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Development and validation of RP-HPLC method for determination of Raloxifene Hydrochloride from pharmaceutical preparation

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ABSTRACT

A simple, efficient, and reproducible method for the determination of raloxifene hydrochloride in tablets has been developed using reverse phase high performance liquid chromatographic method. The elution was done using a mobile phase consisting of buffer and acetonitrile (60:40 v/v) on Symmetry C18, 4.6 x 150mm, 5 μ m, XTerra analytical column with flow rate of 0.8 ml/min with detection at 287 nm. An external standard calibration method was employed for quantitation. The elution time was 4.890 min. The linearity range was 10-50 mg/ml for raloxifene hydrochloride. The present method was validated with respect to system suitability, linearity, precision, limit of detection (LOD) and limit of quantification (LOQ), accuracy (recovery), Robustness. The proposed method can be readily utilized for bulk drug and pharmaceutical formulations.

Key Words: RP-HPLC, Raloxifene hydrochloride, XTERRA Column, method development and validation.

INTRODUCTION

Raloxifene Hydrochloride is chemically 6-hydroxy-2-(4-hydroxyphenyl)- benzothiophen-3-yl]-[4-[2-(1-piperidyl)ethoxy]phenyl] -methanone[1]. A second generation selective estrogen receptor modulator (SERM) used to prevent osteoporosis in postmenopausal women. It has estrogen agonist effects on bone and cholesterol metabolism but behaves as a complete estrogen antagonist on mammary gland and uterine tissue.

The literature survey[2,3,4,5,6] reveals that there is some HPLC methods have been reported. The aim of the present study was to develop and validate a simple, isocratic RP-HPLC[7,8,9] method for the determination of Raloxifene Hydrochloride in tablets. The developed method was validated using ICH guidelines for validation[10].

Today, RP-HPLC is the most popular analytical technique for separating complex mixtures in the chemical, pharmaceutical and biotechnological industry. RP-HPLC is the opposite of normal-phase chromatography, with a nonpolar stationary phase and a polar, largely aqueous mobile phase. The most common stationary phases used are octadecyldimethyl (C₁₈) phases with silica as the solid support. Silica has a small pH range (3 to 8) where mixtures can be separated without degradation of the column performance. Above pH 8, silica supports dissolve and destroy the column. Below pH 3, the silicon-carbon bond is cleaved, and the column is destroyed. The separation is achieved by analytes having different interactions with the stationary phase. In RP-HPLC, solutes are separated using their hydrophobicity. A more hydrophobic solute will be retained on the column longer than a less hydrophobic one. Also, polar solutes will interact with the silica surface to cause peak tailing. The mobile phase is one of the two components involved in the separation process. Water is generally one of the components of a binary mixture in RP-HPLC. Water is considered to be the weak component of the mobile phase and does not interact with the hydrophobic stationary phase chains.

The RP-HPLC method reported in this study was validated in accordance with the International Conference on Harmonization (ICH) guideline[11] and best practice[12,13,14]. Specificity, linearity, precision (repeatability and intermediate precision), accuracy, robustness, limit of detection and limit of quantitation were evaluated.

EXPERIMENTAL SECTION

Acetonitrile (HPLC grade), Raloxifene Hydrochloride as the reference standard was purchased from Matrix laboratories, Distilled water was de-ionised by using a Milli-Q system (Millipore), Ralista tablets and the chemicals of analytical reagent grade purchased from various sources, Pump (Waters Alliance 2695), Detector (UV – Visible Model 2487), Injector Autosampler (20 μ l), The Column C₁₈ XTERRA (150mm), Elio PH – Meter, A & D – Digital Balance.

Chromatographic conditions

The mobile phase consisted of a mixture of buffer- acetonitrile (60:40 v/v). The flow rate was set to 0.8 ml /min, Injection volume 20 μ l, The Column used is C₁₈ XTERRA (150mm). The detection wavelength was set to be at 287 nm. RP-HPLC analysis was performed isocratically at room temperature.

Method Development:[15,16]

Preparation of Standard Solution

Accurately weighed and transferred 10mg of Raloxifene hydrochloride Working standard into a 100 ml volumetric flask and added about 70 mL of Diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further pipetted 1 ml of the stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. Mixed well and filtered through 0.45 μ m filter.

