Gradient RP-HPLC method for the determination of Purity and Assay of Raloxifenehydrochloride in Bulk Drug

Sathyaraj A., Satyanarayana V. and Basaveswara Rao M. V.
Department of Chemistry, Krishna University, Machilipatnam, Andhra Pradesh, INDIA

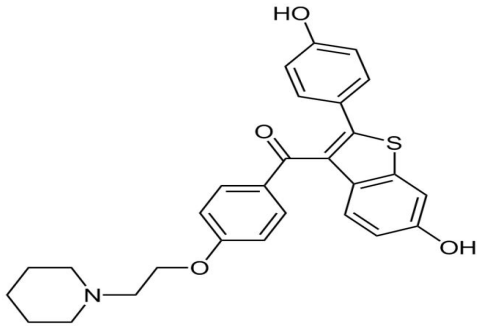
Available online at: www.isca.in
(Received 02nd April 2011, revised 22nd April 2011, accepted 23th April 2011)

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.

Key Words: Gradient, RP HPLC, Raloxifenehydrochloride, acetonitrile

Introduction



Raloxifene

Raloxifene Hydrochloride, a non-steroidal selective estrogen receptor regulator, is currently applied to both the prevention and treatment of postmenopausal osteoporosis[1,2]. Preservation of bone density, suppression 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[3,4]. For either osteoporosis treatment or prevention[5], supplemental calcium and/or vitamin D 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[6]. 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. Characterization of impurities is a crucial aspect of drug development and approval, and is central to quality control.

Material and Methods

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[7]. 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.

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[8].

Estimation of Raloxifene[9]: 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[10]. 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 10 and 20 µg/ml of Raloxifene were subjected to the proposed HPLC method of analysis and the recoveries were noted.

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

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

Preparation of system suitability solution: Weigh accurately about 15 mg of Raloxifenehydrochloride standard to a 50 ml volumetric flask; add 0.5 ml of system suitability stock solution dilute with diluent to volume and mix.

Preparation of standard solution: Weigh accurately about each 10 mg of impurity-A, dimethoxy impurity, Raloxifene hydrochloride standard into a 1000 ml volumetric flask dissolve and dilute to1000 ml with diluent. Take 30 ml of above solution into a 100 ml volumetric flask, dilute to 100 ml with diluent.

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

Sequence of Injections:

Sequence	No. of Injection
Mobile phase	1
System suitability	1
Standard preparation	2
Test preparation	2

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

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%.
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 7.2 g of monobasic potassium phosphate and transfer into 1000 ml of water and mix add 1.5ml of phosphoric acid further adjust with phosphoric acid or potassium hydroxide solution to ph of 2.5 ± 0.1 and 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 50.0 mg of raloxifene hydrochloride standard into 100 ml volumetric flask. Take 5.0 ml of above prepared solution into a 50 ml

volumetric flask dilute to 50 ml with mobile phase (0.05mg/ml)

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

Sequence of injection

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

Sequence	No. of Injection
Mobile phase	1
System suitability solution	1
Standard preparation	6
Test preparation	2

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:

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

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

Results 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%.

Conclusion

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.

References

1. Boss S.M., Huster W.J., Neild J. et al., Effects of raloxifene hydrochloride on the endometrium of postmenopausal women, *Am. J. Obstet. Gynecol.*, **177,** 1458-64 **(1997)**

2. Hardman J.G and Limbird L.E., (Eds.), Goodman and Gilman's, The Pharmacological, Basis of Therapeutics, 10th ed., McGraw-Hill, New York, **(2001)**

3. Smith A., Heckelman P.E., Obenchain J.R., Gallipeau J.A.R., Arecca and Budavari S., The Merck Index, 13th Ed., Merck Research Laboratories,White house station, NJ, 1452. and references cited there in. **(2001)**

