm. All the three methods obey beer lamberts law in concentration range of 1-6µg/ml and distilled water was used as solvent.
Siddartha R et al., developed and validated a reverse phase high performance liquid chromatographic method for the determination of Tamsulosin Hydrochloride in bulk and tablet dosage forms. The HPLC separation was carried out by reverse phase chromatography on Shimadzu HPLC, 10-At detector with hypersil ODS C₁₈ Column 250 X 4.6 mm (particle size of 5µ) and constant flow pump. Injector with 20 µl loop with a mobile phase composed in the ratio acetonitrile: (0.05M) KH₂PO₄ buffer (45:55) at flow rate 1.8 ml /min. The detection was monitored at 240nm. The calibration curve for Tamsulosin Hydrochloride was linear from 10-50 µg/ml and internal standard (Bromhexine) 10 µg/ml were prepared by suitable dilutions of the stock solution with appropriate mobile phase. The interday and intraday precision was found to be within limits. LOD and LOQ for Tamsulosin Hydrochloride were found to be 0.495 and 0.461. Accuracy (recoveries: 98.5-98.55%) and reproducibility were found to be satisfactory.

Blumireddy C et al., developed a reverse phase high performance liquid chromatographic method for the determination of Tamsulosin Hydrochloride 0.2% & Tolterodine tartrate 0.2% combination pellets. An Inertsil ODS 3V (4.6 mm X 150 mm) that contain 5µm packing column, gradient mode, with 30.5ml perchloricacid with 95 ml of water and add 10.5gm sodium hydroxide, made up to 1000ml with water and homogenize as mobile phase. The flow rate is 2ml/minute and effluent is monitored at 220nm.

Dipti B et al., developed a spectrofluorimetric method for the determination of Tamsulosin Hydrochloride in tablet dosage form. The solvent systems and wavelengths of detection were optimized in order to maximize the sensitivity and minimize cost of analysis. The excitation and emission wavelengths were found to be 226 nm 322 nm respectively for Tamsulosin Hydrochloride in methanol. The calibration graph was linear over the range of 5-30 µg/ml with high value of correlation coefficient. The percentage recovery was found to be 99.70%-99.92%.

Kumar S et al., developed and validated a stability-indicating high-performance liquid chromatographic method for the determination of Tamsulosin Hydrochloridein pharmaceutical dosage forms. Celecoxib was used as Internal Standard (IS). The chromatographic conditions comprised of a reversed-phase Lichrocart / Lichrosphere C18 column (250 x 4.0 mm packed with 5 µ) with mobile phase consisting of a mixture of Acetonitrile: T.D.W. in the ratio (40: 60). Flow rate was 0.8 ml/min. Detection was carried out at 275 nm. The retention time of Tamsulosin
Hydrochloride and Celecoxib were found to be 1.608 and 2.767 min respectively and the linear regression analysis data for the calibration plots showed good linear relationship in the concentration range 1 - 200 μg/ml. The value of correlation coefficient, slope and intercept were, 0.9995, 0.7453 and 0.4584, respectively. Tamsulosin Hydrochloride was subjected to stress conditions of degradation in aqueous solutions including acidic, alkaline, oxidation and photolysis.

Mithlesh R et al.,68 developed and validated a analytical method using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) for the determination of trace levels of boron, present as an impurity in Tamsulosin Hydrochloride. Boron was suitably extracted from Tamsulosin Hydrochloride and brought into the solution using ashing technique followed by quantitative determination by ICP-OES. The limit of detection of the validated method was found as 15 μg/L and the limit of quantitation was calculated as 25 μg/L. The method was found to be linear in the wide working range of 25 μg/L to 800 μg/L with correlation coefficient of 0.99978. The recoveries of boron from the spiked samples of Tamsulosin Hydrochloride were found in the acceptable range of 90 to 98% at three different spiking levels.

Sanjay D et al.,69 developed a reverse phase HPLC method for estimation of Tamsulosin Hydrochloride in bulk and capsule dosage form. It was resolved by using a mobile phase of Acetonitrile:water in the ratio of 50:50 v/v at the flow rate of 1.5 ml/min. Elutents were monitored at 214 nm. The retention time of the drug was 1.7 min with this method, linearity was observed between area under curve and concentration of Tamsulosin Hydrochloride in the injected solution, in the range of 5 to 100 μg/ml.

