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Research Article

GRADIENT RP-HPLC METHOD FOR THE DETRMINATION OF PURITY AND ASSAY OF RALOXIFENE HYDROCHLORIDE IN BULK DRUG

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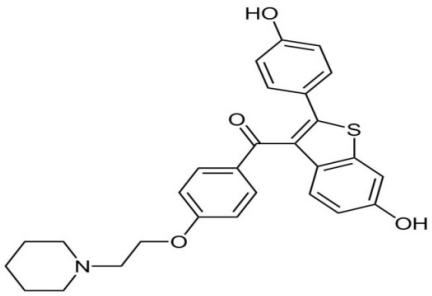
ABSTRACT

A rapid, sensitive, efficient, and reproducible method for the determination of Raloxifene hydrochloride (RLX) has been developed using reverse phase high performance liquid chromatographic method. This method involves separation of RLX on a reversed phase Kromosil C_{18} (150x4.6mm, 5µm) column using UV detection at 280 nm. The elution was done using a mobile phase consisting of acetonitrile and water (30:70 v/v) on SCHIMADZU 2010HT- HPLC equipment. An external standard calibration method was employed for quantitation. A survey of literature revealed spectrophotometric, capillary electrophoresis and a few chromatographic methods for the determination of Raloxifene hydrochloride (RLX) in bulk drug and also in plasma. We are now reporting a gradient RP-HPLC method for the Determination of Purity and Assay of Raloxifene Hydrochloride in bulk drug.

Keywords: Gradient, RP-HPLC, Raloxifene Hydrochloride, Acetonitrile.

INTRODUCTION



Structure of Raloxifene HCl

Raloxifene Hydrochloride, a non-steroidal selective estrogen receptor regulator, is currently applied to both the prevention and treatment of postmenopausal osteoporosis. Preservation of bone density, suppressioln of markers of bone turnover and maintenance of normal bone histology in postmenopausal women has been demonstrated in studies with Raloxifene. In addition to the effects of Raloxifene on bone, a number of beneficial non-skeletal effects have been reported on the breast, uterus and cardiovascular system. For either osteoporosis treatment or prevention, supplemental calcium and/or vitamin should be added to the diet if daily intake is inadequate. Purity determination and assay of Raloxifene hydrochloride was achieved by HPLC using reverse phase.The presence of impurities, even in small amounts, may affect the efficacy and safety of pharmaceuticals. Methods for detecting and controlling impurities are subject to continuous review and improvement. Characterisation of impurities is a crucial aspect of drug development and approval, and is central to quality control.

EQUIPMENT

LC-2010HT

Schimadzu 2010HT- HPLC (Auto Sampler), $BDSL_7$ 250 x 4.6mm, 5µm or equivalent.(Column for Related Substances), Kromosil c_{18}150 x 4.6mm, 5µm or equivalent and UV-VIS detector. System reliability has been improved by standardizing the line arrangement in order to integrate units. Further improvements to method transfer have been achieved by the pre-eminent flow rate accuracy, gradient concentration accuracy and reduction of equipment line capacity differences.

MATREIALS AND METHODS

Materials used

Raloxifene hydrochloride Standard, Raloxifene hydrochloride Sample, N-Oxide impurity, Impurity-H, Dimethoxy impurity, Acetonitrile, Phosphoric acid / Potassium Hydroxide, Phosphate Buffer, Milli-Q water, All the chemicals used were HPLC grade.

Method

Method developed from United States Pharmacopoeia (USP) for analysis of Raloxifene Hydrochloride by HPLC.

Estimation of Raloxifene

About 50 mg of Raloxifene was weighed accurately and transferred into a 50 ml volumetric flask and dissolved in 25 ml of methanol. The solution was sonicated for 15 min and the volume was made up with a further quantity of the methanol to get a 1 mg/ml solution. Subsequent dilutions of this solution ranging from 0.5-50 µg/ml were made in 10 ml volumetric flasks with the mobile phase. 20 µl of the solution was injected each time into the column, at a flow rate of 1ml/min. Each of the dilutions was injected 5 times into the column and the corresponding chromatograms were obtained. From these chromatograms, the retention times and the areas under the peaks of the drug were noted. The regression equation of the drug concentrations was computed. This equation was used to estimate the amount of Raloxifene in pharmaceutical dosage forms. To check the intra-day an inter-day variation of the method, solutions containing 10and 20 µg/ml of Raloxifene were subjected to the proposed HPLC method of analysis and the recoveries were noted.

