Analytical Method Development and Validation of Raloxifene Hydrochloride by RP-HPLC

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ABSTRACT:

A precise, rapid, easy and economical RP-HPLC technique for the detection of Raloxifene HCL was developed by using Acetonitrile and Milli-Q water along with 0.1% Ortho Phosphoric Acid (OPA) 50:50 v/v as mobile phase. The maintained flow rate was kept at 0.5mL/minute. At 287nm respectively by using UV detector detection of Raloxifene HCL was performed. RT of Raloxifene HCL was identified at 2.9 min respectively by this proposed method. The R2 value is 0.996, the LOD value is 1.5µg/ml and the LOQ value is 10g/ml. The proposed method is use of the routine Quality control. The R2 value is 0.996, the LOD value is 0.8µg/ml and the LOQ value is 0.5µg/ml. The accuracy was found to be 98.71%.

KEYWORDS: UV detector, RP-HPLC method, Raloxifene HCL, LOD, LOQ.

INTRODUCTION

Selective Estrogen Receptor Modulators (SERMs) are a class of chemical substances that are not oestrogen. They bind to oestrogen receptors and interact with them, and they have oestrogen agonist or antagonist activities in distinct target tissues[1,2]. In clinical medicine, their role is changing. In the United States, four SERMs are now available. Clomiphene

citrate (Clomid), tamoxifen, and toremifene are three triphenylethylenes, while raloxifene is a benzothiophene. The literal meaning of the term "osteoporosis" is "porous bone." Osteoporosis being a bone-weakening disorder leads the patient to higher risk of unexpected bone fractures[3,4]. Raloxifene is a selective benzothiophene oestrogen receptor modulator (SERM) that has anti-osteoporosis and lipid-lowering properties[3]. Women take raloxifene after menopause to prevent and cure bone loss (osteoporosis)[4]. It helps keep bones healthy and decreases bone loss, making them less prone to shatter. Raloxifene may also reduce the risk of developing a specific kind of breast cancer after menopause (invasive breast cancer). Raloxifene is not an oestrogen hormone, but it has estrogen-like effects in certain regions of the body, such as the bones[7]. Hot flushes or leg cramps are possible adverse effects[7]. Raloxifene hydrochloride is also being researched for use in the treatment of various cancers[8]. It inhibits the actions of the hormone oestrogen in breast tissue, perhaps slowing the growth of breast cancer cells. Low BMD and osteoporosis are more common as chronic kidney disease progresses, and raloxifene is an effective therapy for postmenopausal women with chronic kidney disease[11].

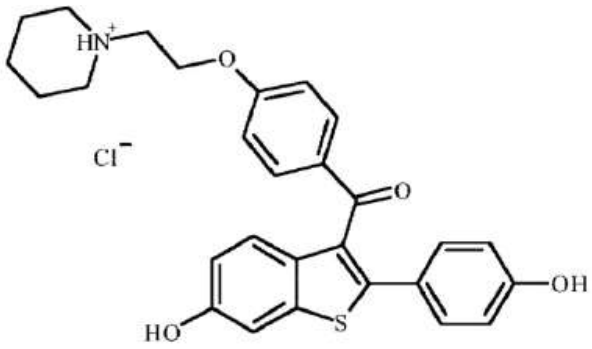


Fig 1:- Chemical Structure of Raloxifene HCl

IUPAC name	[6-hydroxy-2-(4-hydroxyphenyl)-benzothiophen-3-yl]-[4-[2-(1-piperidyl)ethoxy]phenyl]-methanone
Melting point	143 to 147°C
Synonyms	Evista, Keoxifene
Solubility	Freely soluble in acetonitrile and methanol
Molecular formula	$C_{28}H_{27}NO_4S$
Physical appearance	Crystals from acetone
Category	Selective Estrogen Receptoe Modulators(SERMs)
Mechanism of Action	Activation of the estrogenic pathway by binding to estrogen receptors.

MATERIALS & METHODS:

Materials

Raloxifene HCL standard was procured from Apotex Pharmachem, Bengaluru. Evista was purchased from the local market, which is a Raloxifene HCL marketed formulation manufactured by Eli Lilly company. Merck pharmaceuticals was the provider of all the analytical grade chemicals which were used. HPLC grade ACN and 0.1% OPA is used as MP. 0.1% OPA was prepared by using Millipore water and filtration was done to ensure high purity of mobile phase which is free from impurities, by using 0.2 µm membrane filter. Preparation of the solutions were done by using HPLC grade ACN as diluent.

Table 1:List of chemicals and reagents used in project.

S. No	chemical / drug	Make	Grade
1	Raloxifene HCL	Apotex Pharmachem	API
2	Acetonitrile	Merck Ltd	HPLC
3	Ortho phosphoric acid	Merck Ltd	HPLC
4	Milli pore Water	Merck Ltd	HPLC

Table 2: Instruments used during the method development.