Atul H et al.,70 developed and validated a spectrophotometric method for simultaneous estimation of Tamsulosin Hydrochloride (TAM) and Finasteride (FINA) in combined tablet dosage form. The ratio derivative spectroscopic method involves measurement of first derivative amplitude of ratio spectra at 240.01 nm for FINA and 229.91 nm for TAM as two wavelengths for estimation. Beer’s law is obeyed in the concentration range of 2-10 and 25-125 μg/ml for TAM and FINA, respectively.

Nanda K et al.,71 Three UV methods have been developed for the estimation of Tamsulosin Hydrochloride in pure drug and tablet dosage form. Method A is absorbance maxima at 281 nm, method B is the first order derivative spectra of drug showed a sharp peak at 234.5 nm, method C is the area under curve, in the wavelength
range of 286-276 nm. All the three methods obey Beer's law in concentration range of 5-25 µg/ml.

Nithiyananthan T et al.,\textsuperscript{72} describes a HPLC method for estimation of Tamsulosin Hydrochloride in bulk and tablet dosage form. The estimation was carried out on ODS C-18 column using a mobile phase consisting of sodium dihydrogen orthophosphate buffer-Acetonitrile (70:30). The eluent was monitored at 280 nm.

Ravindra K et al.,\textsuperscript{73} developed two methods for the estimation of Tamsulosin Hydrochloride and Tolterodine in its pharmaceutical dosage form. First method is based on the simultaneous equations and wavelengths selected for analysis were 225.5 nm for Tamsulosin Hydrochloride and 284.0 nm for Tolterodine respectively. Second method is Q analysis method, based on absorbance ratio at two selected wavelengths 217.0 nm (iso-absorptive point) and 225.5 nm. The linearity was obtained in the concentration range of 5-25 µg/ml and 10-50 µg/ml for Tamsulosin Hydrochloride and Tolterodine respectively.

Panda V et al.,\textsuperscript{74} reports a spectrophotometric method for the simultaneous estimation of Tamsulosin Hydrochloride and Dutasteride in combined tablet dosage form. Fixed dose combination tablets containing Tamsulosin Hydrochloride & Dutasteride are used to treat the symptoms of an enlarged prostate, a condition technically known as benign prostatic hyperplasia or BPH. The drugs individually and in mixture obey Beer’s law over conc. range 0.0347 mg/ml for Tamsulosin Hydrochloride (TAM) and for Dutasteride 0.012 mg/ml (DUTA). The mean recoveries from tablet by standard addition method were 99.0% and 99.5%.

Sangita Aet al.,\textsuperscript{75} developed a liquid chromatographic tandem mass spectrometric (LC–MS–MS) method was for simultaneous identification and quantification of Tamsulosin Hydrochloride and Dutasteride in human plasma, which was well applied to clinical study. The method was based on liquid–liquid extraction, followed by an LC procedure with a Gemini C-18, 50 mm · 2.0 mm (3 lm) column and using methanol: ammonium formate (97:3, v/v) as the mobile phase. Protonated ions formed by a turbo ion spray in positive mode were used to detect analytes and internal standard. MS–MS detection was by monitoring the fragmentation 228.1 (m/z) for Tamsulosin Hydrochloride, 529.3 (m/z) for Dutasteride and 305.3 (m/z) for Finasteride (IS) on a triple quadrupole mass spectrometer. The lower limit of quantification for both Tamsulosin Hydrochloride and Dutasteride was 1 ng/ml.
4.3 MATERIALS & METHODS

Instrumentation:
Chromatographic separation was performed using Agilent 1120 compact LC system integrated with a variable wavelength programmable UV detector and a Rheodyne injector equipped with 20µl fixed loop. The separation was performed on a reverse phase C_{18} column [Agilent ODS UG 5 column, 250mm x 4.5mm]. Quantitative HPLC was performed on a gradient High Pressure Liquid Chromatography (Shimadzu HPLC class VP series) with two LC-10 AT, VP pumps, variable wavelength programmable UV/Visible detector SPD-10A, VP, CTO-10ASVP column oven (Shimadzu), SCL-10A, VP system controller (Shimadzu) and one column [Phenomenex C18 (250 mm x 4.6 mm) I.D; particle size 5 mm]. The HPLC system was equipped with the software “class VP series version 5.03” (Shimadzu).

