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A NEW VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF RALOXIFENE IN PURE AND TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, rapid, sensitive and precise reverse phase high performance liquid chromatographic method has been developed for the estimation of raloxifene in pure and pharmaceutical dosage form. In this method RP-C_{18} column (100mmx4.6mm I.D., 3µm particle size) with mobile phase consisting of phosphate buffer pH 6.8 and acetonitrile in the ratio of 60:40v/v was used. The detection wavelength is 287nm and the flow rate 1.0ml/min. The linearity was found in the range of 20-120µg/ml and shows a correlation coefficient of 0.993. The optimized method was validated with respect to linearity, precision, accuracy, limit of detection and limit of quantitation. The proposed method is simple, fast, accurate, precise and reproducible hence can be applied for routine quality control analysis of raloxifene in pure and pharmaceutical dosage form.

Key words: Raloxifene, HPLC, Validation.

INTRODUCTION

Raloxifene hydrochloride[1] is an antiosteoporotic drug, first selective estrogen receptor modulator (SERM) for the prevention and treatment of osteoporosis in postmenopausal women. It affects the cycle of bone formation and breakdown in the body and reduces loss of bone tissue. It produces estrogen-like effects on bone, reducing resorption of bone and increasing bone mineral density in postmenopausal women. It is a polyhydroxylated non-steroidal benzothiophene compound[2]. It is chemically[3] [6-Hydroxy-2-(4-hydroxyphenyl)benzo[*b*]thien-3-yl][4-[2-(1-piperidinyl)ethoxy]-phenyl]methanonehydrochloride.

Literature survey reveals that various spectrophotometric[4-10], HPLC[11-17], LC-MS[18-19] and capillary electrophoresis[20] methods have been reported for the determination of raloxifene in pure and pharmaceutical dosage form. In this study a simple, rapid, accurate, sensitive and precise HPLC method was developed for the estimation of raloxifene in pharmaceutical dosage form.

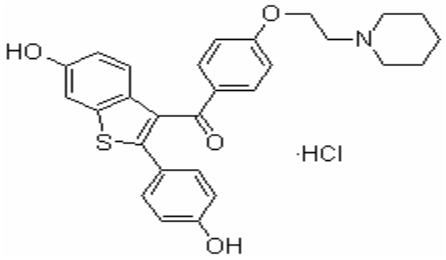

Fig.-1: Chemical structure of raloxifene hydrochloride

EXPERIMENTAL

Instrumentation

The separation was carried out on Waters HPLC system with Waters 2695 binary HPLC pump, Waters 2487 dual absorbance detector, Waters Empower software and RP-C_{18} column (100mmx4.6mm I.D; particle size 3µm).

Chemicals and reagents

Raloxifene hydrochloride was a gift sample by Glochem Industries Limited, Hyderabad. HPLC grade of acetonitrile was purchased from E. Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

HPLC conditions

The mobile phase consisting of phosphate buffer (pH 6.8 adjusted with orthophosphoric acid) and acetonitrile (HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 60:40v/v was pumped into the column at a flow rate of 1.0ml/min. The detection was monitored at 287nm and the run time was 6min. The volume of injection loop was 20µl prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system.

Working standard solution of raloxifene

About 100mg of raloxifene was weighed accurately and transferred into a 100ml volumetric flask. It was dissolved in 50ml of acetonitrile with sonication for 20min. and the volume was made up with a further quantity of the acetonitrile to get a 1mg/ml solution. From this, a working standard solution of the drug (100µg/ml) was prepared by diluting 1ml of the above solution to 10ml in a volumetric flask.

Procedure

A mixture of the phosphate buffer and acetonitrile in a 60:40v/v ratio was found to be the most suitable mobile phase for ideal separation of raloxifene. The solvent mixture was filtered through a 0.45µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0ml/min. The column was maintained at ambient temperature. The pump pressure was set at 800psi. The column was equilibrated by pumping the mobile phase through the column for at least 30min. prior to the injection of the drug solution. The run time was set at 6min. The detection of the drug was monitored at 287nm. 20µl of the working standard solution of raloxifene was injected into the column for six times and the corresponding chromatograms were obtained. Under these optimized chromatographic conditions the average retention time obtained for the drug was 3.814min. A typical chromatogram showing the separation of the drug is given in Fig. 2.