S. No	Name	Maker
1	Analytical Balance	Shimadzu, Japan
2	pH meter	Systronics, India
3	HPLC Prominence I series equipped with an auto sampler and UV detector.	Shimadzu, Japan
4	Sonicator	Antech

Method development

Selection of wavelength: UV-visible spectrophotometer 1800 was used to determine the λ max of Raloxifene HCL.

Selection and preparation of mobile phase: Due to less polarity of Raloxifene HCL, combinations of various ratios of different mobile phase were tried for MP selection. Standard Raloxifene HCL drug with numerous combination of mobile phase of different ratios and flow rate was injected for the peak optimization. A sharp peak was obtained by continuing the procedure. At 50:50(v/v) of ACN and 0.1% OPA sharp peak was acquired. 0.1% -OPA is prepared by diluting 1ml of OPA in 1000ml of Millipore water.

Standard stock solution preparation: Raloxifene HCL stock solution was made by dissolution 10 mg of standard drug into 10ml of HPLC grade acetonitrile and 0.1% OPA in Millipore water to obtain 10µg/ml conc.

Sample Preparation: - The sample solution was prepared by taking 1mhg of crude drug extract in volumetric flask and dissolved using 1ml of Acetonitrile. The sample solution of 1000µg/ml concentration was obtained and sonicated for 15 mins for degassing and from the pipette 1 ml and make up diluent to get 100µg/ml was filtered by using the syringe filters (0.22 µm) to remove any unwanted materials and was kept in the HPLC vial

Method optimization for Raloxifene HCL:- A Study was carried out on the effect of various parameters involved in method development. The solubility of Raloxifene HCL in several solvents was tested initially. Followed by selection of suitable columns for separation for the proposed method was carried out. Chromatographic conditions were optimized so as to attain a satisfactory separation of eluted compounds in HPLC. Different diluent were tested for elution of the drug initially. MP selection and flow rate were obtained on the basis of peak parameters such as tailing factor, run time and resolution. Acetonitrile and 0.1% OPA in Millipore water at ratio 50:50 (v/v) was used in the form of MP keeping the flow rate at 0.5ml/min for Raloxifene HCL. The standard and sample chromatogram of Raloxifene HCL at 2.9 min were shown in the figures. Chromatographic conditions were used for the method.

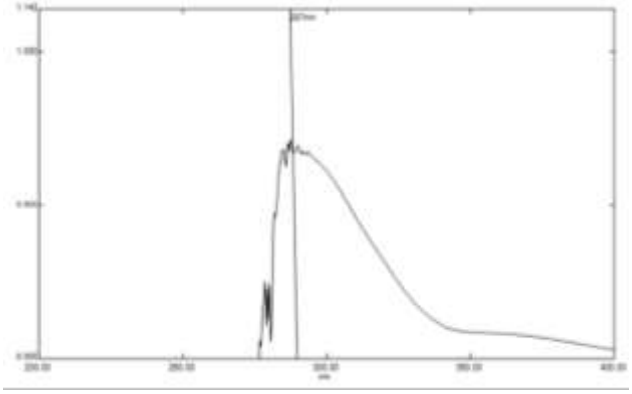
RESULT AND DISCUSSION:

Validation of the given method for the determination of Raloxifene HCL had been done according to the guidelines of ICH.

Method optimization for Raloxifene HCL.

The solubility of Raloxifene HCL in several solvents was tested initially. Followed by selection of suitable columns for separation for the proposed method was carried out. Chromatographic conditions were optimized so as to attain a satisfactory separation of eluted compounds in HPLC. Different diluent were tested for elution of the drug intially. The selection of mobile phase (MP) and the flow rate were obtained by using parameters of peak such as tailing factor, run time and resolution. ACN and 0.1% OPA in Millipore Water of ratio 50:50 (v/v) was used in the form of MP keeping the flow rate at 0.5ml/min for Raloxifene HCL. Fig. 3 shows the blank chromatogram for Raloxifene. Fig. 4 & 5 respectively shows the standard and sample chromatogram for Raloxifene HCl The standard and sample chromatogram of Raloxifene HCL having RT 2.9 min. Table 3 shows the chromatographic conditions considered for the methods.