Table 4.1: Chromatographic conditions for TSH

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary phase (column)</td>
<td>C_{18} column (Agilent ODS UG column, 250mm x 4.5mm)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile and methanol in a ratio of 40:60, v/v</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 ml/min</td>
</tr>
<tr>
<td>Column temperature</td>
<td>Ambient</td>
</tr>
<tr>
<td>Volume of injection</td>
<td>20 µl</td>
</tr>
<tr>
<td>Detection wavelength</td>
<td>280 nm</td>
</tr>
<tr>
<td>Retention time</td>
<td>3.6 min</td>
</tr>
</tbody>
</table>

Reagents used
- Methanol (HPLC grade)
- Double distilled water
- Acetonitrile (HPLC grade)

Preparation of standard stock solution:
25mg of pure drug was weighed accurately and transferred to 25ml volumetric flask and dissolved in 10ml solvent and make up to the mark with solvent to obtain a final concentration of 1000μg/ml (standard stock solution A).

From the standard stock solution 2.5ml of aliquot was pippeted in to 25ml volumetric flask and dissolved in 10ml solvent and make up to the mark with the solvent to obtain a final concentration of 100μg/ml (standard stock solution ‘B’). The resulting solution was filtered through Whatman filter paper (No. 41).
Preparation of sample solution

Marketed tablet formulation (VELTAM) containing 0.4 mg of Tamsulosin Hydrochloride was analyzed by this method. Twenty tablets were accurately weighed and their average weight determined. The tablets were then crushed to fine powder and powder equivalent to 10mg was taken in 100ml volumetric flask and dissolved in 50ml of mobile phase. The solution was kept for sonication for 15min. The solution was made up to the mark with the mobile phase and filtered through 0.45 μ membrane filter to get the concentration of 100µg/ml (sample stock solution A).

1.0ml aliquot of the above solution was transferred to a 10ml volumetric flask and diluted to the mark with the mobile phase to obtain a concentration of 10μg/ml (sample stock solution ‘B’).

METHOD DEVELOPMENT:

Purpose:
The purpose of method development is to optimize the chromatographic conditions by conducting various trails that results in a sensitive, precise, accurate and reliable method that enables the estimation of Tamsulosin Hydrochloride in pharmaceutical formulations.

Procedure:
In developing a HPLC method, a systematic study of effects of various parameters was undertaken by varying one parameter at a time and controlling all other parameters. The following studies are to be conducted for this purpose.

A) Selection of Mode of separation:
The first consideration when developing an HPLC method is to determine the solubility of sample components. As Tamsulosin Hydrochloride was soluble in polar solvents RP-HPLC method was chosen.

B) Selection of Mobile Phase:

Pure drug Tamsulosin Hydrochloride was injected into the HPLC system and run in different mobile phases. Different mobile phases like methanol and water, water and acetonitrile were tried in order to find the best conditions for the separation of Tamsulosin Hydrochloride. It was found that Methanol and Acetonitrile gives satisfactory results as compared to other mobile phases. Finally the optimal composition of the mobile phase was determined to be Acetonitrile and methanol (50:50) at flow rate 1min/ml. This mobile phase produced good resolution, reasonable retention time and acceptable peak symmetry for the drug.
C) Selection of Stationary phase:
Selection of appropriate stationary phase helps to improve the efficiency of the method. In the present study, in order to get better peak resolution with less tailing factor and more theoretical plates, C_{18} column (Agilent ODS UG column, 250mm x 4.5mm) was selected.

**Preparation of Optimized Mobile Phase:**
Mix acetonitrile 400 ml (40%) and 600 ml of methanol HPLC (60%) and degas in ultrasonic water bath for 5 min. Filter through 0.45 \( \mu \) filter under vacuum filtration.

