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Development and validation of stability indicating RP-HPLC method for the determination of related substances in Raloxifene hydrochloride tablets dosage forms

Babu C[1], Suresh Reddy K.V.N[2], Shashi Kumar K.N[*3]

[1]Department of Chemistry, Jawaharlal Nehru Technological University Anantapur, Anantapuramu, Andhra Pradesh, India
[2]Department of Chemistry, GITAM Deemed to be University, Visakapatnam-530045, Andhra Pradesh, India
[3]Department of Humanities and Sciences, Annamacharya Institute of Technology & Sciences, Kadapa-516003, Andhra Pradesh, India

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ABSTRACT

This work is intended to thrive a stability-indicating high performance liquid chromatographic method for the analysis of Raloxifene HCl related compounds in pharmaceutical dosage forms. The separation was achieved Inertsil C8 (150 x 4.6 mm ID, 3.5μm)column using a gradient method. Mobile phase A is 0.01M KH2PO4 buffer (pH4.5), and mobile phase B is acetonitrile used in this work. 1.0 mL/ minute is the flow of rate and at 280nm noticed wavelength is monitored. For specificity, the limit of quantification, the limit of detection, linearity, accuracy, method precision, intermediate precision, robustness and stability this method is validated. The six injection impurities of standard solutions at the 4.0 μg/mL conjecture concentration were confirmed experimentally for LOQ values. The correlation coefficient of the impurities is more than 0.99. All impurities meet the criteria for linearity of both the impurities and raloxifene. The RSD recoveries obtained for impurities are not more than 10%. The achievement of this study demonstrated that the method is selective, linear, precise, rugged, robust and stability-indicating for the determination of related substances in raloxifene HCl tablet dosage form.

*Corresponding Author

Name: Shashi Kumar K.N
Phone: +91-9705364322
Email: sasiphd@gmail.com

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INTRODUCTION

Raloxifene hydrochloride belongs to the benzothiophene class of compounds and used for the osteoporosis in postmenopausal women by its SERM activity(selective estrogen receptor modulator) (Salazar *et al.*, 2015). Raloxifene HCl tablet is given as a dosage form for the administration of oral. 55.7 mg of freebase molar equivalent contains in each 60mg of raloxifene HCl tablet. The excipients used in tablet dose forms are crospovidone, lactose, carnauba wax, hypromellose, magnesium stearate, lactose monohydrate, modified pharmaceutical glaze, magnesium stearate, polysorbate 80, propylene glycol, polyethylene glycol, povidone, and titanium dioxide (Salazar *et al.*, 2015). Literature view report that different spectrophotometric methods are available for raloxifene determination (Sivasubramanian and Pavithra, 2006; Kalyanaramu and Raghubabu, 2011; Basavaiah *et al.*,

2008) and through Reversed-Phase HPLC in dosage and bulk drug form (Salazar *et al.*, 2015; Suneetha and Rao, 2010; Kumar *et al.*, 2011). Plackett–Burman design is used for the evaluation of raloxifene hydrochloride and its impurities by a new LC Validation method (Stojanović *et al.*, 2013). UPLC method to analysis raloxifene and its corresponding impurities in drug and dosage form reported Saini *et al.* (2012). Identification and description of possible impurities through the synthesis of the raloxifene hydrochloride drug in bulk (Reddy, 2012). Structural explanation of viable impurities of raloxifene HCL by LC/ESI-MS (Jagadeesh *et al.*, 2014). Confirmation of raloxifene and glucurovides in urine samples by LC-MS/MS method (Trdan *et al.*, 2011).

