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Stability Indicating Method Development and Validation of Raloxifene HCl in Bulk and Formulation

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ABSTRACT

Raloxifene is a medication used to prevent and treat osteoporosis in postmenopausal women and used to reduce the risk of breast cancer in those at high risk. To developed a rapid, specific, accurate and precise method for Raloxifene HCL by Stability Indicating RP-HPLC. The method was developed using the mobile phase of Water: Acetonitrile (20:80) v/v (pH adjusted to 3.5) by orthophosphoric acid. Raloxifene was separated on Inertsil C18 Column [4.6x250mm, particle size 5µm] at wavelength 284nm. The flow rate is 0.7 mL/minute and the run time of 7 minutes. The Linearity was observed in concentrations ranging from 10 to 60 ppm, and assessed by the chromatographic condition (r^2 0.999). The percentage relative standard deviation in precision (intraday and interday) studies was found to be less than 2%. Stability studies is been carried out which show degradation of drug which is less than 15 %. The method was found to be accurate, precise, robust, and specific as the drug peak did not interfere with the extra peaks during the forced degradation studies.

Keywords: Raloxifene HCL, RP-HPLC, ICH Guidelines, Stability-indicating.

INTRODUCTION

A second-generation selective estrogen receptor modulator (SERM), raloxifene mediates both estrogenic and anti-estrogenic effects on bone, lipid metabolism, and blood coagulation, as well as anti-estrogenic effects on breast and uterine tissues. [1] Raloxifene is used to lower postmenopausal women's risk of breast cancer. For this indication, a daily dosage of 60 mg is utilized.[2],[3],[4] The pharmacological activities of RLFX are primarily mediated by binding to estrogen receptors and appear to work as an estrogen agonist in bone[5],[6],[7]. It is utilized in the prevention of osteoporosis in postmenopausal women.It preserves bone density and reduces bone loss, which lessens the likelihood that bones will break. The prevalence of osteoporosis and low bone mineral density (BMD) increases with age in postmenopausal women with chronic renal disease. Raloxifene is an effective treatment for these women. [7] The objective of our research was to create an isocratic RP-HPLC technique that is fast, reliable, selective, sensitive, and accurate for measuring Raloxifene HCL in tablet dosage forms. The test technique was verified by the use of ICH recommendations.[8]

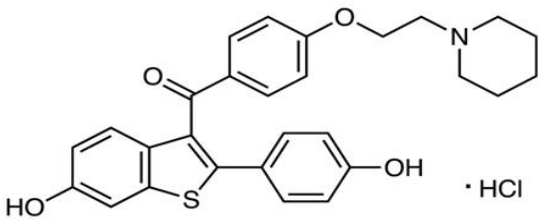

Figure 1: - Chemical Structure of Raloxifene HCl

Table no 1: Drug profile of Raloxifene HCL

Attributes	Description
IUPAC and Chemical name	[6-hydroxy-2-(4-hydroxyphenyl)-benzothiophen-3-yl]-[4-[2-(1-piperidyl)ethoxy]phenyl]-methanone
Molecular weight	510.05 g/mol
Half-life	27 to 32 hours
Physical Description	Crystals from acetone
Melting Point	143 to 147°C
Solubility	Freely soluble in acetonitrile and methanol
Mechanism of Action	Activation of the estrogenic pathway by binding to estrogen receptors. It is a Selective Estrogen Receptor Modulators (SERMs)
Route of administration	Oral Route

Aim - To Study Stability Indicating Assay Method development & Validation for API in Formulation.

Objective –

- To develop new analytical method and to validate.
- To develop rapid, sensitive and selective method.
- Economic and accurate method.
- Method and validation according to ICH guidelines.
- To reveal the degradation mechanisms such as hydrolysis, oxidation, thermolysis, or photolysis of the drug.
- To study stability indicating the method of drug.

MATERIALS AND METHODS

Chemicals

Raloxifene HCL standard was procured from Titan Laboratories Pvt Ltd. Raloxiheal tablet was purchased from the local market, which is a Raloxifene HCL marketed formulation manufactured by Healing Pharma India Pvt Ltd.