**Conditioning of the columns:**
Before the new run of HPLC conditioning of the columns was done by passing HPLC grade methanol at 1 ml/min flow rate for 30 min, so as to remove the remains of the previous run’s present in the column.

**Loading of mobile phase:**
Filtered and degassed mobile phase was filled in the channel. Priming was done for each channel by using freshly prepared mobile phase.

**Recommended procedure**
After systematic and detailed study of the various parameters involved, as described under results and discussion in this chapter, the following procedure was recommended for the determination of TSH in bulk samples and pharmaceutical formulations.

**Method:**
Appropriate aliquots were pipetted out from the working stock solution (100\( \mu \)g/ml) in to a series of 10ml volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range, ranging from 20,40,60,80 and 100 \( \mu \)g/ml. These solutions were filtered through a 0.45\( \mu \) membrane filter and sonicated for 20min.

Triplicate dilutions of each of the above concentrations were prepared separately and from these triplicate solutions, 20\( \mu \)L of each concentration of the drug were injected in to the HPLC system and their chromatograms were recorded under the described chromatographic conditions. A calibration curve of concentration vs. peak area was established. Regression equations were established and the correlation coefficients were determined.
4.4 ANALYTICAL METHOD DEVELOPMENT

To develop a suitable (specific) and robust LC method for the determination of Tamsulosin Hydrochloride, different mobile phases were employed to achieve the best separation and resolution. The method development started with symmetry C18 (50×2.1 mm, 3.5µm) with the following mobile phase: water and methanol in a ratio of 40:60 v/v, sonicated for 10 mins, filtered, and degassed. The flow rate was adjusted at 1.2 ml/min. The column temperature was 25°C and the injection volume was 25µl. UV detection was performed at 280 nm and the sample temperature was maintained at 25°C. Theoretical plates were less than 2000. For the next trial, the mobile phase composition was changed to water and acetonitrile mix in a ratio of 50:50. The mixture was sonicated for 10 mins, filtered, and degassed. The chromatographic conditions remained the same as above. The peak shape was not good. Again, the mobile phase composition was changed and the method development was carried out with a mobile phase containing a mixture of 50 ml of acetonitrile and 50 ml of methanol. The mixture was sonicated for 10 mins, filtered, and degassed. The retention time of Tamsulosin Hydrochloride was 3.6 mins. The peak shape was good. The chromatograms of Tamsulosin Hydrochloride blank, placebo, and standard were shown in the Figures 4.2, 4.3 & 4.4 respectively.

Figure 4.2: A typical Chromatogram of Tamsulosin Hydrochloride Blank
Figure 4.3: A typical Chromatogram of Tamsulosin Hydrochloride Placebo

Figure 4.4: A typical Chromatogram of Tamsulosin Hydrochloride Standard

4.5 METHOD VALIDATION:

The developed LC method is extensively validated for Tamsulosin Hydrochloride using the following parameters

4.5.1 Specificity:

Purpose:
Specificity of a method was determined by testing standard substances against potential interferences. Triplicate injections of 100% test concentration were given to the system for checking the interferences of excipients if any.

Procedure:
Common excipients that are usually present in the formulation such as lactose, microcrystalline cellulose and magnesium stearate have been added to the
samplesolution and injected into the HPLC system by following the test method conditions.

4.5.2 System Suitability:

System suitability tests were carried out on each day of validation to evaluate the components of the analytical system in order to show that the performance of the system meet the standards required by the method.

The HPLC system was equilibrated using the initial mobile phase composition, followed by six replicate injections of the 100% concentration containing 100µg/ml. The chromatogram was representing in the Figure 4.5 and result was reported in the Table 4.2.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No.of theoretical plates (N)</td>
<td>2638</td>
</tr>
<tr>
<td>2</td>
<td>Tailing factor (T)</td>
<td>1.2</td>
</tr>
<tr>
<td>3</td>
<td>Retention time (min)</td>
<td>3.6±0.03</td>
</tr>
<tr>
<td>4</td>
<td>%RSD</td>
<td>0.845</td>
</tr>
</tbody>
</table>

Figure 4.5: Chromatogram showing System suitability of TSH

4.5.3 Linearity:

Purpose:
The purpose of this study is to verify that the detector response is directlyproportional to the concentration.

