International Journal of Pharmaceutics & Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

SIMPLE UV METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF RALOXIFENE IN MARKETED FORMULATION

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***Article History:**

Received: 18/10/2019
Revised: 27/10/2019
Accepted: 07/12/2019

ABSTRACT

The present research work discusses the development and validation of a UV spectrophotometric method for raloxifene (RLX). Simple, accurate and cost efficient spectrophotometric method has been developed for the estimation of RLX in Tablet dosage form. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ max) was found to be 282 nm. The percentage recovery of RLX was in the 99.12±0.68. Beers law was obeyed in the concentration range of 5-25µg/ml. Calibration curves shows a linear relationship between the absorbance and concentration. The line equation y=0.024x+0.002 with r2 of 0.999 was obtained. Validation was performed according to ICH guidelines for Linearity, accuracy, precision, LOD and LOQ. The sample solution was stable up to 36 hours. The proposed method may be suitable for the analysis of RLX in tablet formulation for quality control purposes.

Keywords: Raloxifene, Validation, Precision, Accuracy, LOQ, LOD, ICH guidelines.

INTRODUCTION

Raloxifene (RLX) chemically [6-hydroxy-2-(4- hydroxyphenyl)-benzotiophen-3-yl]-[4-[2-(1-piperidyl) ethoxy] phenyl]-metadon (Figure 1), is a selective oestrogen-receptor modulator (SERM) that has been approved for use in the prevention and treatment of osteoporosis in postmenopausal women. A SERM interacts with oestrogen receptors, functioning as an agonist in some tissues (bone and cardiovascular system) and as antagonist in other tissues (mammary tissue and uterus) (Katzung, 2006; Rang *et al.*, 2006). Recently, raloxifene has been also approved by FDA for the prevention of invasive breast cancer. It was developed by Ely Lilly Company and marketed as Evista® in form of tablets of 60.0 mg. It is generally well tolerated, but it has as most common adverse effects hot flashes and leg cramps. A

serious adverse effect is venous thromboembolism (ANVISA, 2012; Brunton *et al.*, 2010; NCI, 2012). Literature view report that different spectrophotometric methods are available for RLX 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 RLX and its impurities by a new LC Validation method (Stojanović *et al.*, 2013). UPLC method to analysis RLX 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 RLX drug in bulk (Reddy, 2012). Structural explanation of viable impurities of RLX by LC/ESI-MS (Jagadeesh *et al.*, 2014). Confirmation of RLX and glucurovides in urine samples by LC-MS/MS method (Trdan *et al.*, 2011). Most of these methods are uneconomic and involving complex sample preparation. So, there is a need for the development of simple sensitive effective and economic methods and hence the present work was planned to validate the UV spectroscopic method for RLX in tablet formulations by using following parameter like accuracy, precision, linearity and range, limit of detection, limit of quantification, specificity, robustness, ruggedness and system suitability as per ICH guidelines (ICH, 2005; ICH 1995). The aim of present work is to find out a simple, sensitive, specific, spectrophotometric method for the detection of RLX in pharmaceutical tablet formulation.

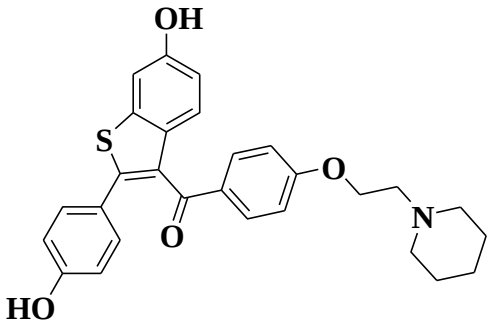

Fig. 1 Chemical structure of raloxifene

EXPERIMENTAL

Reagents and chemicals

The working standard of raloxifene was provided as gift sample from GSN Pharmaceuticals

Pvt., Ltd. Hyderabad, India. The market formulation Evista tablets (RLX 60mg) were procured from local market. Triple distilled water was generated in house. All solvents and reagents were of analytical grade. All the solutions were protected for light and were analyzed on the day of preparations.

Instrument

In UV-spectrophotometric method, Labindia model-3000+ series were used, which is a

wavelength accuracy ±1 nm, with 1cm quartz cells.

Determination of wavelength of maximum absorbance (λ_{max}) of raloxifene

Wavelength of maximum absorption was determined by scanning 10µg/ml solution of raloxifene using UV spectrophotometer from 200 to 400 nm. This showed maximum absorbance at 282 nm (Fig. 2).