Preparation of Sample Solution

Weighed 5 Raloxifene hydrochloride Tablets and calculated the average weight. Accurately weighed and transferred the sample equivalent to 10 mg of raloxifene hydrochloride into a 100 ml volumetric flask. Added about 70 ml of diluent and sonicated to dissolve it completely and made volume up to the mark with diluent. Mixed well and filtered through 0.45 μ m filter. Further pipetted 1ml of the stock solution into a 10ml volumetric flask and diluted up to the mark with diluent. Mixed well and filtered through 0.45 μ m filter

RESULTS AND DISCUSSION

HPLC separation of Raloxifene hydrochloride was carried out on a Xterra C18 column by an isocratic elution with buffer-acetonitrile (60:40 v/v). The flow rate was constant at 0.8 ml/min and the column temperature was at room temperature (24 \pm 1 $^{\circ}$). The UV wavelength was set at 287 nm. No interference from diluents, impurities, or excipients present in the pharmaceutical formulation was observed at this detection wavelength. Before each run LC column was equilibrated with the mobile phase for about 15 min. A sharp, symmetrical peak was obtained for Raloxifene hydrochloride when analyzed under these conditions. This retention time enable rapid determination of the drug, which is important for routine quality control analysis.

System suitability test was established from five replicate injections of a solution containing Raloxifene hydrochloride 10 μ g/ml. The percent relative standard deviation (RSD) of the peak area was calculated. The peak tailing for drug was measured. A useful and practical measurement of peak shape, the peak tailing and theoretical plate count was determined. Column plate number was determined using the formula, $N = 5.54(t_R/w_h)^2$, where w_h is the bandwidth at 50% of peak height. The proposed method met these requirements within the United States Pharmacopoeia (USP) accepted limits (Tailing factor < 1.5, Theoretical plates > 2000). The stability of Raloxifene hydrochloride in solution was investigated in the method development phase. five solutions containing 10 μ g/ml of Raloxifene hydrochloride were tested. The solutions were stable during the investigated time and the RSD was < 1.0% for retention time (min), peak area and height. The solutions were shown to be stable with no significant change in Raloxifene hydrochloride concentration over this period.

Method validation:[17,18,19,20]

Linearity:

Appropriate amounts of Raloxifene hydrochloride stock solutions were diluted with mobile phase to give concentration of 10, 20,30,40 and 50 μ g/ml. Each solution was injected calibration plot was prepared. Linearity was evaluated by linear least-squares regression analysis. Good linearity was observed over the concentration range evaluated (10-50 μ g/ml) as shown in the linearity curve in figure-3. The correlation coefficient was found 0.999.

Precision:

The precision of the method was investigated with respect to repeatability and intermediate precision. The repeatability (intra-day precision) of the method was evaluated by assaying five replicate injections of the Raloxifene hydrochloride at 100% of test concentration (30 μ g/ml) on the same day. The %RSD of the retention time (min) and peak area were calculated.

Table 1: Separation characteristics of Raloxifene Hydrochloride analysed under optimized conditions and method validation of Raloxifene Hydrochloride

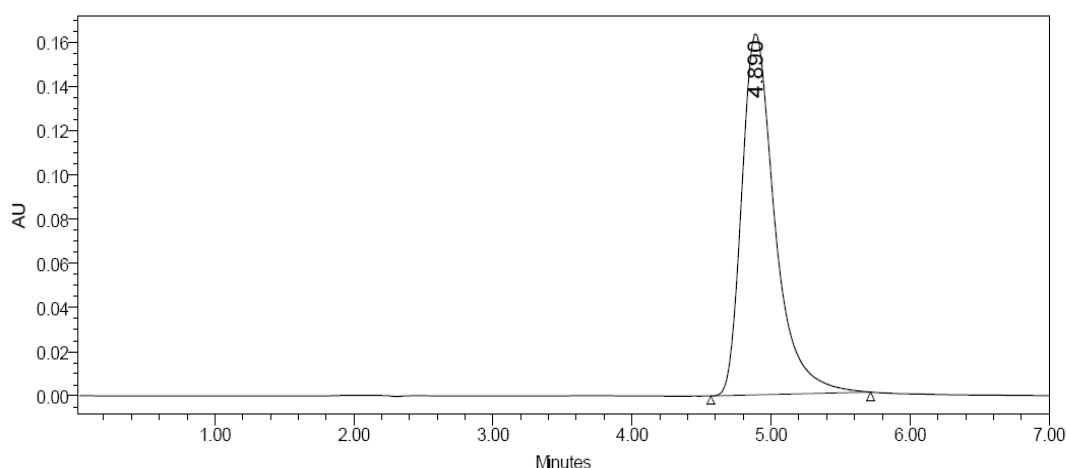
PARAMETERS	LIMIT	OBSERVATION
System suitability	Theoretical Plates should not less than 2000. Tailing factor should not more than 2.0	Theoretical plates: 2215.8 Tailing factor: 1.5
Precision: A) System Precision B) Method precision	RSD NMT 2.0% RSD NMT 2.0%	0.32 0.03
Linearity	Correlation coefficient NLT 0.99	0.999
Accuracy	%Recovery range- 98-102 %	99.8%
Robustness(Flow, Mobile phase)	System suitability parameters should comply	complies
LOD	S:N Ratio should be about 3	2.96
LOQ	S:N ratio should be about 10	9.96

The system is suitable for tailing factor, theoretical plate, and resolution.

