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Spectrophotometric and Chromatographic Methods for the Estimation of Raloxifene Hydrochloride in Bulk and Pharmaceutical Formulations

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ABSTRACT

Aim: To develop simple, accurate and precise spectrophotometric and chromatographic methods for the estimation of Raloxifene Hydrochloride (RXF) in pure and pharmaceutical dosage forms.

Study design: Spectrophotometric and chromatographic methods.

Place of Study: Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, Egypt.

Methodology: The differential spectrophotometric method was based on the measurement of the absorbance difference (ΔA) at 333.4 nm of alkaline raloxifene hydrochloride solutions in 0.1 N NaOH against its acidic solutions in 0.1N HCl. RP-HPLC was developed using benzophenone as an internal standard, where the mobile phase used was acetonitrile: water (50:50, v/v), delivered at a flow rate of 1.2 ml/min on a stationary phase composed of C_{18} column; and the detection was carried out at the λ_{max} of RXF (289 nm).

Results: The recovery percentage for RXF was found to be 100.46 ±0.65 and 99.96 ±0.83 for the two methods, respectively. The methods were validated as per ICH guidelines regarding accuracy, precision and system suitability.

Conclusion: All the results obtained were found to be within the acceptable limits. The methods were successful to estimate RXF in bulk powder and pharmaceutical preparation Evista ®.

Keywords: (Raloxifene Hydrochloride; differential spectrophotometry; absorbance difference; HPLC; benzophenone).

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1. INTRODUCTION

Raloxifene hydrochloride (RXF) [6-hydroxy-2-(4-hydroxyphenyl)- benzothiophen-3-yl]- [4-[2-(1-piperidyl)ethoxy]phenyl] –methanone hydrochloride] is a selective estrogen receptor modulator acting as an estrogen agonist in bone and in liver it reduces total cholesterol and LDL, but does not increase HDL. It is an estrogen receptor antagonist in uterine and breast tissues [1]. The chemical structure of RXF is shown in Figure 1. The official monograph for RXF in Ph. Eur. 7 [2] described HPLC method for the analysis of RXF in presence of its related substances using C8 as stationary phase and a movile phase of a solution of 9.0 g/L potassium dihydrogen phosphate adjusted to pH 3.0 with phosphoric acid and acetonitrile in the ratio of (70: 30 v/v). Several methods have been reported for the analysis of raloxifene hydrochloride in pharmaceutical dosage form including spectrophotometry [3-7], chromatography [8-15], electrochemistry [16] and capillary electrophoresis [17] .

The aim of this work is to develop and validate simple, accurate and precise spectrophotometric and chromatographic methods for the estimation of Raloxifene Hydrochloride (RXF) in pure and pharmaceutical dosage forms.

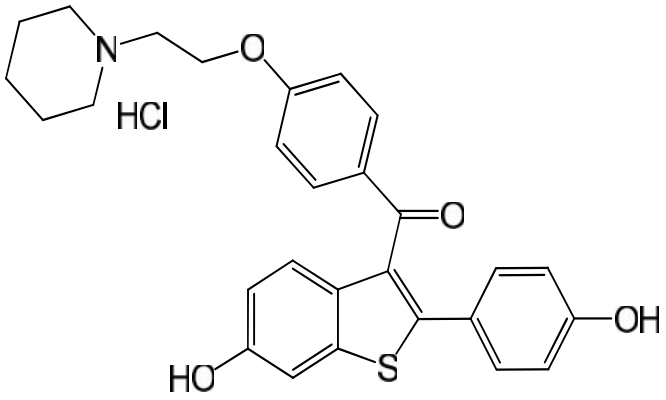

Fig. 1. The chemical structure of Raloxifene hydrochloride (RXF).

2. METHODOLOGY

2.1 Chemicals and reagents

Methanol, acetonitrile (Merck-USA) and deionized water were used.

2.2 Materials

Raloxifene hydrochloride was kindly supplied by Eli Lilly Co and its purity was checked by the HPLC method and found to be 99.85±0.81 % [18] and Evista® tablets labeled to contain raloxifene hydrochloride 60 mg /tablet, Eli Lilly Co.

2.3 Apparatus

UV-1650PC, UV - Visible Shimadzu Spectrophotometer (Japan) with matched 1 cm quartz cells was used for all absorbance measurements. Spectra were automatically obtained by Shimadzu UV-Probe 2.32 system software.

HPLC system, Shimadzu series (Japan) chromatographic system equipped with quaternary pump, microvacuum degasser, thermostatted column compartment and equipped with PDA detector was used. Sample injections were made through an autosampler. Zorbax Column SB-C_{18} (150mm×4.6 mm, 5 µm particle size i.d.) was used.

