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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TAMSULOSIN HCL AND TOLTERODINE TARTRATE IN COMBINED PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple and selective RP-HPLC method was developed for the simultaneous determination of Tamsulosin hydrochloride and Tolterodine tartrate tablet dosage forms. Chromatographic separation was achieved on an Inertsil ODS C18 column (4.6 mm × 250 mm, 5 µm) using mobile phase consisting of a mixture of phosphate buffer (KH_2PO_4) and Acetonitrile (CH_3CN) in the ratio 50:50 v/v, with detection of 214 nm. Retention time was estimated to be 2.81 min and 4.28 min for Tamsulosin Hydrochloride & Tolterodine tartrate respectively. Accuracy was found to be 100.46% and 99.6% for Tamsulosin HCL and Tolterodine tartrate respectively. The linearity was observed over a range of 0.25-0.75µg/ml for Tamsulosin HCl and 2.5-7.5µg/ml for Tolterodine tartrate. In precision relative standard deviation values for both was found to be less than 2.0%. The designed method was found to be simple, specific, accurate, linear and precise. It can be used for regular analysis for the simultaneous estimation of Tamsulosin hydrochloride and Tolterodine tartrate tablet dosage forms.

KEYWORDS: Tamsulosin HCl, Tolterodine tartrate, RP-HPLC, Method development, Validation.

INTRODUCTION

Tamsulosin is chemically 5 – [(2R) – 2- [[2-(2-ethoxy phenoxy) ethyl] amino] propyl] – 2– methoxybenzene-1-sulfonamide hydrochloride, a selective antagonist at α_{1A} and α_{1B} adrenoceptors in the prostate, prostatic capsule, prostatic urethra and bladder neck. At least three discrete α_1-adrenoceptor subtypes have been identified such as: α_{1A}, α_{1B} and α_{1D}; their distribution differs between human organs and tissues. Approximately 70% of the α-1 receptors in human prostate are of the α_{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.[1] Tamsulosin hydrochloride is extensively metabolized by cytochrome P_{450} 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. On administration of the radio labeled dose of tamsulosin hydrochloride to four healthy volunteers, 97% of the administered radioactivity was recovered, with urine (76%) representing the primary route of excretion compared to faeces (21%) over 168 hours. Used in the treatment of signs and symptoms of benign prostatic hyperplasia.

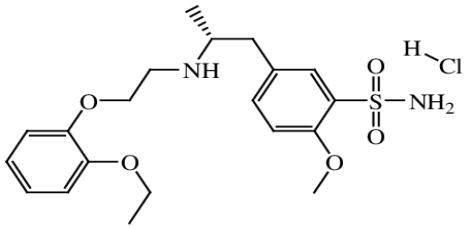

Fig 1: Chemical Structure of Tamsulosin hydrochloride.

Tolterodine is chemically (R)-N, N-di-isopropyl- 3-(2- hydroxy-5-methylphenyl)-3-phenylpropanamine L-hydrogen Tartrate, an antimuscarinic drug that is used to treat urinary incontinence. Tolterodine acts on M_2 and M_3 subtypes of muscarinic receptors.[2] Tolterodine and its active metabolite, 5-hydroxymethyltolterodine, acts as competitive antagonist at muscarinic receptors. This antagonism results in inhibition of bladder contraction, decrease in detrusor pressure, and an incomplete emptying of the bladder. Following administration of a 5-mg oral dose of 14C-tolterodine solution to healthy volunteers, 77% of radioactivity was recovered in urine and 17% was recovered in faeces in 7 days. It is used for the treatment of overactive bladder.

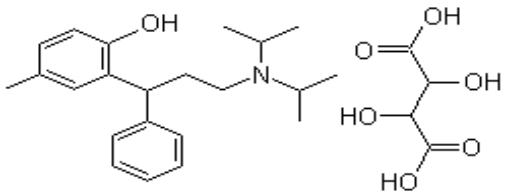

Fig 2: Chemical Structure of Tolterodine tartrate.

Our present study aimed at developing and validating a RP-HPLC method, being simple, accurate and selective and the proposed method can be used for the estimation of these drugs in combined dosage forms.

MATERIALS AND METHODS

Chemicals and Reagents

Tamsulosin hydrochloride and Tolterodine tartrate were purchased from Sigma-Aldrich. Acetonitrile was purchased from Merck. Water (HPLC Grade) was obtained from a Milli-QRO water purification system. Potassium dihydrogen ortho phosphate (A.R. grade) was procured from Merck.