The Chromatograph is Programmed as Follows

Time	Solution A (%)	Solution B (%)	Elution
0.00	75	25	Isocratic
9.00	75	25	Linear gradient
40.25	50	20	Linear gradient
42.25	75	25	Linear gradient
49.00	75	25	Equilibration

PREPARATION OF MOBILE PHASE

Preparation of solution-A Weigh accurately about 9.0gm of monobasic potassium phosphate and transfer into 1000ml of water and mix add 0.6ml of phosphoric acid further adjust with phosphoric acid or potassium hydroxide solution to a pH of 3.0±0.1 and mix.

Preparation of solution-B

Acetonitrile was used.

Mobile phase

Use variable mixtures of solution A and solution B as directed for chromatographic system. Diluent is repare a mixture of solution A and acetonitrile.

Preparation of system suitability stock solution

Weigh accurately about 6mg of Raloxifene hydrochloride into standard 50ml volumetric flask and add 15ml of acetonitrile, 3.0ml of water and 5.0ml of 30 percent hydrogen peroxide let it stand at 30^{0}C for atleast 6 hours. Dilute with solution A 50ml.

Preparation of system suitability solution

Weigh accurately about 15mg of Raloxifenehydrochloride standard to a50ml volumetric flask; add 0.5ml of system suitability stock solution dilute with diluent to volume and mix.

Preparation of standard solution

Weigh accurately about each 10mg of impurity-A, dimethoxy impurity, Raloxifene hydrochloride standard into a 1000ml volumetric flask dissolve and dilute to1000ml with diluent. Take 30ml of above solution into a 100ml volumetric flask, dilute to 100ml with diluent.

Preparation of test solution

Weigh accurately about 30mg of test sample into a 10ml volumetric flask dissolve and dilute to volume with diluent.

Sequence of Injections

SEQUENCE	NO.OF INJ
Mobile phase	1
System suitability	1
Standard preparation	2
Test preparation	2

When the system has reached equilibrium make sequence of injections
Determine the peak areas by integration
Disregard any peak that is less than 0.05%
System suitability for related substance
Resolution between raloxifene and raloxifene N-oxide peaks should not less than 3.0.
Tailing factor of raloxifene peak should not be more than 2.0.

Calculation for related substance

1. **For impurity-H, dimethoxy Impurity, raloxifene -N-oxide**

$$\frac{\gamma_i}{\gamma_R} \times \frac{W_R}{W_i} \times 100$$

2. **For other individual impurity**

$$\frac{\gamma_u}{\gamma_{RU}} \times \frac{W_{RU}}{W_i} \times 100$$

Where
γ_i =average area of each (impurity –H, dimethoxy, raloxifene N-oxide) impurity in sample preparation. γ_R=average area of corresponding (impurity –H, dimethoxy, raloxifene N-oxide) impurity in standard solution. W_R=concentration of corresponding impurity in the standard solution in mg/ml, W_i =concentration of sample preparation in mg/ml, γ_u = average area of other impurity in sample preparation.W_{RU}=concentration of raloxifene in standard solution in mg/ml, W_i =concentration of sample in mg/ml.

Specifications
Raloxifene impurity I[1]: should not be more than 0.20%.

Related compound	Relative retention time
4-2-(1-pipritinyl)ethaoxy benzoic acid	**0.16**
Raloxifene impurity I	**0.74**
Raloxifene-N-oxide	**1.17**
Raloxifene	**1.00**
Dimethoxy impurity	**1.82**

4-2-(1-pipritinyl) ethoxy benzoic acid: should not be more than 0.10%.
Dimethoxy impurity: should not be more than 0.10%.
Raloxifene-N-oxide: should not be more than 0.10%.
Any other individual impurity: should not be more than 0.10%
Total impurity: should not be more than 0.50%.

ASSAY BY RP-HPLC (on dried basis)
Preparation of buffer solution
Weigh accurately about 7.2g of monobasic potassium phosphate and transfer into 1000ml of water and mix add 1.5ml of phosphoric acid further adjust with phosphoric acid or potassium hydroxide solution to ph of 2.5±0.1and mix.

Preparation of mobile phase
Prepare a filtered and degassed mixture of buffer solution and acetonitrile (67:33).

Preparation of standard solution
Weigh accurately about 50.0mg of raloxifene hydrochloride standard into 100ml volumetric flask. Take 5.0ml of above prepared solution into a 50ml volumetric flask dilute to 50ml with mobile phase (0.05mg/ml).

Preparation of test solution
Weigh accurately about 50.0mg of raloxifene hydrochloride into 100 ml volumetric flask. Dissolve and dilute to the volume with diluents. Take about 5.0ml of above prepared solutioninto a 50.0ml volumetric flask. Dilute to 50ml with mobile phase (0.05mg/ml).

Sequence of injection

SEQUENCE	NO.OF INJ
Mobile phase	1
System suitability solution	1
Standard preparation	6
Test preparation	2

PROCEDURE

When the system has reached equilibrium make sequence of injections.