2.4 Procedure

2.4.1 Preparation of standard solutions

Twenty mg of pure RXF were accurately weighed, introduced into a 100 ml volumetric flask and dissolved in methanol to obtain a concentration of (200µg/ml).

An accurate weight of benzophenone (200 mg) was introduced into a 100 ml volumetric flask and dissolved in mixture of acetonitrile: water (50:50, v/v) to obtain a concentration of (2mg/ml).

2.4.2 Linearity of differential spectrophotometric method

Ten ml aliquot of the standard solution was transferred into two volumetric flask (100 ml), where the first flask was completed to the mark using 0.1 N NaOH, while the second one was completed to the mark with 0.1 N HCl, to prepare two working solutions of concentration (20 µg/ml). Different aliquots of both the alkaline and the acidic solutions (2-10 ml) were transferred into two separate series of 10 ml volumetric flasks. The volume was completed with 0.1 N NaOH for the alkaline solutions and 0.1 N HCl for the acidic solutions. The absorbance difference (ΔA) of the alkaline solutions of RXF in the sample cell was measured at 333.4 nm relative to that of the acidic solutions of RXF in the reference cell. A calibration curve was plotted, representing the concentration of RXF versus the corresponding absorbance difference (ΔA), from the average of three experiments.

2.4.3 Chromatographic conditions for RP-HPLC method

The mobile phase used was acetonitrile: water (50:50 v/v) on a stationary phase composed of C18 column. It was filtered through 0.45 um membrane filter and degassed using ultrasonic path. The system was equilibrated and saturated with the mobile phase for half an hour before the injection of the samples. The flow rate was maintained at 1.2 ml/min. Detection was carried out at the λ_{max} of RXF (289 nm). Benzophenone was used as an internal standard. System suitability parameters including resolution, number of theoretical plates, tailing and capacity factors were calculated.

2.4.4 Linearity of RP-HPLC method

Different aliquots (2-8 ml) of RXF standard solution (200 µg/ml) were introduced into a series of 10 ml volumetric flasks, then two ml aliquot of benzophenone solution (internal standard) was added to each flask and the volume was completed to the mark with the mobile phase. Twenty microliters aliquot of each flask was injected and the previous chromatographic conditions were adopted. The resulting chromatograms, retention times (t_R) of the eluted peaks and the area under the peaks (AUPs) were recorded. The ratios (R) of the recorded relative AUPs of RXF to that of benzophenone were plotted against the concentration of RXF to obtain the calibration curve, from the average of three experiments.

2.4.5 Application to pharmaceutical preparation (Evista® tablets)

An accurate weight of the powdered tablets equivalent to 20 mg of RXF was transferred into a beaker. Twenty ml aliquot of methanol was added and the beaker was stirred for 5 minutes with a magnetic stirrer then filtered into a 100 ml volumetric flask and the volume was completed with methanol to prepare a working solution of (200 µg/ml). Different aliquots (2- 4 ml) of this solution were transferred into a series of 10 ml volumetric flasks and completed to the mobile phase. The chromatographic conditions for RP-HPLC method were applied. The standard addition technique was applied and the regression equation was used to calculate the recovered concentrations of the labeled and the added RXF. Further dilution was performed by transferring five ml from the working solution of (200 µg/ml) into two 50 ml volumetric flasks, the first flask was completed to the mark using 0.1 N NaOH and the second one was completed to the mark with 0.1 N HCl to prepare two solutions of concentration (20 µg/ml). Different aliquots of both the alkaline and acidic solutions of RXF equivalent to (60-140 µg) was transferred into 2 sets of 10 ml volumetric flasks and completed with the same alkaline or acidic solution. The difference absorbance (ΔA) of the alkaline solutions of raloxifene hydrochloride in the sample cell was measured at 333.4 nm relative to that of the acidic solution of raloxifene hydrochloride in the reference cell. The same procedure was repeated using the standard addition technique and the concentrations of the labeled and added standard RXF could be calculated using the regression equation.

3. RESULTS AND DISCUSSION

3.1 Differential spectrophotometric method

The reported spectrophotometric methods of RXF included colorimetric methods utilizing different reagents [3-7]. Those methods are considered to be tedious, time consuming and of lower reproducibility. So the aim of this work was to develop and validate simple, accurate and precise spectrophotometric method based the absorbance difference (ΔA) of UV absorption. The specifity of the difference spectrophotometric determination RXF is due to its phenolic group which exhibits different spectral changes upon alteration of pH. RXF exhibits a bathochromic shift of its λmax at 289 nm in acidic solution to 333.4 nm in alkaline solution. The difference absorption spectrum (ΔA) of an alkaline RXF solution in 0.1 N NaOH was relative to an identical concentration in 0.1N HCl in the reference cell shows that the maximum difference occurs at 333.4 nm, Fig. 2.