TABLE 2: Recovery studies of Raloxifene Hydrochloride from samples with known concentrations

%Concentration (at specification Level)	Area	Amount Added ($\mu\text{g/ml}$)	Amount Found ($\mu\text{g/ml}$)	% Recovery	Mean Recovery
50%	1368358	5.0	5.05	101.0%	99.8%
100%	2702064	10.0	9.97	99.7%	
150%	3979124	14.9	14.7	14.7%	

*n=3

Figure 1: Typical LC chromatogram of the Raloxifene hydrochloride sample for the system suitability

Intermediate precision (inter-day precision) was demonstrated by evaluating the relative peak area percent data the LC system at three different concentration levels (50%, 100%, and 150%) that cover the assay method range (5-25 $\mu\text{g/ml}$). The %RSD of the system was calculated from the individual relative percent peak area mean values at the 50%, 100%, and 150% of the test concentration. The intra-day (n= 5) and inter-day (n= 3) %RSD are given in table. All the data are within the acceptance criteria of 2% .

Figure 2: Typical LC chromatograms obtained for precision (a),(b) are for precision (c),(d) are for intermediate precision

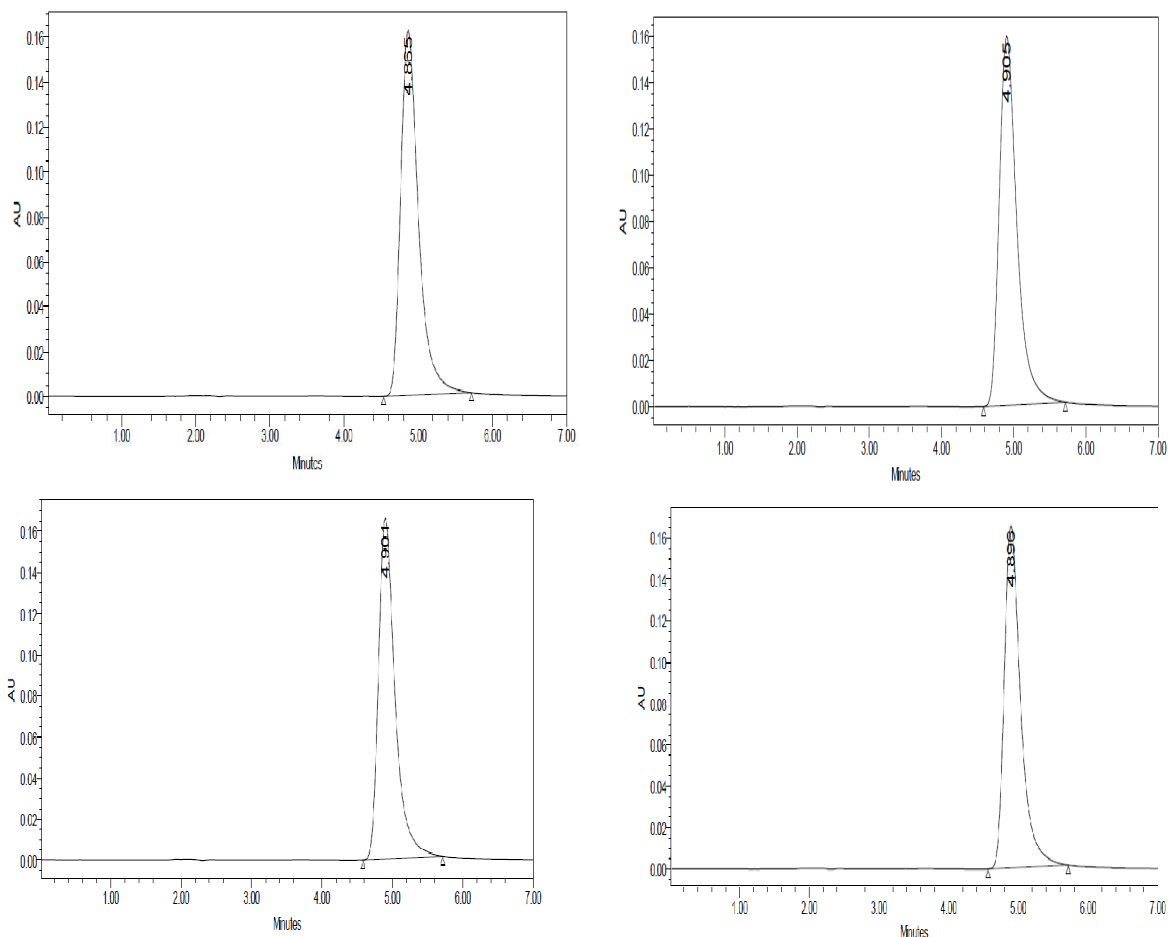
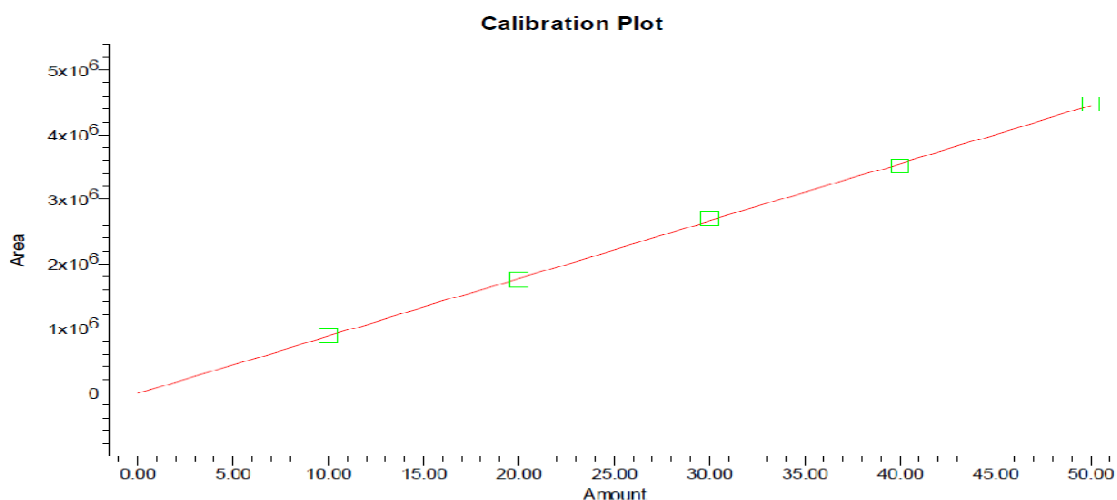


