Research paper

Spectrophotometric and Chromatographic Methods for the Estimation of Raloxifene Hydrochloride in pure form and pharmaceutical preparation

ABSTRACT

Aim: To develop simple, accurate and precise spectrophotometric and chromatographic methods were developed and validated 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 $\pm$0.65 and 99.96 $\pm$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).

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. Several methods have been reported for the analysis of raloxifene hydrochloride in pharmaceutical dosage form including spectrophotometry [2-6], chromatography [7-11], electrochemistry [12] and capillary electrophoresis [13].

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

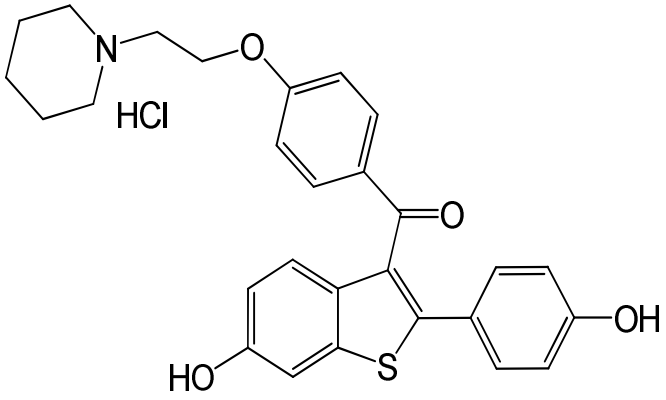

Fig. 1. The chemical structure of Raloxifene hydrochloride (RXF).

2. METHODOLOGY

2.1 Chemicals and reagents

Methanol, acetonitrile (Merck) 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 % [14] 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), equipped with PDA detector.

2.4 Procedure of the reaction of raloxifene hydrochloride with TCNQ

2.4.1 Preparation of raloxifene base 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 transferred into two 100 ml volumetric flask, the first 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 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 solution and 0.1 N HCl for the acidic solution. 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 solution of raloxifene hydrochloride 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). 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 on a stationary phase composed of C18 column. 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.

2.4.4 *Linearity of RP-HPLC method*

Different aliquots (2-8 ml) of RXF standard solution (200 µg/ml) equivalent to (400-1600 µg) were introduced into a series of 10 ml volumetric flasks. Two ml aliquot of benzophenone solution (internal standard) was added and the volume was completed with the mobile phase. Twenty micro liter aliquot of each flask was injected into the column. The previous chromatographic conditions were adopted. The resulting chromatograms, retention times (t_R) of the peaks and the areas under the peaks (AUPs) were recorded. The ratios (R) of the recorded 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 (∆A) 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. 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 (∆A) 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 (∆A) 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.