Determine the peak areas by integration.

System suitability for related substance

Tailing factor of raloxifene peak should not be more than 2.0.

Resolution between raloxifene and raloxifene N-oxide peaks should not less than 2.0.

Relative standard deviation for raloxifene hydrochloride should not be more than 0.7%.

Calculation for assay

$$\frac{Cs}{Cu} \times \frac{\gamma_u}{\gamma_s} \times 100$$

Where

Cs=concentration of standard preparation in mg/ml

Cu= concentration of test preparation in mg/ml

γ_u =average area of main peak in the test solution

γ_s = average area of main peak in the standard solution.

Specifications

Assay of the test sample should not be less than 97.50% and more than 102.00%.

RELATED SUBSTANCES BY RP-HPLC

Preparation of buffer solution

Solution A

Phosphate buffer

Solution B

Acetonitrile procured from Rankem

Mobile phase

Use variable mixtures of solution A and solution B as shown below

Time (min)	Solution A (%)	Solution B (%)	Elution
0.00	75	25	Isocratic
9.00	75	25	Linear gradient
40.25	50	50	Linear gradient
42.25	75	25	Linear gradient
49.00	75	25	Equilibration

Specific individuals

Unknown impurity I = 0.04%
Unknown impurity II = 0.03%
Unknown impurity III = 0.02%
Unknown impurity IV = 0.02%
Unknown impurity V = 0.06%
Unknown impurity VI = 0.03%
Unknown impurity VII = 0.03%

Specifications

Raloxifene impurity I[1]: should not be more than 0.20%.

4-2-(1-pipritinyl) ethoxy benzoic acid: should not be more than 0.10%.

Dimethoxy impurity: should not be more than 0.10%.

Raloxifene-N-oxide: should not be more than 0.10%.

Any other individual impurity: should not be more than 0.10%.

Total impurity: should not be more than 0.50%.

RESULT

Test passed

Assay By RP-HPLC

No of INJ	Areas of Raloxifene In Test Solution
01	1225951
02	1225246
03	1225645
04	1225234
05	1225678
06	1225461
AVG	1225536
SD	277.5604
%RSD	NMT 0.70%

No of times test solution injected = 2

No. of Injections	Areas of raloxifene in test solution
01	1225216
02	1225216
AVG	1225216

Assay of sample preparation

$$\frac{1225216}{1225536} \times \frac{50.02}{.50.08} \times 100 = 99.85$$

Specification

Assay of the test sample should not be less than 97.00% and more than 102.00%

RESULT AND DISCUSSION

The Gradient RP-HPLC method was used to determine the Raloxifene Hydrochloride Related substance and developed the sensitive, precise and accuracy of dosage forms. For this, a binary mixture of acetonitrile and phophate buffer (30:70 v/v) portion was found to be the most suitable mobile phase as the chromatographic peaks obtained with this system were better defined and resolved and all almost free from tailing. The assay of drug content and related substance was quantified using the proposed method of analysis which was mention. This reveals that the method is quite precise, sensitive and reproducible for the analysis of Raloxifene in bulk drug in short analysis time. Under the above mentioned conditions, the retention time obtained for Raloxifene was 2.860 min. A model chromatogram was shown in Figure D. A good linear relationship was observed between the concentration of Raloxifene and respective peak areas. Total impurities are not more than 0.50% and assay of the drug was found to be 99.85%