4. Delmas P.D., Bjarnason N.H., Mitlak B.H. et al. Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine

endometrium in postmenopausal women, *N Engl J Med.,* **337,** 1641-7 **(1997)**

5. Baker V.L. and Jaffe R.B., Clinical uses of antiestrogens, *Obstet. Gynecol. Surv.,* **51**, 45-59 **(1996)**

6. Kauffman R. F., and Bryant H. U., Selective estrogen receptor modulators, *Drug News Perspect.,* **8**, 531-9 **(1995)**

7. Lim H. K., Stellingweif S., Sisenwine S. and K. W., *J. Chromatogram. A,* **831**, 227-241 **(1999)**

8. Delmas P., Bjarnason N. H., Mitlak B. H., Ravoux A. C., Shah A. S., Huster W. J., Draper M. and Christiansen C. N., *Engl. J. Med*., **337,** 1641–1647 **(1997)**

9. Zweigenbaum J. and Henion J., *Anal. Chem*., **72**, 2446–2454 **(2000)**

10. United State Pharmacopoeia, *USP/ NF* **(2003)**

11. Nandini P. and Jayant W. P., *Indian Drugs*, **38,** 591–592 **(2001)**

12. Wang Q., Zhang H. M., Yu Z. G. and Shenyang Yaoke Daxue Xuebao, 19, 105 **(2002)**

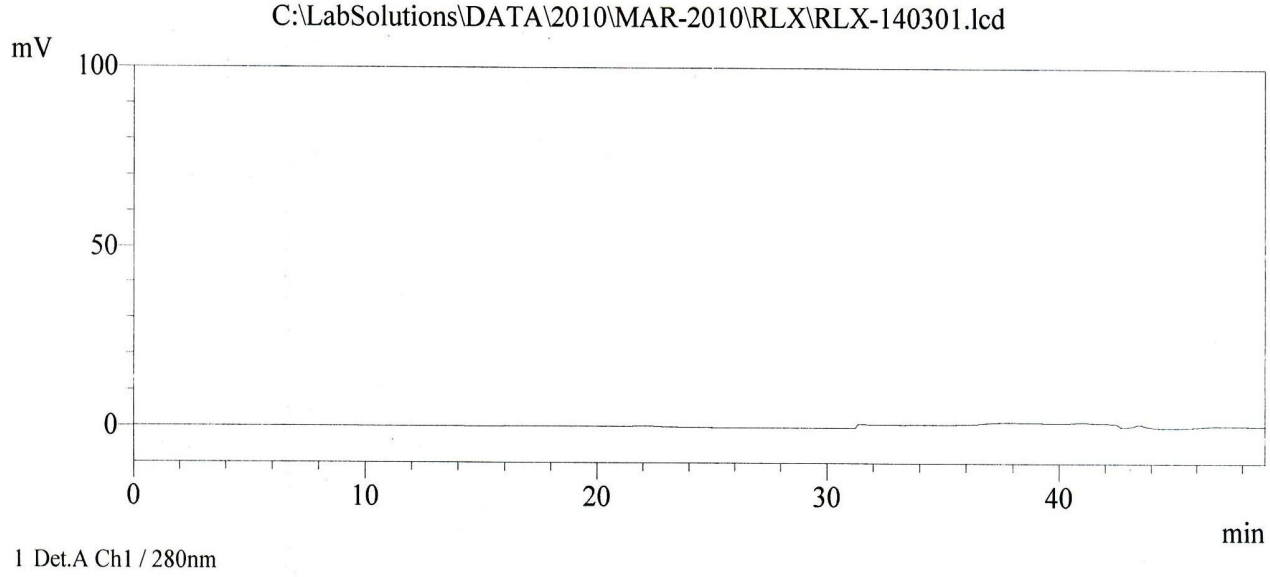


Figure-A: Blank Chromatograms of Raloxifene Hydrochloride Related substance and Assay