Procedure:
Appropriate aliquots of standard Tamsulosin Hydrochloridestock solutions (100µg/ml) were taken in different 10 ml volumetric flasks and resultant solution was diluted up to the mark with diluent to obtain final concentration of 20-100µg/ml. These solutions were injected into chromatographic system. The chromatograms were
obtained and peak area was determined for each concentration of drug solution. Calibration curve of Tamsulosin Hydrochloridewas constructed by plotting peak area vs. applied concentration. The slope, intercept, and correlation coefficient were also determined. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range. Calibration curve was represented in the Figure 4.6. The result was reported in the Table 4.3. Chromatograms representing the linearity were shown in the Figures 4.7-4.9.

### Table 4.3: Calibration data of TSH

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Peak area Injection 1</th>
<th>Peak area Injection 2</th>
<th>Peak area Injection 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>4566676</td>
<td>4566745</td>
<td>4566664</td>
</tr>
<tr>
<td>40</td>
<td>7996842</td>
<td>7996792</td>
<td>7997542</td>
</tr>
<tr>
<td>60</td>
<td>11874318</td>
<td>11793416</td>
<td>11864314</td>
</tr>
<tr>
<td>80</td>
<td>15986698</td>
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</tr>
<tr>
<td>100</td>
<td>20103213</td>
<td>20104327</td>
<td>20105634</td>
</tr>
</tbody>
</table>

| Slope(m) | 18412 |
| Intercept(c) | 19807 |
| Correlation coefficient($R^2$) | 0.999 |

$$y = 19807x + 18412$$

$$R^2 = 0.999$$

![Figure 4.6: Standard Calibration Curve of TSH](chart.png)
Figure 4.7: Chromatogram showing Linearity of TSH Inj-1

Figure 4.8: Chromatogram showing Linearity of TSH Inj-2

Figure 4.9: Chromatogram showing Linearity of TSH Inj-3
4.5.4 Precision:

Purpose:
The purpose of this study is to establish that the developed RP-HPLC method is precise for the analysis of TSH in Pharmaceutical formulations. The precision of the method was verified by performing repeatability and intermediate precision studies.

Repeatability:
The precision of the method was assessed by the six replicate injections of the 100% test concentration (100μg/ml) and analyzed at 280nm on the same day and %relative standard deviation (%RSD) was calculated. The result was reported in the Table 4.4. Chromatogram representing the precision was shown in the Figure 4.10.

Intermediate Precision:
The precision of the method was assessed by the six replicate injections of the 100% test concentration (100μg/ml) and analyzed at same time on three different days at their selected analytical wave length of 280nm. The variation of the results on different days was analyzed and %RSD was calculated. The result was reported in the Table 4.5. Chromatogram representing the precision was shown in the Figure 4.11.

Table 4.4: Repeatability data of TSH

<table>
<thead>
<tr>
<th>S.no</th>
<th>Retention time (min)</th>
<th>Peak area</th>
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<tbody>
<tr>
<td>1</td>
<td>3.64</td>
<td>20614851</td>
</tr>
<tr>
<td>2</td>
<td>3.63</td>
<td>20595671</td>
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<td>3</td>
<td>3.64</td>
<td>20706573</td>
</tr>
<tr>
<td>4</td>
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<td>21021132</td>
</tr>
<tr>
<td>5</td>
<td>3.60</td>
<td>20615841</td>
</tr>
<tr>
<td>6</td>
<td>3.64</td>
<td>20537856</td>
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<tr>
<td>Mean</td>
<td>3.64</td>
<td>20681987</td>
</tr>
<tr>
<td>%RSD</td>
<td>-</td>
<td>0.845</td>
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</table>

Figure 4.10: Chromatogram Showing Repeatability of TSH
Table 4.5: Intermediate Precision Data of TSH

<table>
<thead>
<tr>
<th>S.No</th>
<th>Peak area</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
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<td>20615849</td>
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<td>6</td>
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<td>Mean</td>
<td></td>
<td>371825</td>
<td>379674</td>
<td>381143</td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td>1.30</td>
<td>1.38</td>
<td>1.42</td>
</tr>
</tbody>
</table>

Figure 4.11: Chromatogram Showing Intermediate Precision of TSH

4.5.5 Accuracy:

Purpose:
The purpose of this study is to express the extent of closeness of the results obtained by the proposed method to that of true value. Recovery studies were performed by standard addition method for verifying the accuracy of the proposed method. A known quantity of pure drug was added to pre-analysed sample and contents were reanalysed by the proposed method and the percentage recovery was reported.