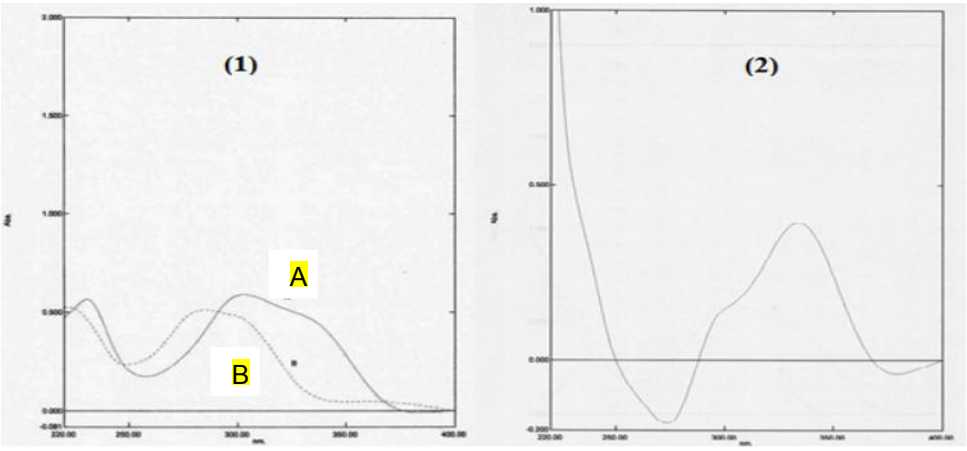

Fig. 2. (1) Absorption spectra of RFX 10ug/ml, A: in 0.1 N NaOH [__], B: in 0.1 N HCl […], (2) absorbance difference ΔA showing maximum at 333.4 nm.

3.2 RP-HPLC method

Several chromatographic methods were reported for the analysis of RXF, but all those methods suffered from critical conditions, either by applying gradient elution [9, 12, 13]; or by using a mobile phase containing controlled pH buffer [8, 10, 15] which may affect the life time of the column. So the aim of this work was to develop and validate accurate and precise HPLC method using isocratic elution and a simple mobile phase. In this study, RFX was analyzed using a mobile phase composed of (acetonitrile: water, 50: 50 v/v), where it showed good resolution between the eluted peaks of RXF and the internal standard benzophenone. Detection was carried at 289 nm. The flow rate was 1.2 ml/ min on Waters C18 column.

To optimize the proposed HPLC method, different binary mixtures of water and methanol were tried, including the (50:50) (water: methanol) failed to achieve resolution of the eluted peaks with these systems and the detector response was markedly low. Use of water and acetonitrile achieved good resolution. Increasing the water ratio decreased the retention times and resulted in peak broadening. Different authentic were tried as internal standard but benzophenone was chosen as an internal standard, due to the similarity between its structure and that of RXF; and the complete separation between their eluted peaks. The elution order was found to be: raloxifene hydrochloride (t_R = 2.75) and benzophenone (t_R = 8.86). The chromatogram shows complete separation and good resolution, Fig. 3.