TABLE 3: Robustness of the method

conditions	Value	System suitability Results	
		USP Plate count	USP Tailing
Mobile phase ($\pm 10\%$)	50:50	2916	1.3
	60:40	2208	1.5
	70:30	2697	1.4
Flow rate(ml/min)	0.6	2828	1.5
	0.8	2208	1.5
	0.4	2661	1.4

Accuracy:

Accuracy of the method was evaluated by fortifying a Raloxifene hydrochloride sample solution (with respect to the target assay concentration) with three known concentrations of reference standard (10, 20 and 30 $\mu\text{g/ml}$). Percent recoveries were calculated from differences between the peak areas obtained for fortified and unfortified solutions. Good recoveries were obtained within the acceptance criteria (98.0-102.0%) as shown in Table-2. No significant differences were observed between amounts of Lamivudine added and the amounts found.

Figure 3: The linearity curve for the Raloxifene hydrochloride sample (10 to 50 μ g/ml)

LOD & LOQ:

The limit of detection (LOD) and limit of quantitation (LOQ) tests for the procedure were evaluated by serial dilutions of Raloxifene hydrochloride stock solutions in order to obtain signal-to-noise ratios (s/n) of $\approx 3:1$ and $\approx 10:1$, respectively. The LOD value for Raloxifene hydrochloride was found to be 0.03 μ g/ml (s/n = 2.96,) and LOQ (n =6) was 0.10 μ g/ml (s/n = 9.96) as shown in Table-1