Chromatograms of Raloxifene Hydrochloride Related substance and Assay

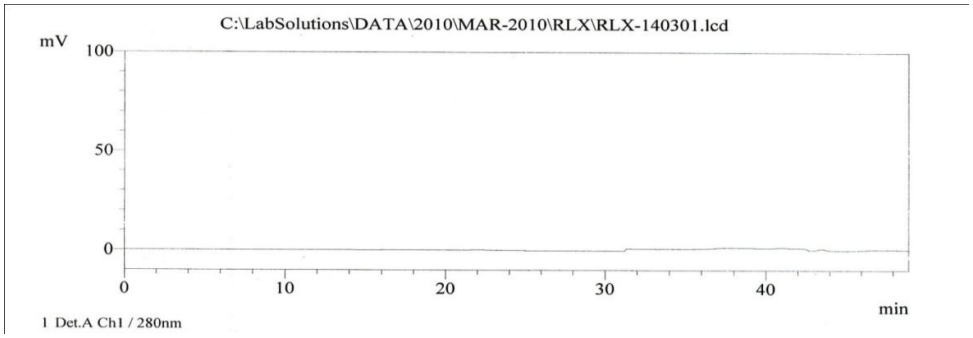


Fig. A: Blank

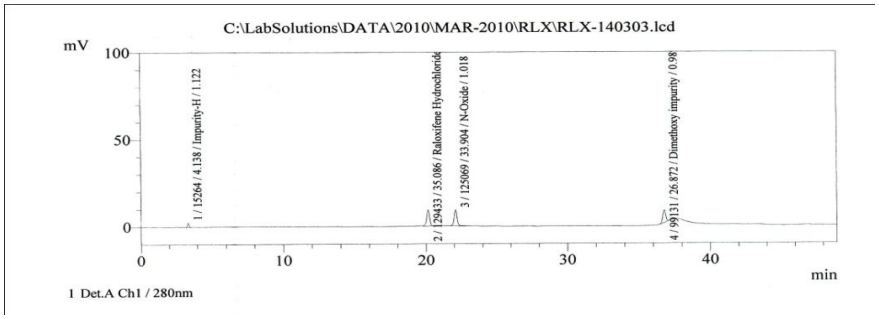


Fig. B: Standard for Raloxifene Hydrochloride Related Substance

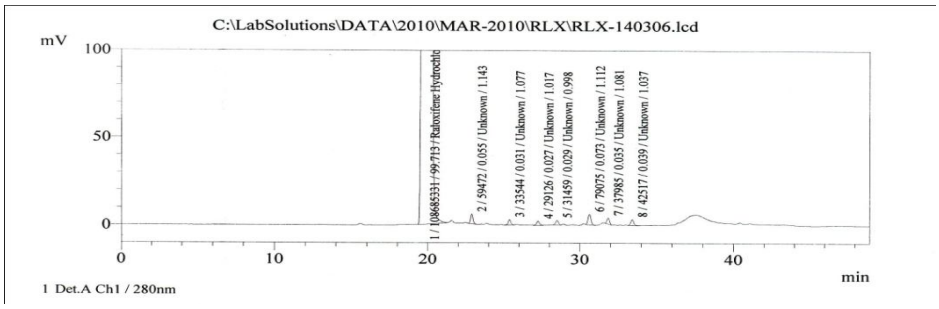


Fig. D: Standard for Assay of Raloxifene Hydrochloride

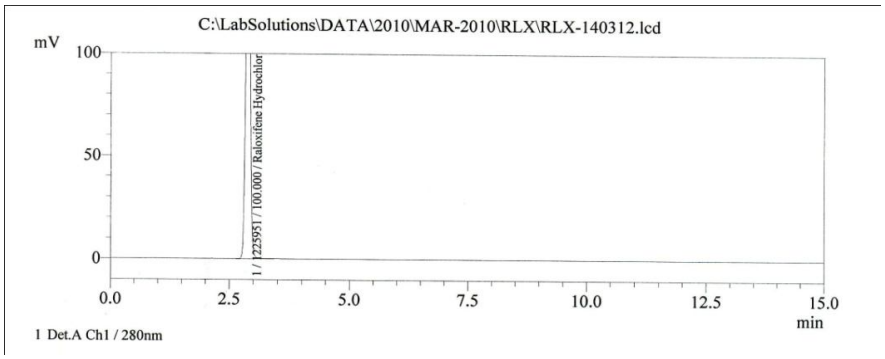
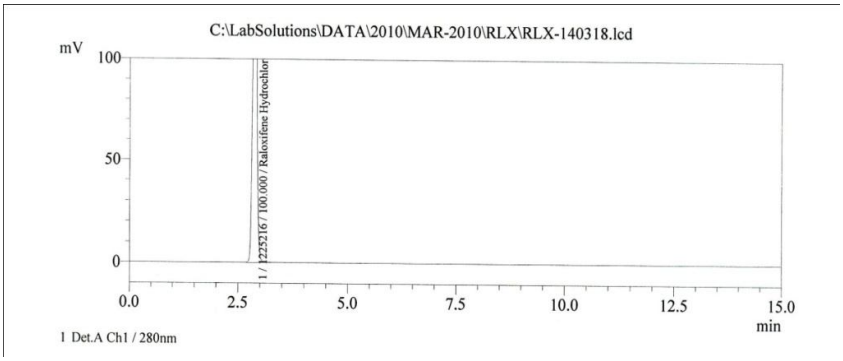


Fig. C: Raloxifene Sample for Substance



B. Assay of Raloxifene Hydrochloride Bulk sample

CONCLUSIONS

The RP-HPLC method developed for quantitative and related substance determination of Raloxifene hydrochloride is linear, accurate, precise, rapid and specific. The method was fully validated showing satisfactory data for all method validation parameters tested. The developed method is stability indicating and can be conveniently used by quality control department to determine the related substance and assay in regular Raloxifene Hydrochloride production samples and also stability samples. The Gradient RP-HPLC technique is a latest of its kind and the impurities were separated in a short time.

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