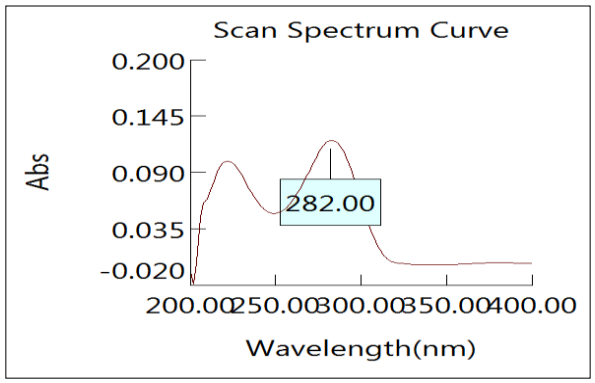

Fig. 2Determination of λ max of raloxifene

Preparation of standard stock solution (Stock-A)

Standard stock solutions were prepared by dissolving 100 mg of drug in 50 ml of water and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark with water to get a concentration of 1000 µg/ml (Stock-A) for drug.

Preparation of Standard Stock Solution

10mg of raloxifene was weighed accurately and transferred to 10 ml volumetric flask, and the volume was adjusted to the mark with the 0.1 N HCl to give a stock solution of 1000 ppm or µg/ml.

Preparation of Working Standard Solution

From stock solutions of raloxifene 1 ml was taken and diluted up to 10 ml separate volumetric flask. From this solution 0.5 1.0, 1.5, 2.0, and 2.5ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with 0.1 N HCl, gives standard drug solution of 5-25µg/ml concentrations of raloxifene.

Preparation of the calibration curves of the drug

The calibration curve was prepared by scanning test samples ranging from 5-25µg/ml at 282 nm for raloxifene. The calibration curve was tested by validating it with inter-day and intra-day measurements. Mean of n =5 determinations was plotted as the standard curve (Fig.3).

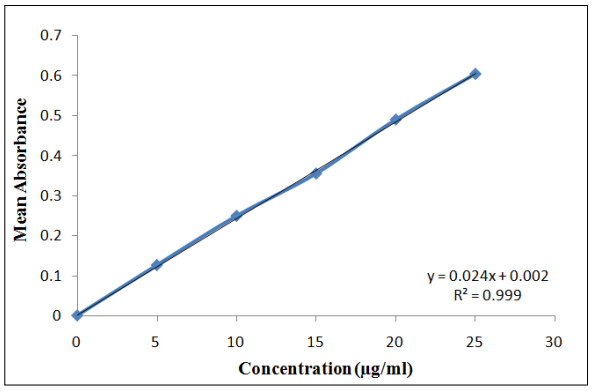

Fig. 3 Calibration curve of raloxifene

Validation of calibration curve method

Linearity

Linearity of drug was established by response ratios of drug. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio table 1.

Table 1 Response ratio of raloxifene

S. No.	Raloxifene		
	Conc. (µg/ml)	ABS	Response Ratio
1.	0	0	0
2.	5	0.1263	0.02526
3.	10	0.2493	0.02493
4.	15	0.3559	0.02372
5.	20	0.4903	0.02451
6.	25	0.6043	0.02417

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of raloxifene to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels table 2.

Table 2 Results of recovery studies

Recovery Level %	% Recovery (Mean±SD)*
80	98.85±0.15
100	99.12±0.68
120	98.56±0.41

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week. The results are shown in table 3.

Table 3 Results of precision (%R.S.D.)

Parameter		Mean±SD
Precision (%R.S.D.) *	Repeatability	99.45±0074
	Day to Day	98.74±0.125
	Analyst to Analyst	98.25±0.45
	Reproducibility	99.74±0.56

Analysis of tablet sample

Twenty marketed tablets of raloxifene were weighed and ground to a fine powder; amount equal to 60 mg of raloxifene was taken in 100 ml volumetric flask and sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with 0.1 N HCl. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and

further diluted with 0.1 N HCl to get the final concentrations of drug in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from calibration curve method. The procedure was repeated for five times table 4.

Table 4 Analysis of tablet formulation

Conc. Present (µg/ml)	Replicate	
	Conc. Found (µg/ml)	% Conc. Found
Raloxifene	Raloxifene	Raloxifene
5	4.98	99.00
10	9.95	98.75
15	14.96	99.33
20	19.99	99.88
25	24.94	99.40

Conclusion

The results and the statistical parameters demonstrate that the proposed UV spectrophotometric method is simple, rapid, specific, accurate and precise. Therefore, this method can be used for the determination of raloxifene either in bulk or in the dosage formulations without interference with commonly used excipients and related substances.

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