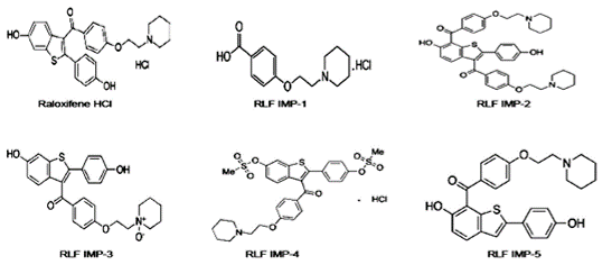

Figure 1: Chemical Structure of raloxifene and its impurities

The main object of this work is an advanced, fast, accurate, precise and simple method for the decide and quantification of 5 related substances in raloxifene. The structures of the drug substances and related substances are presented in Figure 1. The specification limits of all impurities are tabulated on the support of ICH thresholds and official monographs (USP, Ph.Eur and BP). According to the guidelines of FDA (Food and Drug Administration) and ICH (International Conference on Harmonization), this method was demonstrated with respect to the limit of detection, the limit of quantification, linearity, precision, accuracy, specificity and stability studies.

MATERIALS AND METHODS

Reagents and Standards

Reference standards and related compounds of drug substances are obtained by GSN Pharmaceuticals Pvt., Ltd. Hyderabad. Raloxifene tablets dosage forms were brought from the local market. Acetonitrile at HPLC class was acquired from Qualigens, India. Water is purified through Milli-Q Millipore for HPLC analysis. 0.45 μm filter nylon used to filter the mobile phase all the solutions.

Instrumentation

The HPLC system used in the current method was Waters 2695 separation module includes of binary pump plus autosampler, degasser, column oven and 2996 photodiode array detector. The Empower software of Waters Corporation was used to detect and process the output signals. Inertsil C8 150 x 4.6 mm ID, 3.5μm column was used for LC studies. The flow rate of the mobile phase is 1.0 mL/ minute. The column temperature was continued at 300C and the detection observed at a wavelength 280nm. 10 μL was used for injection.

Impurity stock solutions and Standard preparation

Finely powdered after weighing the tablets, with the mortar and pestle. 100mg equivalent of raloxifene was taken into a volumetric flask of 50ml. Extract all the active compounds and their related impurities add 35ml of diluent and fill up after sonication. By using Nylon 0.45 μm syringe, discard the first 2ml of the filtrate and transfer the remaining into 2ml HPLC vial.

RESULTS AND DISCUSSION

Analytical method development

As raloxifene hydrochloride shows a basic character, it was hypothesized that acidic pH could improve the ionization capacity of the drug. Therefore, a pH of 4.5 was maintained and consequently, good separation of peaks in the column was obtained. The mobile phase composition was finalized after examines with different solvent systems. It was found that the use of methanol with water or pH 4.5 phosphate buffer caused peak broadening with substantial loss of peak area. Mobile phase A (0.05 mM KH2PO4 buffer at pH 4.5) and mobile phase B (acetonitrile) are optimal for good separation and for obtaining a sharp peak of raloxifene HCL and its five impurities. Peak tailing was a major criterion for selection of the stationary phase. Initial trials with C18 columns of two different makes, (Phenomenex® C18, 250 mm long and 4.6 mm internal diameter, particle size 5 μm and Kromasil C18, 250 mm long and 4.6 mm internal diameter, particle size 5 μm) showed peak tailing less than 1.5. Finally, good separation and taling was found in Inertsil C8 column. Based on the trials, an optimum column temperature of 30 ºC was selected to avoid a shift in retention time and the best peak shape. Wavelength depends on λmax, which was selected as 280 nm, based on the below given UV-Vis spectra of raloxifene.