Instrumentation

HPLC instrument Waters e-2695 equipped with photodiode array detector. Using a digital pH meter (Mettler Toledo) checked the pH of the solution.

Chromatographic Conditions

In RP-HPLC, the stationary phase is non - polar and the mobile phase is polar, nonpolar compounds are retained for longer periods as they have more affinity towards the stationary phase. Hence, polar compounds travel faster and are eluted first.[3-6] Chromatographic separation was achieved on an Inertsil ODS C18 (4.6 mm × 250 mm, 5 µm) column using mobile phase consisting of a mixture of phosphate buffer (KH_2PO_4) and Acetonitrile (CH_3CN) in the ratio 50:50 v/v, with detection of 214 nm.

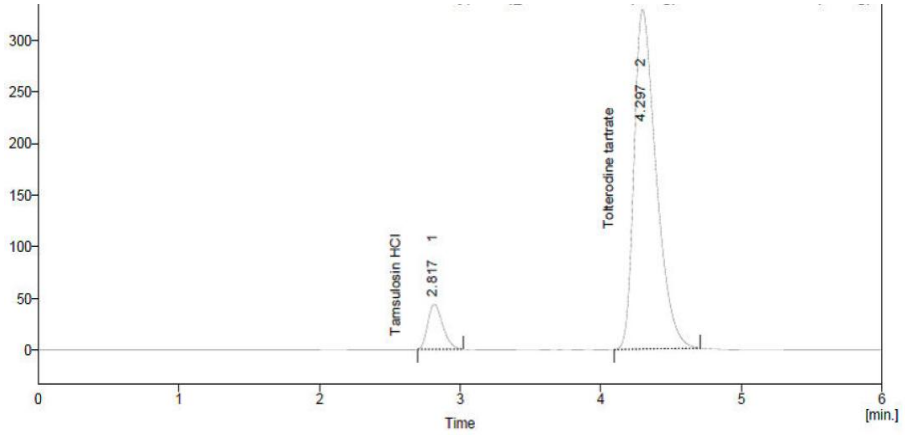

Fig 3: Chromatogram of Tamsulosin hydrochloride and Tolterodine tartrate.

Preparation of stock solution

Weigh accurately 100 mg each of Tamsulosin hydrochloride and Tolterodine tartrate and transfer to a clean, dry 100 ml of volumetric flask, dissolved and diluted up to the mark by using mobile phase as diluent. This results in 1.0mg/mL solution.

Preparation of standard solution

Take 0.1mL of stock solution in a 10mL volumetric flask containing 5mL of diluent, dissolve and dilute to 10ml with diluent to make 1µg/mL of standard solution.

Preparation of sample solution

20 tablets of each containing 0.4mg and 4mg of Tamsulosin hydrochloride and Tolterodine tartrate respectively were weighed and taken into a mortar uniformly mixed. From the uniform mixture, 100mg of the sample was transferred to a volumetric flask, dissolved and diluted to volume with diluent. This solution results in 0.5µg/ml and 5 µg/ml of Tamsulosin hydrochloride and Tolterodine tartrate respectively. Different sample solutions ranging from 50-150% were prepared.

Method validation

The method was validated as per ICH and FDA guidelines, and the validation parameters included specificity, linearity, range, accuracy and precision.

Specificity

Specificity is the ability to assess accurately the analyte in the presence of components which may be expected to be present in the sample matrix. Typically, these might include impurities, degradants, matrix, etc. It is a measure of the degree of interference from such

other things such as other active ingredients, excipients, impurities, and degradation products, ensuring that a peak response is due to a single component only. Specificity is divided into two separate categories: identification and assay/ impurity tests. For identification purpose, specificity is demonstrated by the ability to discriminate between compounds of closely related structures or comparison to a known reference standard. For assay/ impurity tests, specificity is demonstrated by the resolution of the two closely eluting compounds. These compounds are usually the major component or the active ingredient and an impurity.[7-9]

Linearity and range

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line. Range is the (inclusive) interval between the upper and lower levels of analyte concentration that have been demonstrated to be determined with precision, accuracy, and linearity using the method. The range is normally expressed in the same units as the test results obtained by the method. A minimum of five concentration levels, along with certain minimum specified ranges are to be determined. For assay tests, the minimum specified range is 80-120% of the target concentration. For impurity tests, the minimum range is from the reporting level of each impurity to 120% of the specification.[7-9] The relationship between the % concentration and area of drug in sample should be linear in the specified range and the correlation should not be less than 0.9.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy should be established across the specified range of the analytical procedure.[9] Accuracy is measured as the percentage of the analyte recovered by assay, spiking samples in a blind study. Accuracy studies for drug substance and drug product are recommended to be performed at the 80, 100 and 120% levels of label claim as stated in the Guidelines for Submitting Samples and Analytical Data for Methods Validation Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g., three concentrations / three replicates each of the total analytical procedure). Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.[10-12] The acceptance criterion for

accuracy is the Relative Standard Deviation (RSD) for all the recovery values should not be more than 2.0%.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.[7-9] The relative standard deviation (RSD) for the assay of six sample preparations should not be more than 2.0%.