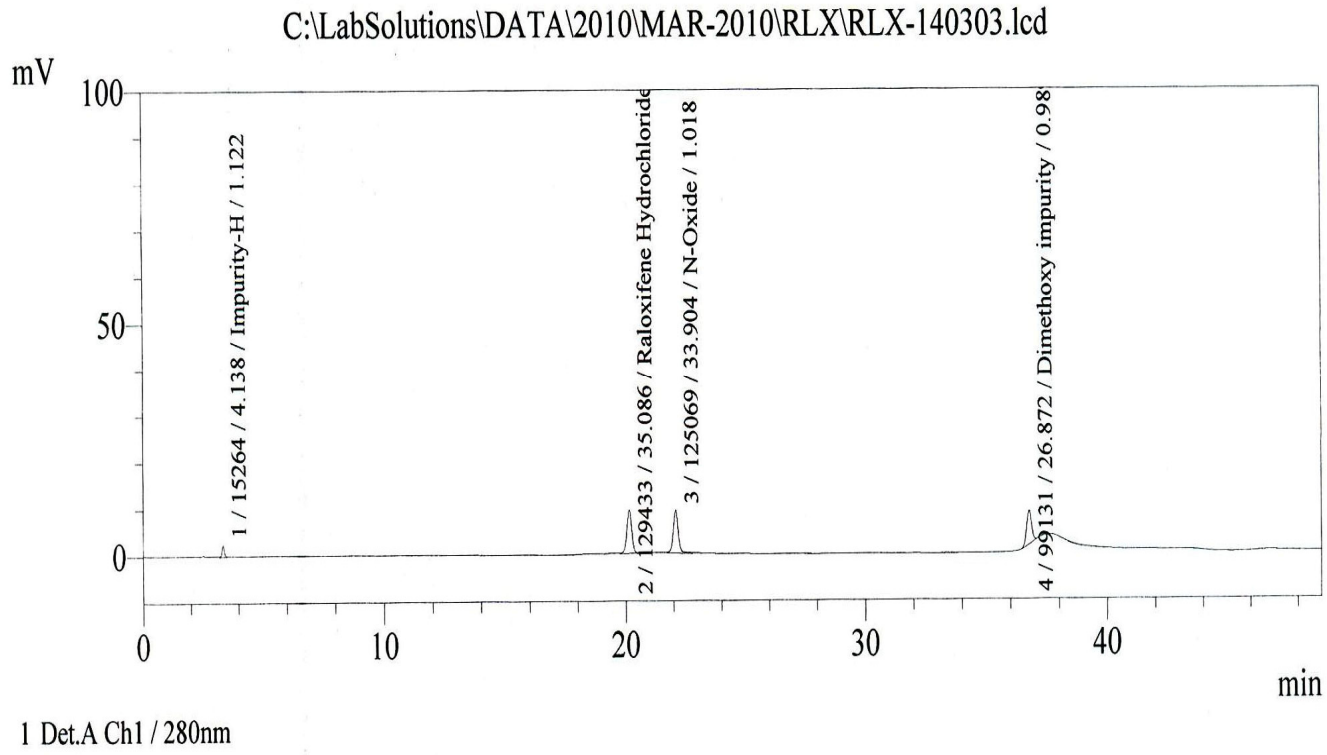


Figure-B: Standard for Raloxifene Hydrochloride related substance

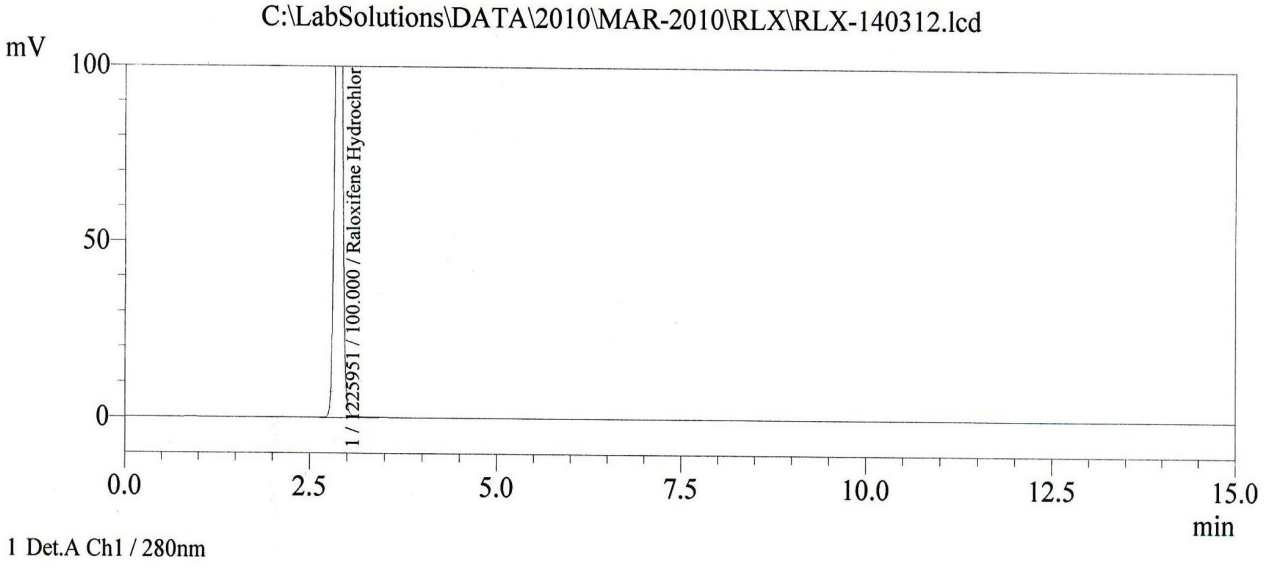