Fig 2: UV spectrum of Raloxifene HCL



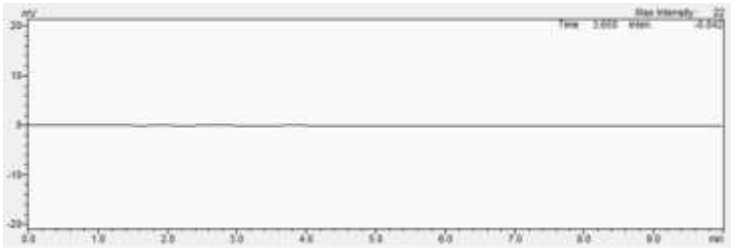


Fig 3: Blank Chromatogram

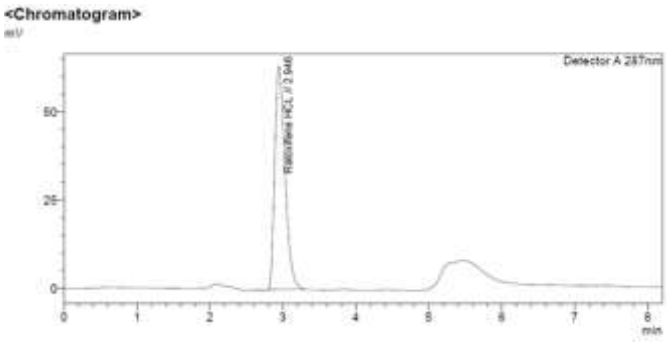


Fig.4: Raloxifene HCL Std. chromatogram.

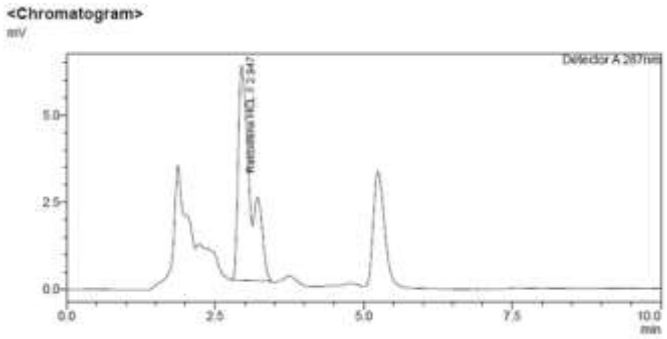


Fig. 5: Raloxifene HCL Sample Chromatogram.

System Suitability

6 injections of standard solution of Raloxifene HCL were injected for the testing of system suitability parameters. The table below displays the results for system suitability

Table 3: System Suitability results

Parameter	**Acceptance criteria**	**Result**
Theoretical plates	NLT 2000	2831
Tailing factor	NMT 2.0	1.473

Data interpretation: according to the above Table no. 3 it can be concluded that system suitability parameters were passing.

Linearity: This refers to the ability of obtaining experimental result that is in proportion to the analyte's conc. available in the sample. Five different concentrations (i.e. 2, 4, 8, 16, 32 and 64µg/ml) in triplicate were used to obtain the calibration curve and linearity expression y=mx+c was applied to establish the linearity and thus the slope was calculated. Fig 6 shows the calibration curve of Raloxifene HCL respectively. Table no. 4 shows the concentration and peak area.

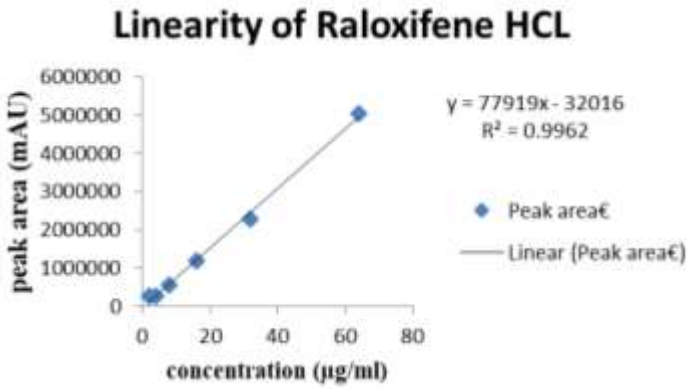


Fig. 6: Calibration curve for Raloxifene HCL

Table 4: Results for Linearity

Level	Conc (µg/ml)	Area
1	2	270449
2	4	326004
3	8	550730
4	16	1192850
5	32	2180427
6	64	5051223
Regression Equation		Y=77919+32016
Correlation Coefficient (R^2)		0.996
Slope		77919
Intercept		811206

Data interpretation:

As per the linearity data obtained from Table 4 Raloxifene HCL, it is giving that response of Raloxifene HCL is linear at a range of 2 to 64µg/ml of working concentration. Linearity parameter passed as the correlation was not less than 0.95.

Precision

Evaluation of repeatability of the following process was done by using different conc. of the drug 10, 30 and 50µg/ml. Preparation of the above solutions was done from stock solution and the evaluation of precision was done by injecting them intraday and interday. For interday studies preparation of the concentrations was done thrice a day. Interday and Intraday precision results were shown in table 5,6 respectively.

