



VISIBLE SPECTROPHOTOMETRIC DETERMINATION OF RALOXIFENE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS USING 4-AMINOPHENAZONE AND POTASSIUM FERRICYANIDE REAGENT

BURIDI.KALYANARAMU* AND K.RAGHUBABU

Department of Engineering Chemistry, AU College of Engineering (A), Andhra University, Visakhapatnam, Andhrapradesh-530003 (India)
Email: kalyanaramubrd@gmail.com

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ABSTRACT

A simple and sensitive visible spectrophotometric method for the determination of Raloxifene hydrochloride using 4- Amino phenazone reagent has been developed in bulk and tablet dosage forms. It is based on the formation of red colored anti- pyrine dye by treating the drug with 4-AP and potassium ferri cyanide used as an oxidant in the presence of weak base with an absorption maximum of 540.3nm. The Regression analysis of Beer's Law plot showed good correlation in a general concentration range of 4-12µg/ml. The proposed method is validated with respect to accuracy, precision, linearity and limit of detection. The suggested procedure is successfully applied to the determination of the drug in pharmaceutical preparation, with high percentage of recovery, good accuracy and precision. The results of analysis have been validated statistically by repeatability and recovery studies. The results are found satisfactory and reproducible. The method is applied successfully for the estimation of raloxifene in tablet form without the interference of excipients.

Keywords: Beer's Law, 4-AP, Potassium ferri cyanide, SERM, Estimation, Validation.

INTRODUCTION

Raloxifene hydrochloride (RLX) is a second generation selective estrogen receptor modulator (SERM) non steroidal compound that belongs to the benzothiopyene class of compounds. The chemical designation is methanone, [6-hydroxy-2-(4-hydroxyphenyl) benzo [b] thien-3-yl]-[4-[2-(1-piperidiny) ethoxy] phenyl]-, hydrochloride.

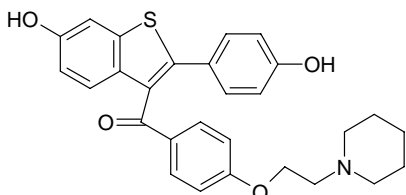


Fig.1 Showing Chemical structure of RLX

RLX is used in the treatment and prevention of osteoporosis and invasive breast cancer in post-menopausal women and also reduces the risk of hormone-positive breast cancer and vertebral fractures. It is an estrogen agonist in bone, where it exerts an anti-resorptive effect. The drug is listed in Merck Index¹. Some analytical methods which include HPLC²⁻¹⁰, LC- MS-MS¹¹⁻¹², Capillary electrophoresis¹³, Resonance Rayleigh Scattering (RRS)¹⁴, UV¹⁵⁻¹⁶ and visible spectrophotometric¹⁷⁻²² have been reported in the literature for the determination of RLX in pharmaceutical preparations. The main purpose of the present study was to establish a relatively simple, sensitive and validated visible spectrophotometric method for the determination of RLX in pure form and in pharmaceutical dosage forms, since most of the previous methods involve critical reaction conditions or tedious sample preparations and less specificity. So the authors have made some attempts in this direction and succeeded in developing a method based on the oxidative coupling reaction with 4-AP in the presence of an alkaline potassium ferricyanide oxidizing agent and gives an N-substituted quinoneimine (red colored anti-pyrine dye)²³. The method can be extended for the routine assay of RLX formulations.

MATERIALS & METHODS

A Systronics UV/Visible spectrophotometer model -2203 with 10 mm matched quartz cells was used for all spectral measurements. All the chemicals used were of analytical grade. 4-AP (Fluka , 0.5 % , 2.46x10⁻²M, solution prepared by dissolving 500mg of 4-AP in 100ml distilled water on slightly warming in hot water bath and

cooled to room temperature), Potassium ferricyanide (BDH, 2%, 6.07x10⁻²M solution prepared by dissolving 2g of Potassium ferricyanide in 100ml of distilled water) and Na₂CO₃ solution (Ranbaxy, 1.0%, 9.43x10⁻²M prepared by dissolving 1.0gm of sodium carbonate in 100ml of distilled water) were prepared.

Preparation of Standard stock solution: 100mg RLX was dissolved initially in 10ml of 0.1M sodium hydroxide and then followed by dilution to 100 ml with distilled water to get 1mg/ml stock solution. This solution was further diluted stepwise with the same solvent to obtain working standard solution concentration of 100µg/ml.

Sample solution: About 20 tablets were weighted to get the average tablet weight and pulverized and the powder equivalent to 100mg of RLX was weighed, dispersed in 25ml of isopropyl alcohol (IPA), sonicated for 30minutes and filtered through whatman filter paper no.41. The filtrate was evaporated and the residue was used for the preparation of working sample solution in the same way as under working standard solution.

Determination of wavelength maximum (λ_{max})

The 2.5 ml of working standard solution of RLX (100µg/ml) was taken in 25ml calibrated tube. To this, 0.6ml Na₂CO₃, 1.0 ml of 4-AP and 1.0ml of potassium ferricyanide were added successively and the total volume in each tube brought to 9.0ml with distilled water and kept for 5 min. at room temperature for complete color development. The volume was made up to the mark with distilled water. In order to investigate the wavelength maximum, the above colored solution was scanned in the range of 400-760 nm UV-Visible spectrophotometers against a reagent blank. From the absorption spectra (Fig.2), it was concluded that 540.3nm is the most appropriate wavelength for analyzing RLX with suitable sensitivity.