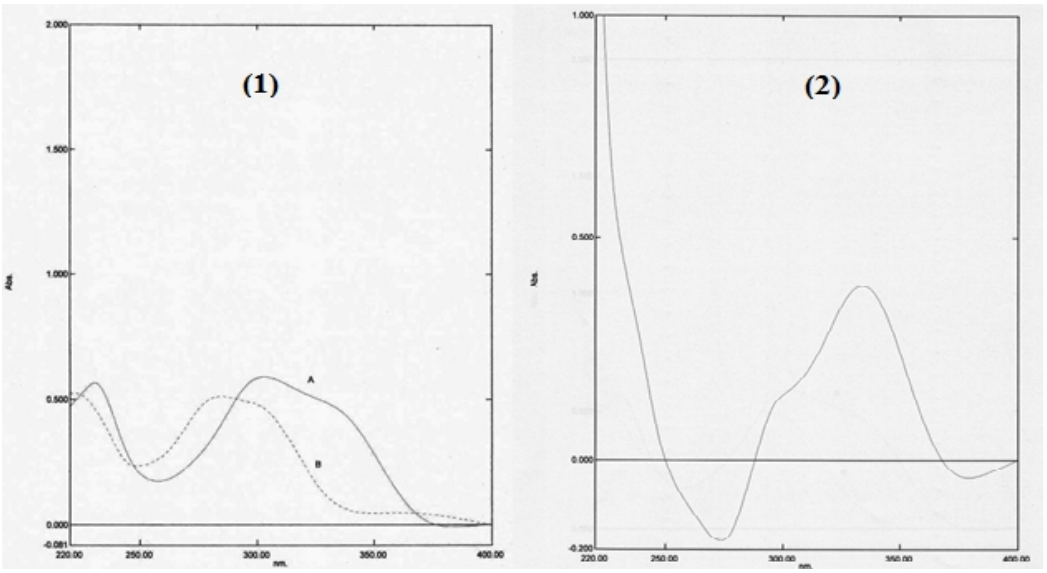

Fig. 2. (1) Absorption spectra of RFX 10ug/ml, A: in 0.1 N NaOH [__], B: in 0.1 N HCl […], (2) absorbance difference ∆A showing maximum at 333.4 nm.

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 [8, 11]; or by using a mobile phase containing controlled pH buffer [7, 9] 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. Detection was carried at 289 nm. The flow rate was 1.2 ml/ min on Waters C18 column.

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 used as internal standard with the elution order: raloxifene hydrochloride (tR = 2.75) and benzophenone (tR = 8.86). The chromatogram shows complete separation and good resolution, Fig. 3.

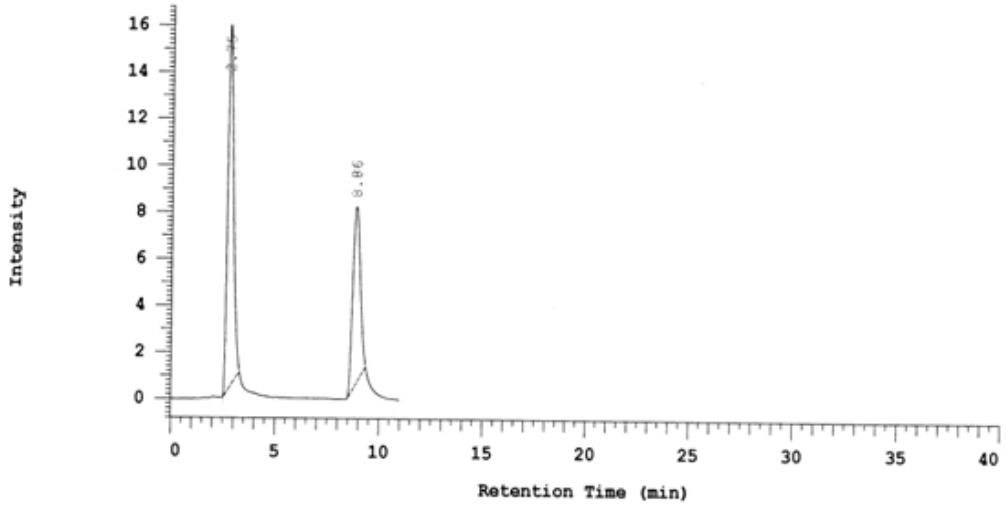

Fig. 3. Chromatogram for RP-HPLC showing retention time of RXF (t_R = 2.75) and internal standard benzophenone (t_R = 8.86).

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 [15] for the proposed methods including: linearity, range, accuracy, precision. The results were listed in Table 2. System suitability parameters were calculated and compared to USP guidelines [16]. The results were listed in Table 3.

5. CONCLUSION

The proposed spectrophotometric and chromatographic methods showed several advantages over the reported methods. The methods were applied successfully for the estimation of raloxifene hydrochloride in pure form and pharmaceutical preparation.

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	4- 20	40- 140

(µg/mL)		
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
r^2	0.9993	0.9998

[a] Average of three experiments.

[b] Mean ± SD of five blind concentrations of RXF within concentration range.

[c] Mean / RSD of triplicate determination of three concentrations of RXF (n=9).

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

Parameter	RP-HPLC method for RXF	Reference value [16]
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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