Figure-C: Standard for Assay of Raloxifene Hydrochloride

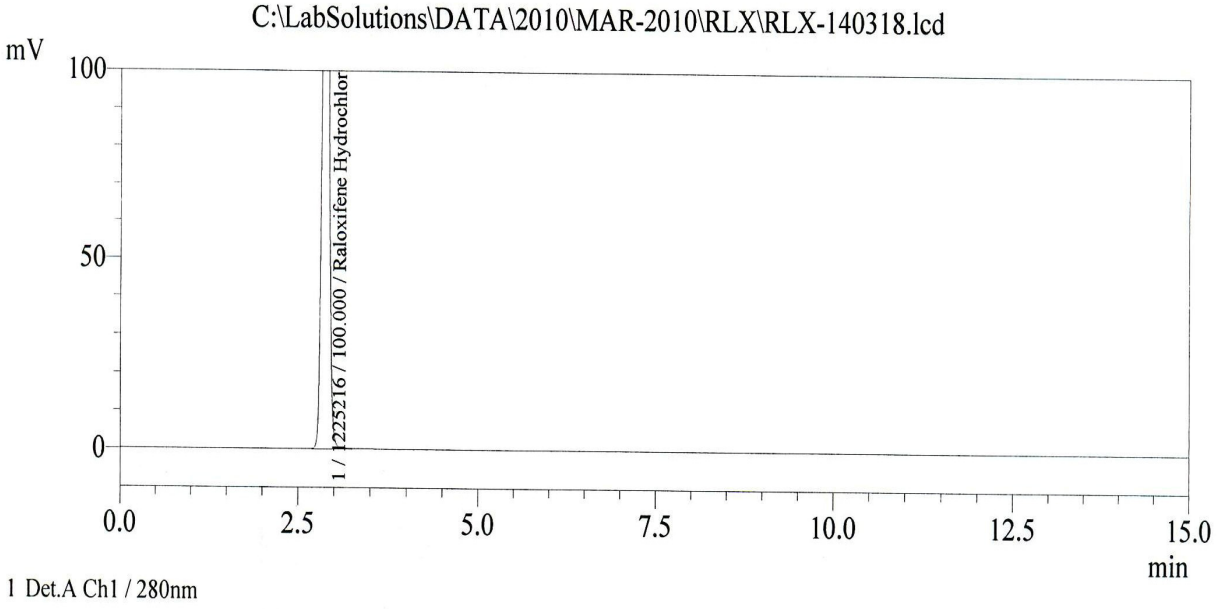


Figure-D: Assay of Raloxifene Hydrochloride bulk sample