Procedure:

Preparation of 80% recovery sample:

3.2ml of standard stock solution ‘B’ (32μg/ml) was added to the 4.0ml (40μg/ml) of sample stock solution in a 10ml volumetric flask and volume was made up to the mark with mobile phase to get 80% recovery sample (72μg/ml).
Preparation of 100% recovery sample

4.0ml of standard stock solution ‘B’ (40µg/ml) was added to the 4.0ml (40µg/ml) of sample stock solution in a 10ml volumetric flask and diluted up to the mark with mobile phase to get 100% recovery sample (80µg/ml).

Preparation of 120% recovery sample

4.8ml of standard stock solution ‘B’ (48µg/ml) was added to the 4.0ml (40µg/ml) of sample stock solution in a 10ml volumetric flask and diluted up to the mark with mobile phase to get 120% recovery sample (88µg/ml).

The solutions were filtered through 0.45µ membrane filter and then they were subjected to analysis by RP-HPLC method under the described chromatographic conditions. Recovery studies were carried out in triplicate at each level. The results obtained were compared with expected results and were statistically validated. The result was reported in the Table 4.6. Chromatograms representing the % recovery were shown in the Figures 4.12-4.14.

Table 4.6: Accuracy Study Data of TSH

<table>
<thead>
<tr>
<th>Recovery Level</th>
<th>Amount added(µg/ml)</th>
<th>Amount found(µg/ml)</th>
<th>% recovery (% w/w)</th>
<th>SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard Test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80%</td>
<td>32 40</td>
<td>72.016</td>
<td>100.02%</td>
<td>0.100</td>
<td>0.139</td>
</tr>
<tr>
<td>100%</td>
<td>40 40</td>
<td>79.963</td>
<td>99.95%</td>
<td>0.213</td>
<td>0.267</td>
</tr>
<tr>
<td>120%</td>
<td>48 40</td>
<td>88.056</td>
<td>100.06%</td>
<td>0.162</td>
<td>0.184</td>
</tr>
<tr>
<td>Mean recovery</td>
<td></td>
<td>99.95-100.06% w/w</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.12: Chromatogram Showing Accuracy - 80%

Figure 4.13: Chromatogram Showing Accuracy - 100%

Figure 4.14: Chromatogram Showing Accuracy - 120%
4.5.6 Robustness:

**Purpose:**

The evaluation of robustness should be considered during the development phase and depends upon the type of procedure under study. The robustness of a method is its ability to remain unaffected by the deliberate variations in method parameters such as column temperature, analytical wavelength and flow rate.

**Procedure:**

Small changes in the operational conditions were allowed and the extent to which the method was robust was estimated. Deviations of ±2nm in the detection wavelength and ±0.2 ml/min in the flow rate were tried individually, and percentage Relative standard deviation was calculated statistically. The results were reported in the Tables 4.7-4.8. Chromatograms representing the robustness (variation in parameters) were shown in the Figures 4.15-4.18.

Solutions of 100% test concentration with the specified changes in the operational conditions were injected to the instrument in triplicate and the % RSD of the chromatographic peak area was separately for each variable.