Table 5: Results for Method Intra-Day Precision for Raloxifene HCL

Method precision						
Injection	**RT**	**Tailing**	**Plate count**	**Peak Area**	**Drug in mg**	**Drug in %**
1	2.907	1.443	2033	616409	10.18	101.88
2	2.906	1.442	2012	618214	9.91	99.17
3	2.907	1.428	2015	625017	10.06	100.60
4	2.907	1.434	2040	628017	10.18	101.88
5	2.907	1.422	2016	612487	9.91	99.18
Average	2.906	1.4338	2023.2	620028.8	10.04	100.54
Std dev.	1.704			787.419		
RSD %	1.695			1.05		
Limit	1.0	2.0		2.0		90-110%

Table 6: Results for Method Precision Inter-day for Raloxifene HCL

Intermediate precision						
Injection	**RT**	**Tailing**	**Plate count**	**Peak Area**	**Drug in mg**	**Drug in %**
1	2.908	1.412	2025	542799	10.13	97.09
2	2.909	1.416	1997	554039	10.05	94.94
3	2.909	1.438	2010	550117	9.93	96.70
4	2.909	1.411	2001	552987	9.92	97.76
5	2.908	1.426	2020	552620	9.83	98.26
Average	2.9086	1.4206	2010.6	550512.4	**9.98**	**96.95**
Std dev.	1.705462			741.965		
RSD %	0.05			1.75		
Limit	1.0	2.0		2.0		90-110%

Acceptance criteria:

The calculated Relative Standard Deviation must be below or equal to 2% (≤ 2.0%).

LOD and LOQ:

Calibration standards were used to identify LOD (limit of detection) and LOQ(limit of quantification) for following RP-UFLC method. LOD=3.3×N÷S was the formula used to calculate LOD, where N stands for Standard Deviation and S for the slope. Whereas the formula used for calculation of LOQ is given by LOQ=10×N÷S, where N stands for Standard Deviation and S for the slope. 0.8 and 0.4 µg/ml were the found values of LOD and LOQ of Raloxine HCl.

Table 7: LOD and LOQ for Raloxifene HCL.

LOD	0.8µg/ml
LOQ	0.5µg/ml

Data Interpretation: According to the above table no. 8 a conclusion can be made that at LOD level conc. acceptable peaks were obtained, The Limit of Detection and Limit of Quantification for Raloxifene HCL are 0.25, 0.50, 0.75 µg/ml respectively.

Accuracy: An analytical method's accuracy can be defined as its closeness to the actual value of the sample obtained by the following method. Formulations were laced with standards of 50%, 100% and 150% Raloxifene HCL in order to confirm the technique's accuracy. Analysis of the results were done in order to find the % recovery of the Raloxifene HCL.

$$\% \text{ Recovery} = \frac{\text{Amount of drug recovered}}{\text{Amount of drug added}} * 100$$

Table 8: Accuracy study for Raloxifene HCL

Analyte	**Nominal concentration µg/ml**	**Found concentration µg/ml**	**% Accuracy**	**Avg % Bias**	**%RSD**
Raloxifene Hydrochloride	1	0.98	98.74%	2.3%	98.71
	8	7.88	98.55%	2.5%	99.08
	64	63.84	99.75%	1.3%	99.62

Acceptance criteria: At each level mean % recovery and individual should range from 98.0 - 102.0%

Data interpretation: A conclusion can be made from table 9 i.e. the recovery was within the limit. Therefore, the method was found to be accurate.

Robustness: It is considered the ability of any method to remain unchanged even when slight alterations are made. Checking of the robustness of the particular proposed method was done by increasing and decreasing the following parameters such as detection wavelength, flow rate, column temperature and injecting 10µg/mL. Table 9, 10, 11 respectively shows the robustness results.

Table 9: Robustness result for Raloxifene HCL

WL	Conc.	Area
287	10	2204802
	10	2162473
	10	2096929
Average		2154734.6
ST DEV		1.152422
%RSD		1.155824

Table 11: Robustness Decrease of Wavelength

WL	**Conc.**	**Area**
286	10	2060400
	10	2136265
	10	1956256
Average		2050973.6
ST DEV		1.126354
%RSD		1.128456

Table 12: Robustness Increase of Wavelength

WL	**Conc.**	**Area**
288	10	2401409
	10	2336754
	10	2251236
Average		2329799.6
ST DEV		1.162345
%RSD		1.154623

Acceptance criteria:

The calculated Relative Standard Deviation must be ≤2.0%.

CONCLUSION:

To detect Raloxifene HCl, the proposed RP-HPLC technique was developed. The validation of this following techniques are done as per ICH guidelines. The proposed technique is reliable, which was assured by its linearity, precision, accuracy of the technique. Due to its short analysis time, it can be concluded that the developed method showed superiority over other reported methods. Thus, it can be concluded that the routine detection of Raloxifene HCL in the formulations can be accomplished using the suggested RP-HPLC approach.

CONFLICT OF INTEREST:

Regarding this investigation, there are no conflicts of interest for the authors.

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