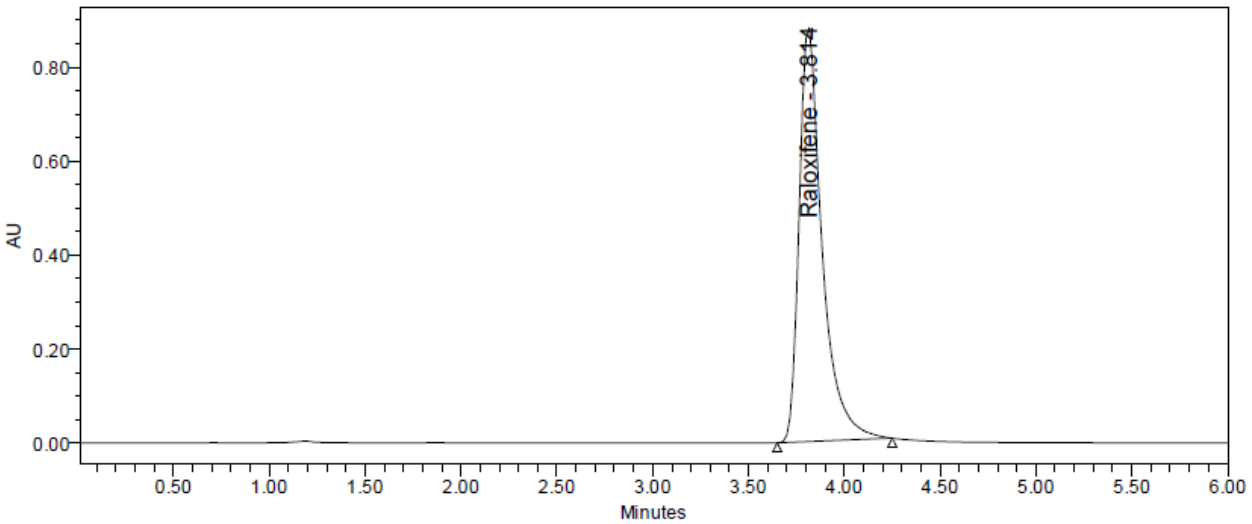

Fig.-2: Typical chromatogram of raloxifene

Calibration plot

From the working standard solution further dilutions ranging from 20-120µg/ml of the drug were prepared in 10ml volumetric flasks using the mobile phase. 20µl of each dilution was injected six times into the column at a flow rate of 1ml/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against corresponding peak area was found to be

linear in the concentration range of 20-120µg/ml of the drug. The relevant data are furnished in Table-1. The regression equation of this curve was computed. This equation was later used to estimate the amount of raloxifene in tablet dosage forms.

Table-1: Linearity data of the method

Concentration (µg/ml)	Mean peak area (n=6)
20	1524955
40	3297039
60	4369617
80	5803627
100	7488413
120	9897957

Validation of the proposed method

The system suitability parameters like selectivity, specificity, linearity, precision, accuracy, limit of detection and limit of quantitation were studied systematically to validate the proposed HPLC method for the determination of raloxifene. Selectivity of the method was assessed on the basis of elution of raloxifene using the above mentioned chromatographic conditions. The linearity was evaluated by linear regression analysis using the least square method. The precision was determined in terms of intra-day and inter-day precision. Solutions containing 25, 50 and 100µg/ml of raloxifene were subjected to the proposed HPLC analysis to check for intra-day and inter-day variation of the method. The intra-day and inter-day variation in the peak area of drug solution was calculated in terms of coefficient of variation (C.V.) obtained by multiplying the ratio of standard deviation to mean with 100. The relevant results are furnished in Table-2.

Table-2: Precision of the proposed HPLC method

Concentration of raloxifene (µg/ml)	Measured concentration of raloxifene (µg/ml)			
	Intra-day		Inter-day	
	Mean (n=3)	% C.V.	Mean (n=3)	%C.V.
25	25.14	0.38	25.12	0.87
50	49.09	0.27	50.40	0.64
100	100.15	0.64	100.25	0.93

The accuracy of the method was assessed by adding known amounts of solutions of raloxifene at 50, 100 and 150% levels of the standard drug solution and analyzing them by the proposed method. The recovery results are furnished in Table-3.

Table-3: Accuracy studies

Concentration	Amount added (mg)	Amount found (mg)	% Recovery	Mean
50%	50	50.23	100.50	100.13
100%	100	101.12	100.10	
150%	150	149.66	99.80	

The system suitability parameters computed for the method are given in Table-4.

Estimation of raloxifene in tablet dosage form

Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate raloxifene in tablet formulation. For this, twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 100mg of raloxifene was transferred to a 100ml volumetric flask. A 30ml quantity of acetonitrile was added to the flask and the contents of the flask were sonicated for 20 min. to ensure complete solubility of the drug. The volume was made up with the

acetonitrile and then the mixture was filtered through a 0.45µ membrane filter. From the filtrate, 1ml of aliquot was taken in a separate 10ml volumetric flask, the contents made up to the volume. This solution containing 100µg/ml of the drug was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table-5.

Table-4: System suitability parameters

Parameter	Result
Linearity ((µg/ml)	20-120
Correlation coefficient	0.993
Theoretical plates (N)	5270
Tailing factor	1.5
LOD (µg/ml)	0.006
LOQ (µg/ml)	0.021
Percentage recovery	100.13

Table-5: Assay and recovery studies

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Brand-1	60	60.78	101.3
Brand-2	60	59.49	99.15

RESULTS AND DISCUSSION

By applying the proposed method, the retention time of raloxifene in a typical chromatogram was found to be 3.814min., which indicates a good base line. The use of the mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 60:40v/v resulted in peaks with good shape and resolution. Linearity range was observed in concentration range of 20-120µg/ml. The regression equation of the linearity plot of concentration of raloxifene over its peak area was found to be y=-190379.53+79818.77x (r^2=0.993), where x is the concentration of raloxifene (µg/ml) and y is the corresponding peak area. The proposed HPLC method was also validated for intra-day and inter-day variation. When the solution containing 100µg/ml of raloxifene were repeatedly injected on the same day, the coefficient of variation in the peak area of drug for three replicate injections was found to be less than 1%. Also, the inter-day variation on three different days was found to be less than 1%. The number of theoretical plates was found to be 5270, which indicates efficient performance of the column. The limit of detection and limit of quantitation were found to be 0.006 and 0.021µg/ml respectively, which indicates that the method is quite sensitive. The high percentage of recovery of raloxifene ranging from 99.80 to 100.50 indicates that the proposed method is highly accurate. In the case of tablet formulation no interfering peaks were found in the chromatogram within the run time indicating that the excipients used did not interfere with the estimation of the drug by this method.

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the determination of raloxifene in pharmaceutical dosage form. Hence, this method can easily and conveniently adopt for routine quality control analysis of raloxifene in pure and its pharmaceutical dosage form.

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