Method validation

The specificity of the method verified by well resolution of both the impurities and any degradants

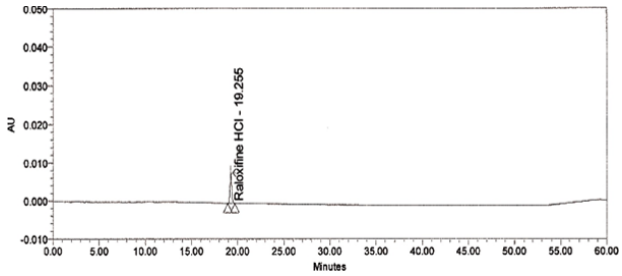

Figure 2: Raloxifene standard solution

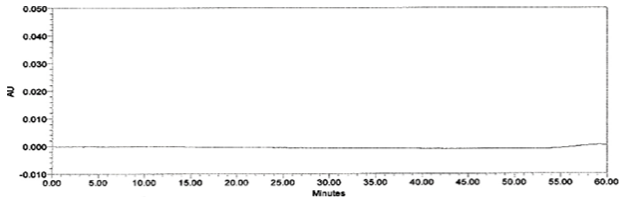

Figure 3: Blank chromatogram

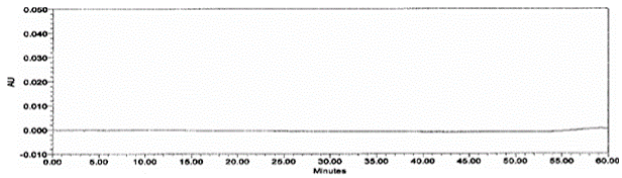

Figure 4: Placebo chromatogram

(by forced degradation study) from raloxifene peak. The method sensitivity was established by the limit of quantification (LOQ) and limit of detection (LOD). The LOQ values were investigated and confirmed by six injections of the impurities at the estimated concentrations at 4.0 ppm. Linearity was assessed by making and injecting the standards in between of LOQ (0.4 ppm) to 150% (6 ppm) of raloxifene working concentration (2000 ppm). Method Accuracy was carry out by having the impurities at a known concentration at LOQ, 2, 4 and 6 ppm levels in triplicate preparations. The precision was checked by method precision and ruggedness. Method precision was checked by injecting six freshly prepared spiked raloxifene impurities at specification level (4 ppm w.r.t 2000 ppm raloxifene working concentration) on the same day. The same experimental procedure was take on for the studies of ruggedness in different days with different columns and reagents. The robustness process was investigated with a slight adjustment in the mobile phase flow rate, Column temperature. The actual flow rate of the mobile phase was 1.0 mL. This was changed by 10%, i.e. from 0.9 to 1.1 mL/min. The column temperature studied on the resolution at 350C and 250C (±50C) was effective, mobile phase pH altered ±0.2 to the actual mobile phase pH of 4.5. At the room temperature and different time gaps as well as refrigerator condition (2-8°C) through standard and spiked sample solution stability of impurities in the raloxifene sample solution was investigated. System suitability was evaluated by injecting the system suitability solution and raloxifene by a series of six injections of the standard solution. The corresponding standard chromatograms are shown in Figures 2, 3 and 4 and system suitability results are shown in Table 1.