System Suitability

System suitability tests are an integral part of gas and liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as a whole. System suitability is the checking of a system to ensure system performance before or drying the analysis unknowns. Parameters such as plate count, tailing factor, resolution and reproducibility (% RSD retention time and area for repetitive injections) are determined and compared against the specifications set for the method. These parameters are measured during the analysis of a system suitability sample that is a mixture of main components and expected by-products.

Optimized chromatographic conditions

Mobile phase: KH_2PO_4(30mM) of P^H 3.5 and Acetonitrile in the ration of 50:50(v/v), Inertsil ODS C18 (250×4.6mm× 5µ) column maintained at a temperature of 25^0C, Flow rate 1.2ml/min and UV detection at 214nm

Observation

The peak Asymmetry factor was less than 2 for both Tamsulosin hydrochloride and Tolterodine tartrate and the efficiency was also good (>2000). The retention time was also satisfactory for both Tamsulosin hydrochloride and Tolterodine tartrate.

RESULTS AND DISCUSSION

The validation of the designed chromatographic method was performed as ICH and USP guidelines. System suitability was performed to demonstrate the repeatability, linearity was performed to demonstrate the concentration range around which the method is applicable for. Accuracy was demonstrated by running 2 concentration levels above and below the test concentration and the recovery was established. Precision and assay were also performed and the results were tabulated.

System suitability

System suitability and chromatographic parameters were validated such as retention time, area, tailing factor and number of theoretical plates were calculated.

Linearity

Linearity was evaluated by linear regression analysis and calculated by least square method by preparing standard solutions of Tamsulosin and Tolterodine at different concentration levels. Absorbance of the resulting solutions were measured and the calibration curve was plotted between absorbance Vs concentration of drug. The results were found to be linear in the range 0.25-0.75µg/ml and 2.5-7.5µg/ml for Tamsulosin and Tolterodine respectively.

Accuracy

Accuracy was performed for various concentrations of Tamsulosin and Tolterodine equivalent to 50%, 75%, 125% and 150% of the standard were injected into the HPLC system.

Precision

Sample and standard solutions were prepared and injected into the HPLC system as per the test procedure.

Robustness

Robustness was performed by small deliberate changes in the chromatographic conditions and retention time was noted. The factors selected were flow rate and variation in the column temperature. The results remained unaffected by small variations in these parameters.

The data is given in the table 1.

Table 1: Validation Results.

S. No	Parameter		Tamsulosin	Tolterodine
1	System suitability	Retention time	2.81	4.29
		Area	330.045	3631.93
		Plate Height	3546	3025
		Tailing Factor	1.481	1.886
2	Linearity	Range	0.25-0.75µg/ml	2.5-7.5µg/ml
		Correlation	0.999	0.999
3	Accuracy	50%	100.93	99.96
		75%	100.39	99.89
		125%	100.42	99.96
		150%	100.46	99.89
4	Precision	% RSD	0.46	0.11
5	Assay	% Assay	99.25	98.15
6	Robustness	Flow Rate	1ml/min	1ml/min
		Std. Deviation	4208.36	4298.1
		% RSD	0.504	0.922
		Flow Rate	1.1 ml/min	1.1 ml/min
		Std. Deviation	18.06	394
		% RSD	0.002	0.007
		Column Temperature	25°C	25°C
		Std. Deviation	7.6	34.4
		% RSD	0.001	0.001
		Column Temperature	30°C	30°C
		Std. Deviation	51.4	450.1
		% RSD	0.006	0.009

CONCLUSION

From the above experimental results and parameters, it was concluded that, this newly developed RP-HPLC method for the simultaneous estimation of Tamsulosin hydrochloride and Tolterodine tartrate was found to be simple, sensitive, accurate, precise and rapid. The mobile phases were simple to prepare and economical. High resolution and shorter retention time made this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries and in approved testing laboratories.

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