Table no 2: Chemicals and Reagent

Sr no	Chemical / Reagent	Make	Grade
1	Raloxifene HCL	Titan Laboratories Pvt Ltd	API
2	Acetonitrile	Merck Ltd	HPLC
3	Ortho phosphoric acid	Merck Ltd	HPLC

Equipment

The equipment which is used for method development is shown in Table no 3

Table no 3: Equipments used for method development

Sr no	Name	Maker
1	Analytical Balance	Shimadzu
2	pH meter	Equip-tronics
3	Sonicator	Labline
4	HPLC	Jasco-Extrema 4100

Method Development

- Selection of wavelength
  Standard solution of the drug was scanned over the range of 200-400nm wavelength against blank in UV spectroscopy. And the working wavelength was determined is 284nm.
- Selection of mobile phase
  Raloxifene HCL has less polarity and various combinations of different ratios were conducted for Mobile Phase selection. Thus Water: Acetonitrile (20:80) v/v (pH adjusted to 3.5) by orthophosphoric acid was selected as mobile phase for further development and validation process.
- Preparation of standard stock solutions
  Raloxifene hydrochloride stock solution (1000µg/mL) was prepared by accurately weighing 100mg of Raloxifene hydrochloride in a 100mL amber volumetric flask and making up to volume with methanol.
- Preparation of sample stock solutions
  Three tablets were precisely weighed and crushed into a fine powder for the sample stock solution. A volume equivalent to 10 mg was calculated, transferred, diluted with methanol, and sonicated for 15 minutes in a amber volumetric flask with a total volume of 100 mL. To prepare the solution with a 100 µg/mL concentration, 1 mL of the sample stock solution mentioned above was transferred into a 10 mL volumetric flask and diluted with methanol.
- Method optimization for Raloxifene HCL
  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. The HPLC method was developed using the mobile phase of Water: Acetonitrile (20:80) v/v (pH adjusted to 3.5 by orthophosphoric acid). Raloxifene was separated on Inertsil C18 Column [4.6x250mm, particle size 5µm] at wavelength 284nm. The flow rate is 0.7 mL/minute and the run time of 7 minutes.

Chromatogram

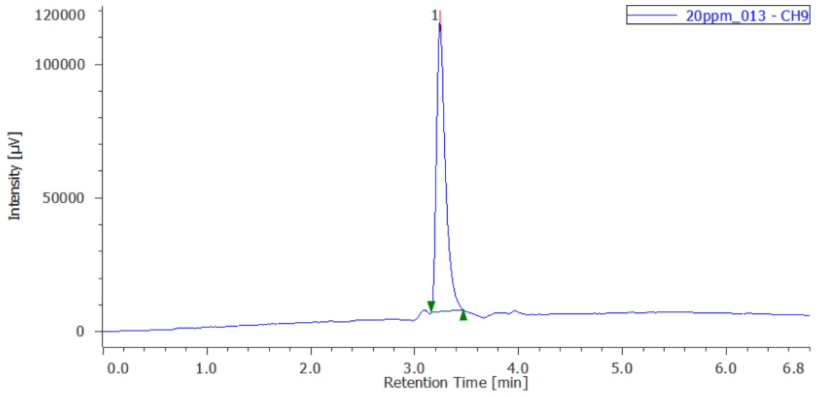

Figure 2: Standard chromatogram of Raloxifene HCL

Table no 4: Optimized chromatographic conditions

Sr no.	Specification	Description
1	Equipment	JASCO Extrema lC system-4000
2	Software	CHROMNAV
3	Column	HiQ SiL C_{18} (250 x 4.6 mm, 5µm)
4	Wavelength	284 nm
5	Column temperature	25°C
6	Flowrate	0.7 mL/min
7	Injection volume	08 µL
8	Run time	7 min
9	Mobile phase	Water: ACN (20:80) v/v [pH adjusted to 3.5 by OPA]
10	Diluent	Methanol
11	Elusion mode	Isocratic

RESULTS AND DISCUSSION

In this article, we present the results of a comprehensive method validation study for a HPLC method. The method was validated based on key performance parameters including linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and forced degradation studies.

Linearity

The linearity of the HPLC method was evaluated by constructing a calibration curve using standard solutions with known concentrations. Using different concentrations of Raloxifene HCL standard solutions, the linearity was assessed. The curve was obtained at a concentration range of 10-60 µg/mL. By plotting the concentration of reference solutions against absorbance, which results in a straight line, the calibration curve was produced. The correlation coefficient (r^2) obtained from the calibration curve was found to be 0.999, indicating a strong linear relationship between the concentration of the analyte and the measured absorbance.

Table no 5: Calibration of Raloxifene HCL

Concentration (µg/mL)	Peak Area
10	325658
20	621200
30	960689
40	1286400
50	1641295
60	2002311