Forced degradation

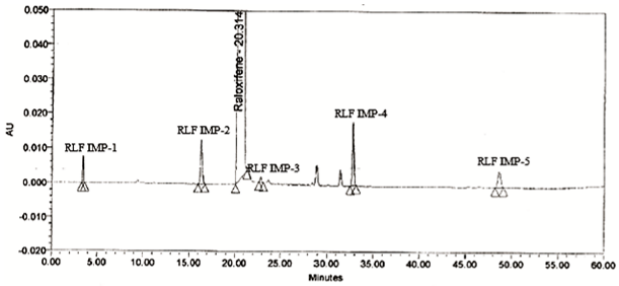

Figure 5: Impurity spiked sample

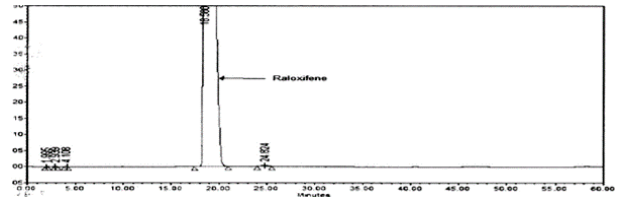

Figure 6: Photolytic degradation chromatogram

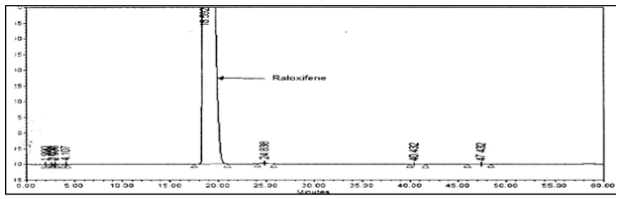

Figure 7: Thermal degradation chromatogram

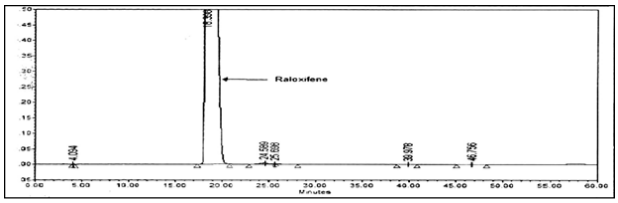

Figure 8: Base degradation chromatogram

Degradation samples were injected into the HPLC system and % degradation of the drug sample, and the purity of peaks obtained from stress samples were checked using a PDA detector.

Results in Table 2 shows that the purity angle is less than the purity threshold and demonstrated that the peak homogeneity of the analytes is confirmed. Assay of stressed samples was tested and check the performance by the comparison with standard references and the mass balance (%impurities + % assay) for stressed samples.

Table 1: System suitability results

S. No	RT (mins)	Peak Area	Tailing Factor	Theoretical Plates
1	19.255	49073	1.3	12505
2	19.245	48499	1.3	12474
3	19.256	49970	1.3	12443
4	19.324	49392	1.3	12440
5	19.512	50068	1.3	12472
6	19.551	48855	1.3	12513
Mean		49310		
SD		622.54		
%RSD		0.10		

Table 2: Summary of retention time(RT), relative retention time (RRT) and the peak purity values forraloxifene HCl and known impurities

Peak	Retention Time (mins)	Relative retention Time	Resolution	Purity angle	Purity threshold
RLF IMP-1	3.412	0.17	–	0.015	0.213
RLF IMP-2	16.292	0.80	15.5	0.324	0.510
Raloxifene	20.314	1.00	6.5	0.149	0.619
RLF IMP-4	22.817	1.12	3.5	1.212	2.367
RLF IMP-5	32.765	1.61	10.3	0.367	0.419
RLF IMP-6	48.654	2.40	16.9	0.425	0.716

Table 3: Results from forced degradation

Sample details	% of degradation	% of Assay	Mass balance	Peak Purity
As such sample	0.05	99.5	-	Pass
Photolytic sample (1.2 million lux hrs)	0.36	99.6	100.4	Pass
Thermal sample (at 60°C for 7 days)	0.15	99.1	99.7	Pass
Base degradation (2N NaOH Solution)	6.12	93.5	100.1	Pass
Acid degradation (2N HCl Solution)	1.52	97.2	99.2	Pass
Peroxide degradation (10% H2O2)	5.12	94.2	99.8	Pass
Humidity sample (90% Humidity for 7 days)	0.12	99.5	100.1	Pass

Table 4: LOD and LOQ

Name of the analyte	Limit of detection		Limit of quantification	
	Concentration (ppm)	S/N Ratio	Concentration (ppm)	S/N Ratio
RLF IMP-1	0.14	2.6	0.41	9.9
RLF IMP -2	0.13	3.8	0.39	9.9
RaloxifeneHCl	0.12	3.5	0.35	10.1
RLF IMP -3	0.13	2.7	0.38	10.0
RLF IMP -4	0.14	2.4	0.42	10.0
RLF IMP -5	0.15	3.0	0.44	9.8

Conditions of the forced degradation studies are included in Table 3, and degradation chromatograms were shown inFigures 5, 6, 7, 8, 9 and 10.