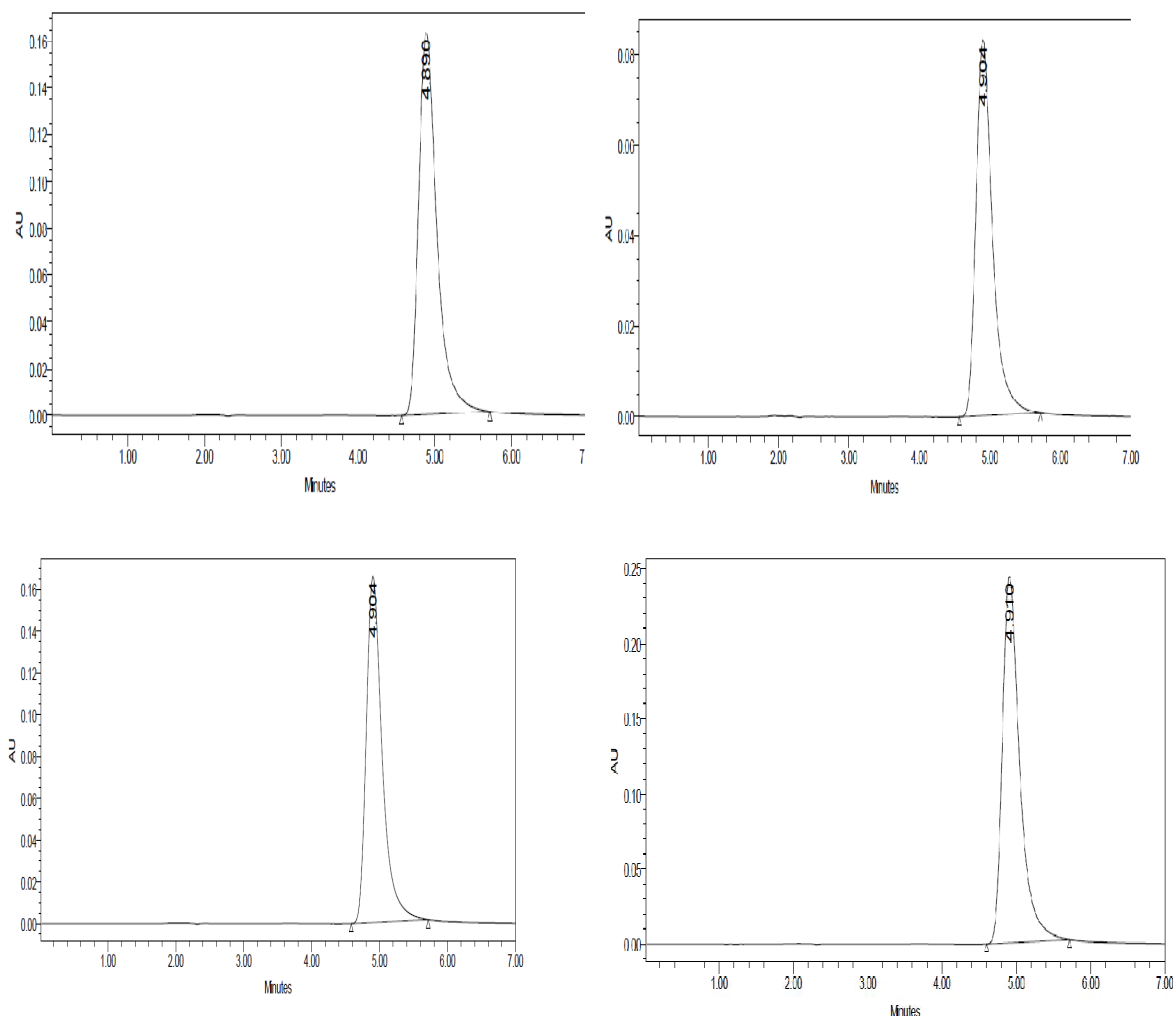
Robustness

Robustness of the method was evaluated by the analysis of Raloxifene hydrochloride under different experimental conditions such as changes in the organic composition of the mobile phase and flow rate. The percentage of methanol in the mobile phase was varied $\pm 10\%$, the flow rate was varied ± 0.2 ml/min. Their effects on the USP plate count, USP tailing at 10%, recovery and repeatability were studied. Deliberate variation of the method conditions had no significant effect on assay data or on chromatographic performance, indicating the robustness of method and its suitability for routine use and transfer to other laboratories. The results from robustness testing are presented in Table-3

CONCLUSION

A RP-HPLC method with UV detection for the assay of Raloxifene hydrochloride was developed and validated. The results showed that the method is very selective, no significant interfering peak was detected; accurate, with the percentage recoveries $> 99\%$; and reproducible, with the %RSD $< 1\%$. The method was sensitive; a little as 0.03 μ g/ml could be detected with the LOQ of 0.10 μ g/ml. The method involves use of a simple buffer with the acetonitrile and minimum sample preparation, encouraging its application in quality control for analysis of Raloxifene hydrochloride in bulk samples, raw materials and final dosage forms.

Figure 4: Typical chromatogram of accuracy for standard and sample, (a) standard; (b),(c) and (d) are for sample concentrations of 50%, 100% and 150% respectively



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