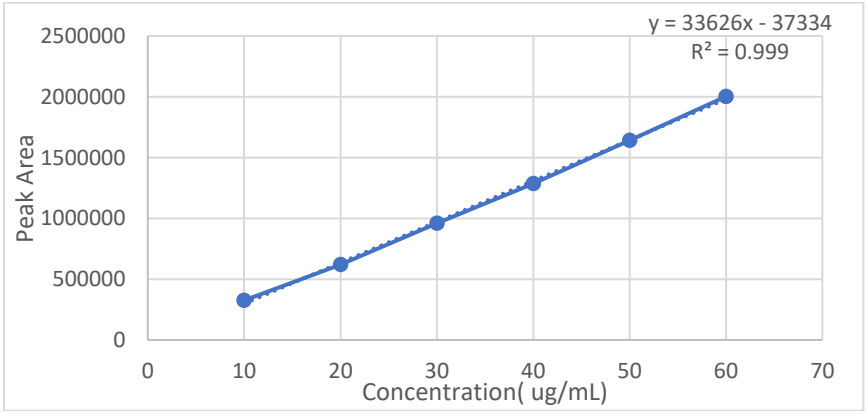

Figure 3: Calibration Curve of Raloxifene HCL

Accuracy

Accuracy of the test method was carried out by determining recovery using standard addition method. The solutions of 80%, 100% and 120% was prepared. Every solution was prepared three times, and the mean percentage of recovery of Raloxifene HCL was determined from each. At 284 nm, these samples were analyzed.

Table no 6 – Result of Accuracy

Concentration (µg/mL)	Area	Mean Area	% Recovery
80%	501896	503253	101.09%
80%	503214		
80%	504649		
100%	635012	634849	99.89%
100%	634719		
100%	634816		
120%	768210	768143	99.60%
120%	767881		
120%	768339		

Precision

The precision method was determined by Intra-day precision and Inter-day precision of the Raloxifene hydrochloride standard solutions. Under the same experimental conditions, six distinct preparations with the same concentration (30 µg/mL) were analysed at 284 nm on the same and separate days. As shown in Table no 7

Table no 7: Intraday Precision Results

Sr no	Concentration	Absorbance	
		Intraday	Interday
1	12	994557	984557
2	12	988253	978119
3	12	991193	991193
4	12	973107	972003
5	12	985252	984252
6	12	997933	994233
Mean		988382.5	985726.17
Standard Deviation		8722.31	9192.43
% Relative Standard Deviation		0.88	0.93

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 no 5,6 & 7 i.e. the recovery was within the limit. Therefore, the method was found to be accurate and precise.

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 no 8

Table no 8: Robustness Result

SR NO.			1	2	3	MEAN	SD	%RSD
FLOW RATE	0.9ml/min	AREA	282293	289483	284330	285369	3705.83	1.30
		RT	2.523	2.5	2.497	3	0.01	0.57
		NTP	6248	6420	6311	6326	87.02	1.38
	0.5ml/min	AREA	455602	467126	454085	458938	7131.75	1.55
		RT	4.553	4.58	4.51	5	0.04	0.78
		NTP	4331	4347	4354	4344	11.79	0.27
TEMP	20°C	AREA	394749	400312	391905	395655	4276.15	1.08
		RT	3.26	3.21	3.25	3	0.03	0.82
		NTP	8376	8319	8427	8374	54.03	0.65
	30°C	AREA	387546	380487	383418	383817	3546.37	0.92
		RT	3.207	3.2	3.24	3	0.02	0.66
		NTP	9822	9804	9764	9797	29.69	0.30

WAVELENTH	282nm	AREA	357192	351185	344687	351021	6254.11	1.78
		RT	3.19	3.253	3.203	3	0.03	1.03
		NTP	7320	7280	7254	7285	33.25	0.46
	286nm	AREA	351813	356416	355333	354521	2406.62	0.68
		RT	3.24	3.26	3.21	3	0.03	0.78
		NTP	8117	7961	8249	8109	144.17	1.78

LOD and LOQ

Calibration standards were used to identify LOD (limit of detection) and LOQ (limit of quantification) for following RP-HPLC 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. 2.02 and 6.14 µg/ml were the found values of LOD and LOQ of Raloxifene HCl.

Table no 9: LOD and LOQ for Raloxifene HCL.

LOD	2.02 µg/mL
LOQ	6.14 µg/mL

Forced Degradation studies

Degradation studies was performed for the drug via., Acid, Alkaline, Neutral, Thermal, Oxidation and Photolytic degradation to get the stability of the drug under given conditions and to determine the degradation of the drug by HPLC.

Table no 10: Forced Degradation studies

Sr no.	Degradation Condition	Procedure
1	Acid	To 1 ml standard stock solution, 1 ml of 0.1N HCl was added and diluted up to mark with solvent. Kept for 24 hrs and then take reading.
2	Alkaline	To 1 ml standard stock solution, 1 ml of 0.1N NaOH was added and diluted up to mark with solvent. Kept for 24 hrs and then take reading.
3	Oxidative	To 1 ml standard stock solution, 1 ml of 3% H2O2 was added and diluted up to mark with solvent. Kept for 6 hrs and then take reading.
4	Thermal	100mg drug was kept in hot air oven at 70°C for 6 hours and further dilution of was made .
5	Photolysis	100mg drug was kept in UV Chamber for 6 hours and further dilution was made.