Limit of detection and quantification

The limit of detection (LOD) and limit of quantitation (LOQ) were checked by injecting diluted solutions having known concentration of known impurities and raloxifene to obtain a signal- to- noise ratio close to 3 for LOD and close to 10 for LOQ. List of Table 4 contains results of analyte peak and each impurity.

Linearity

Table 5: Linearity results of raloxifene

Linearit level	Level	Concentration (ppm)	Area response
1	LOQ	0.314	10171
2	50%	2.063	63252
3	100%	4.125	133503
4	120%	4.95	157004
5	150%	6.188	199755
		Slope	32327.94
		Y-Intercept	-1315.99
		Correlation coefficient	0.9997
		Y-Intercept at 100% level	-0.99

The linearity of the method in multiple reaction monitoring modes was satisfactorily investigated by injecting dissimilar concentrations i.e., LOQ (0.4 ppm), 50% (2 ppm), 100% (4 ppm), 120% (5 ppm) and 150% (6 ppm) w.r.t 0.2% of specification level (specification level 4 ppm of sample concentration 2000 ppm). The calibration curve was obtained by drawing the graph with concentration and peak areas. The slope, intercept, correlation coefficient values were presented in Table 5, which represents an excellent correlation between peak areas and concentrations.

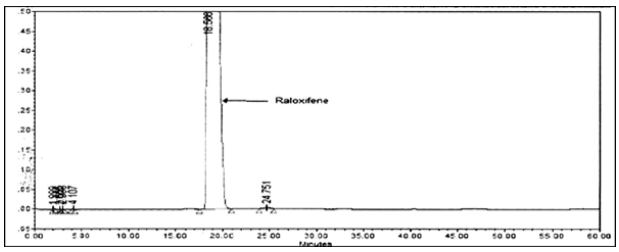

Figure 9: Acid degradation chromatogram

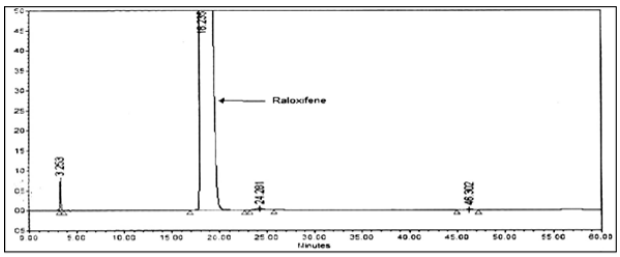

Figure 10: Peroxide degradation chromatogram

Accuracy

Accuracy was conducted by the standard addition method. Therefore, the accuracy of the method was determined by spiking three-levels, i.e. LOQ (0.4 ppm), 50% (2.0 ppm), 100% (4.0 ppm) and 150% (6.0 ppm) of the specification limit (0.2% w.r.t sample concentration 2000 ppm). Recoveries are calculated the content of all impurities in spiked sample preparation and determined the % recovery in raloxifene sample.

Precision

The present method was verified through method precision and intermediate precision. Method precision was checked by raloxifene spiked sample by injecting six individual preparations (2000 ppm of raloxifene HCl, 4.0 ppm of RLF IMP-1 to RLF IMP-5 in diluent). Precision results are given in Table 6, and %RSD was found to below 10%. Results of intermediate at separate columns and reagents on sepa-

Table 6: Summary results of precision

Sample No.	RLF IMP-1 (ppm)	RLF IMP-2 (ppm)	RLF IMP-3 (ppm)	RLF IMP-4 (ppm)	RLF IMP-5 (ppm)
1	4.015	3.965	3.891	4.158	4.015
2	4.152	3.994	4.105	4.109	4.125
3	3.987	3.789	3.875	4.156	4.001
4	4.012	4.021	3.915	4.052	3.894
5	4.002	4.001	4.025	3.984	3.991
6	4.102	3.914	4.003	3.891	3.845
Average ± SD	4.045 ± 0.066	3.947 ± 0.086	3.969 ± 0.090	4.058 ± 0.105	3.979 ± 0.098
% RSD	1.64	2.18	2.27	2.60	2.47