<table>
<thead>
<tr>
<th>Flow rate</th>
<th>Peak area</th>
<th>Mean</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8ml/min</td>
<td>20718734</td>
<td>20714307</td>
<td>1387.912</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>20725432</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20698754</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2ml/min</td>
<td>19887646</td>
<td>1980822</td>
<td>165212.92</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>19769834</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>19864987</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.15: Chromatogram showing variation in Flow rate - 0.8ml/min
Figure 4.16: Chromatogram showing variation in Flow rate - 1.2ml/min

Table 4.8: Robustness Data of Change in Detection Wavelength

<table>
<thead>
<tr>
<th>Wave length</th>
<th>Peak area</th>
<th>Mean</th>
<th>SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>278nm</td>
<td>20498657</td>
<td>20488427</td>
<td>85006.83</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>20398768</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20567856</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>282nm</td>
<td>20925417</td>
<td>20853086</td>
<td>186697.35</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>20876858</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20756934</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.17: Chromatogram showing variation in Wavelength – 278nm
4.5.7 Limit of Detection (LOD):
It is the lowest concentration of analyte that can be detected, but not necessarily quantified, by the analytical method. In chromatography the detection limit is the injected amount that results in a peak with a height at least twice or three times as high as the baseline noise level (S/N ratio~3).

4.5.8 Limit of Quantitation (LOQ):
It is the lowest concentration of analyte that can be determined with acceptable accuracy and precision by the analytical method. LOQ was expressed as concentration of analyte generating an instrument response equivalent to ten times the noise (S/N ratio~10).

Limit of detection (LOD) and limit of quantitation (LOQ) were manually calculated from the slope of the calibration curve and standard deviation. The statistical validation data of LOD and LOQ were reported in the Table 4.9.

Table 4.9: LOD & LOQ data of TSH

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of detection (LOD)</td>
<td>1.12μg/ml</td>
</tr>
<tr>
<td>Limit of quantitation (LOQ)</td>
<td>3.55μg/ml</td>
</tr>
</tbody>
</table>
4.5.9 Assay of Marketed Formulation of Tamsulosin Hydrochloride

**Purpose:**
The purpose is to estimate the amount of TSH present in pharmaceutical formulations.

**Procedure**

20µL of 80µg/ml sample solution of Tamsulosin Hydrochloridewas injected into the chromatographic system and the chromatogram was recorded. Peak area was measured. The assay values were calculated using the regression equation.

The result was reported in the **Table 4.10.** Chromatogram representing the assay was shown in the **Figure 4.19.**

**Table 4.10: Assay Data of Tamsulosin Hydrochloride Marketed Formulation**

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Lable claim (mg)</th>
<th>Amount recovered (mg)</th>
<th>% Amount found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veltam</td>
<td>0.4</td>
<td>0.395</td>
<td>98.75</td>
</tr>
</tbody>
</table>

**Figure 4.19: Chromatogram showing assay of TSH**

4.6 RESULTS AND DISCUSSION:

A RP-HPLC method has been developed and validated by the author for estimation of Tamsulosin Hydrochloride. The proposed RP-HPLC method were validated as per the International Conference on Harmonisation (ICH) Q2B Guidelines, and was found to be applicable for routine quantitative analysis of Tamsulosin Hydrochlorideby RP-HPLC using UV detector in pharmaceutical dosage forms. The author has developed this method based on the use of Agilent C18 column and mobile phase composition of Acetonitrile and methanol in ratio 40:60 v/v. The method was validated over a linear concentration range of 20-100 µg/ml and with a correlation coefficient of \( R^2 \) - 0.999. The %RSD of the peak response of five replicate injections of standard concentration...
was found to be below 2, indicating that the proposed method is precise. The percentage recoveries of active pharmaceutical ingredient (API) from dosage forms ranged from 98.3%-100.4% w/w for Tamsulosin Hydrochloride indicating that the proposed method to be accurate. The LOD & LOQ values were found to 1.12µg/ml and 3.55µg/ml respectively for Tamsulosin Hydrochloride indicating the sensitivity of the method. Thus the results of analysis of pharmaceutical formulations reveal that the proposed method is suitable for their analysis with virtually no interference of the usual additives presented in pharmaceutical formulations. The proposed method (RP-HPLC) is simple, sensitive and reliable and can be used for the routine determination of Tamsulosin Hydrochloride in bulk samples and pharmaceutical formulations depending upon the need of the specific and arising situation.

4.7 CONCLUSION
A simple, specific, accurate and precise reverse phase high performance liquid chromatography method has been developed which can be used for accurately quantitative estimation of Tamsulosin Hydrochloride for routine analysis of individual and combination of drugs. Method was validated as per ICH Q2 (R1) so it can be used by pharmaceutical industries.