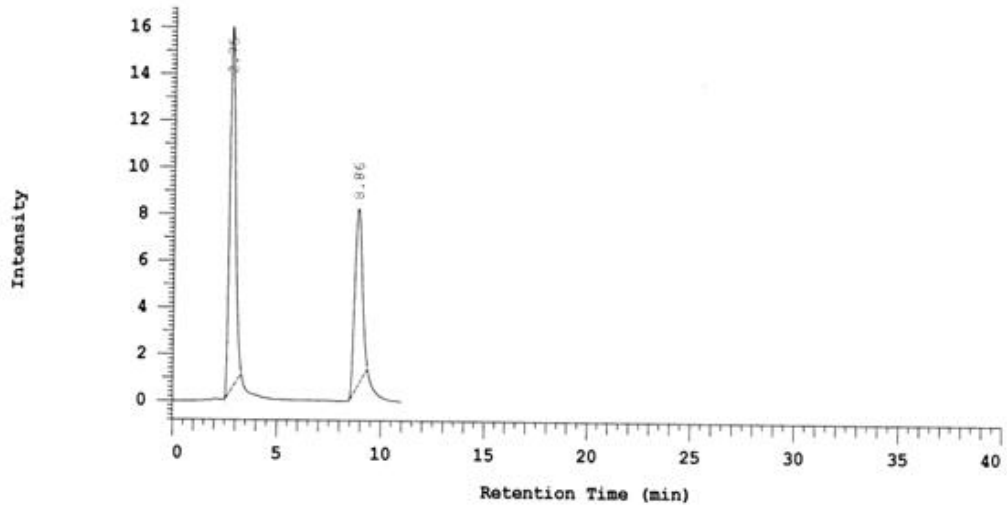

Fig. 3. Chromatogram for RP-HPLC showing retention time of RXF (t_R = 2.75) and internal standard benzophenone (t_R = 8.86).

The advantage of the proposed methods over the reported methods is their simplicity. Both methods were applied for the determination of the drug in its pure form and pharmaceutical dosage form. The validity of the methods was assessed by applying the standard addition technique and the results were shown in Table 1.

Table 1. Application of standard addition technique to the analysis of RXF in Evista ® tablets by the proposed methods.

Differential spectrophotometric method (ΔA)						RP-HPLC method					
Tablet			Standard addition			Tablet			Standard addition		
*Claimed**	*Found**	*Recovery%*	*Taken**	*Found**	*Recovery%*	*Claimed**	*Found**	*Recovery%*	*Taken**	*Found**	*Recovery%*
6	6.13	102.17	9.6	9.71	101.15	40	39.87	99.68	60	60.29	100.48
6	6.13	102.17	12	12.01	100.17	60	59.07	98.45	40	39.32	98.30
8	8.21	102.63	7.2	7.23	100.42	60	59.07	98.45	60	60.52	100.87
8	8.21	102.63	12	12.06	100.50	60	59.07	98.45	80	80.49	100.61
10	10.10	101.00	7.2	7.12	98.89	80	78.58	98.23	40	40.39	100.89
14	14.21	101.50	4.8	4.89	101.88	80	78.58	98.23	60	59.36	98.93
Mean		102.02			100.45			98.58			100.01
S.D.		± 0.65			± 0.91			± 0.55			± 1.11
S.E.		± 0.27			± 0.37			± 0.22			± 0.45

* in µg/ml.

4. METHOD VALIDATION

Method validation was performed according to ICH guidelines [19] for the proposed methods including: linearity, range, accuracy, precision, robustness. The results were listed in Table 2. System suitability parameters were calculated and compared to USP guidelines [20] . The results were listed in Table 3.

5. CONCLUSION

The proposed spectrophotometric and chromatographic methods showed several advantages over the reported methods regarding their simplicity, saving time and of less cost. The methods were applied successfully for the estimation of raloxifene hydrochloride in pure form and pharmaceutical preparation using standard addition technique where SD values were less than 2. The methods were validated via ICH guidelines and all results obtained were within acceptable range.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

All the authors have equal share in this manuscript. All authors read and approved the final manuscript.

Table 2. Assay parameters and validation sheet by applying the proposed methods for determination of RXF.

Method	(∆A) method	RP-HPLC method
Calibration range (µg/mL)	4- 20	40- 140
Slope	0.0399	0.0130
Intercept	- 0.0108	- 0.1862
Mean [a]	100.15	100.07
SD	± 0.65	± 0.65
Accuracy [b]	100.46 ± 0.65	99.96 ± 0.83
Intra-day precision [c]	100.89 / 0.802	100.22 / 0.568
Inter-day precision [c]	99.76 / 0.85	100.81 / 0.71
Robustness [d]	100.16 / 0.65	100.36 / 0.62
r^2	0.9993	0.9998

[a] Average of three experiments.

[b] Mean ± SD of five blind concentrations (5, 8, 11, 14, 17 µg/mL) of RXF within the concentration range.

[c] Mean / RSD of triplicate determination of three concentrations (4, 9, 14 µg/mL) of RXF (n=9).

[d] Mean / RSD of triplicate determination of three concentrations (4, 9, 14 µg/mL) of RXF by 5% change in acetonitrile ratio in mobile phase.

Table 3. System suitability parameters for the RP-HPLC method.

Parameter	RP-HPLC method for RXF	Reference value [19]
t_R (Relative retention time)	2.36	
N (Column efficiency)	2232.89	N > 2000
K' (Capacity factor)	5.00	> 2
T (tailing factor)	1.25	T < 2 T=1 for symmetric peak
Rs (Experimental Resolution)	6.81	Rs >2

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