Table 7: Summary results of intermediate precision

Sample Prep No	RLF IMP-1 (ppm)	RLF IMP-2 (ppm)	RLF IMP-3 (ppm)	RLF IMP-4 (ppm)	RLF IMP-5 (ppm)
1	3.917	4.105	4.001	3.879	3.917
2	3.965	4.258	4.011	3.912	4.101
3	3.891	4.158	4.025	3.869	4.009
4	4.158	4.185	4.125	4.012	4.158
5	4.015	4.201	4.112	4.005	4.217
6	3.875	3.987	4.025	4.105	4.126
Average ± SD	3.970 ± 0.105	4.149 ± 0.094	4.050 ± 0.054	3.964 ± 0.093	4.088 ± 0.108
% RSD	2.65	2.26	1.34	2.33	2.65

Table 8: Robustness parameters

Parameter	Actual	Low level	High level
Flow variation	1.0 mL/min	0.8 mL/min	1.2 mL/min
Column oven temperature	30 ºC	25 ºC	35 ºC
Mobile phase-A pH	4.5	4.3	4.7

rate days included in Table 7 and %RSD was found to below 10%

Robustness

Table 8 report the parameters of the method that were altered to test the robustness of the method. Prepared a standard solution (4.0 ppm of raloxifene hydrochloride) and spiked sample solution (RLF IMP-1 to RLF IMP-5 in diluent), these solutions were run to assess the robustness method whether these changes had a significant effect on the chromatography of the method or not. All system suitability results provide proof of its positive during normal usage.

As per the ICH guidelines, robustness parameters are performed at 10% of the actual conditions. Of course, during the method development, the flow rate was altered up to 20% variation of the actual flow rate, i.e. at 0.8 mL/min, and 1.2 mL/min and not much variation observed. If the flow rates go beyond the 20% of the actual flow, peaks were not eluted properly.

Solution stability

The solution stability investigations were executed systematically to assess the stability of raloxifene standard solution and spiked solution at room temperature (25oC) as well as cooler temperature (2-8oC). Each sample was injected and studies the % recovery of the impurities with respect to the fresh standard solutions. Table 9 reports the results. The results demonstrated that the raloxifene standard solution and spiked solution were stable both at 250C and refrigerator conditions for 24 hrs, which

was evidenced by its good recovery values in the between of 97.6% to 99.9%. The difference between the recoveries at 0th hr and 24th hr was 95% to 105%, which indicates that the standard and spiked solutions prepared in diluent were stable for '24' hrs.

Application of Method

The results obtained in this study demonstrated that the present HPLC method is selective, linear, precise, rugged, robust and stability specify for the confirmation of related in raloxifene HCl tablet dosage forms. Therefore, By using the below-mentioned equation, it is suitable for the proposed method. Local market samples analyzed as per proposed impurity method results found within in the criteria

CONCLUSION

A basic, selective, highly sensitive, more accurate and rapid analytical method was enhanced for the determination and quantification of 5 related substances in Raloxifene. Raloxifene is a non-polar molecule, which is slightly soluble in water. Satisfactory extraction of tablet dosage forms found in the mixture of pH 4.5 buffer and acetonitrile is 50:50 (% v/v). Good separation and quantification of 5 related substances in raloxifene was achieved by selecting KH2PO4 buffer with pH 4.5 and acetonitrile as mobile phase B, stationary phase used is Inertsil C8 with the dimensions 150 x 4.6 mm ID, 3.5μm. 1.0 mL/min was the flow rate in liquid chromatography. The more accurate value of the method was proved by the recovery by the range 80.0% to 120.% with %RSD not more than 5.0, with a correlation of 0.999. The method is validated for specificity, the limit of quantification, the limit of detection, linearity, accuracy, method precision, intermediate precision, robustness and stability this method is validated.

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