Table no 11: Forced Degradation Result

Stress condition	TIME	% Degradation
Acidic(0.1 M HCL)	24 hrs	3.35%
Basic (0.1 M NaOH)	24 hrs	7.92%
Oxidative (3% H_2O_2)	30 min	11.27%
Photolysis (UV Chamber)	6 hrs	5.03%
Thermal (Hot air oven)	6hrs	1.59%

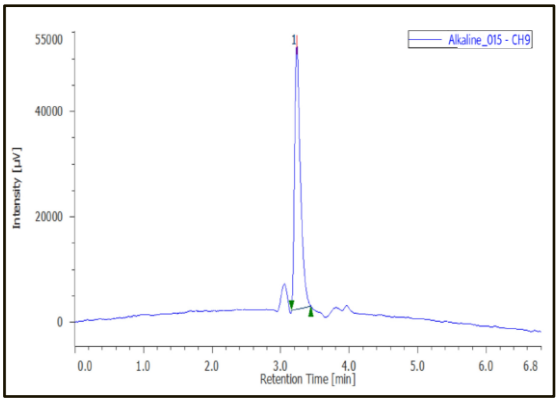

Figure 4: Chromatogram of Alkaline Degradation

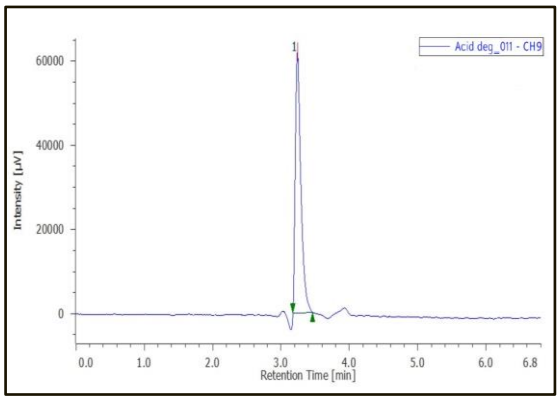

Figure 5: Chromatogram of Acid Degradation

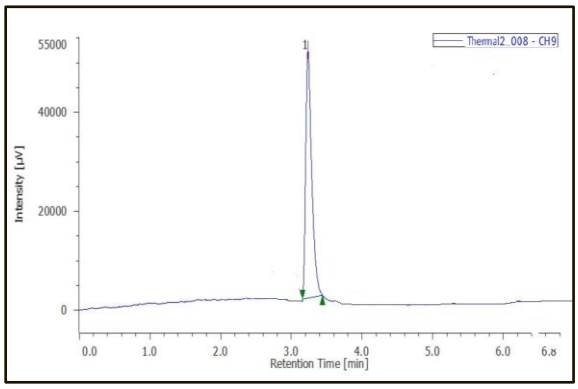

Figure 6: Chromatogram of Thermal Degradation

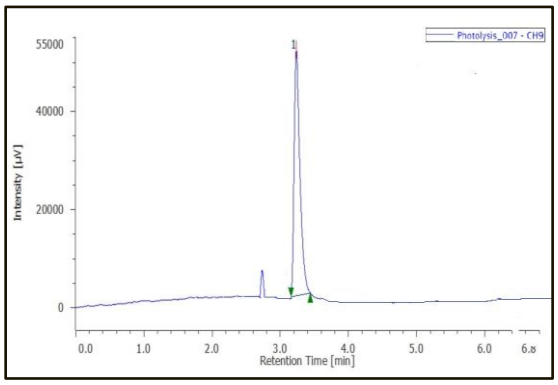

Figure 7: Chromatogram of Photolytic Degradation

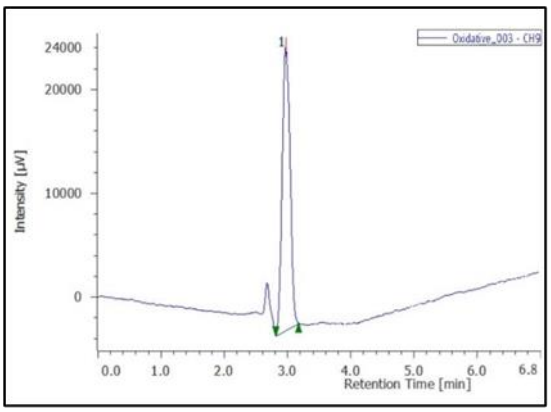

Figure 8: Chromatogram of Oxidative Degradation

CONCLUSION

An isocratic RP-HPLC method was developed for the estimation of Raloxifene HCL in pharmaceutical dosage forms. The method showed satisfactory results for all the method validation parameters tested and indicated that, the developed method is linear, precise, accurate and specific as the drug peak did not interfere with the extra peaks during the forced degradation studies.

Declaration by Authors

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Conflict of Interest: The authors declare no conflict of interest.

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