Preparation of calibration graph

Aliquots of working standard RLX solution (100µg/ml) such as 1.0, 1.5, 2.0, 2.5, 3.0 ml were taken separately in a series of 25ml graduated test tubes, to get a concentration of 4, 6, 8, 10 and 12 µg/ml respectively. A 0.6 ml portion of Na₂CO₃ (2.26x10⁻³M) solution, 1.0ml of (9.84x10⁻⁴M) 4-AP and 1.0 ml of (2.43x10⁻³M) potassium ferricyanide were added successively to each test tube and the total volume in each tube brought to 9.0ml with distilled water and kept for 5 min. at room temperature for complete color development. The volume was made up to the mark with distilled water. The absorbance was measured at 540.3 nm against a similar reagent blank within 30 min. The calibration graph was constructed by plotting the drug concentration versus absorbance (Fig.3).

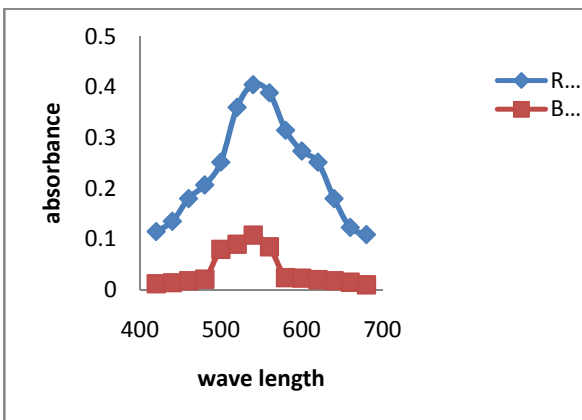


Fig.2: Determination of maximum wavelength for the detection

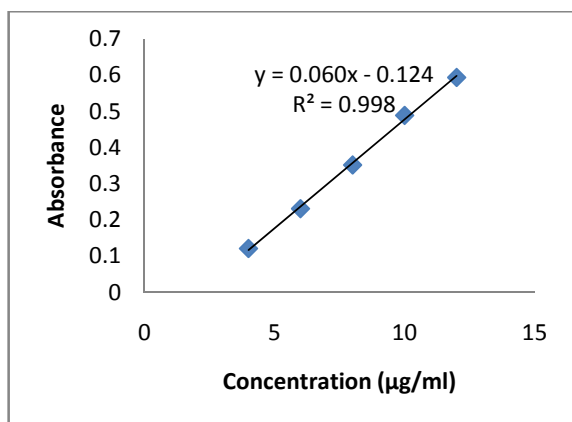


Fig.3: Calibration curve of RLX

RESULTS AND DISCUSSIONS

In developing a method, systematic studies of the effects of various parameters were undertaken by varying one parameter at a time and controlling all others fixed. The effect of various parameters such as time, temperature, nature and concentration of oxidant, volume and strength of 4-AP reagent, potassium ferricyanide, and order of addition of reagents on color development and solvent for final dilution on the intensity and stability of the colored species were studied and the optimum conditions were established. Among the various oxidants (NaIO_4 , potassium ferricyanide or $\text{K}_2\text{S}_2\text{O}_8$) tried in combination with 4-AP for the oxidative coupling reaction, potassium ferricyanide-4-AP was found to be the best suited with regard to sensitivity and stability of the colored species formed. Various basic substances (pyridine, Na_2CO_3 or NaOH) were tried for maintenance of alkalinity for the oxidative coupling reaction. Among these, pyridine and Na_2CO_3 were responded. But Na_2CO_3 was found to be the best by virtue of high ϵ_{max} values and stability considerations. Other water miscible solvents like methanol, ethanol, propan-2-ol and acetonitrile were found to provide no additional advantage. So distilled water is selected as a solvent for final dilution of the colored species. The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation (calculated from the six measurements containing 3/4th of the amount of the upper Beer's law limits), Regression characteristics like standard deviation of slope (S_b),

standard deviation of intercept (S_a), standard error of estimation (S_e) and % range of error (0.05 and 0.01 confidence limits) were calculated and are shown in Table-1.

Commercial formulations containing RLX were successfully analyzed by the proposed method. The values obtained by the proposed and reference method (reported UV method in methanol λ_{max} 289nm) for formulations were compared statistically by the t-and f-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre analyzed formulations at three different concentration levels. These results are summarized in Table-2. The ingredients usually present in formulations of RLX did not interfere with the proposed analytical method.

Chemistry of colored species

In 1940 the highly sensitive color reaction of phenols with 4-aminophenazone was introduced into analytical practice by Emerson²⁴. Emerson²⁵ postulated that the treatment of phenol, enol or amine with 4-AP and an oxidant under neutral or alkaline condition results in the formation of an oxidative coupling product (anti-pyrine dye) which is used for the determination of RLX in the present investigation, as RLX possesses Para substituted phenol group. The formation of oxidative coupling product may be represented in scheme (Fig.4).

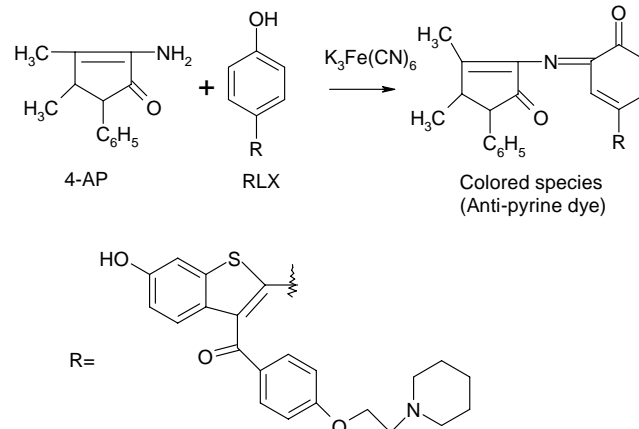


Fig.4 Scheme for the colored reaction of the drug analyte with 4-AP

Table 1: Optical Characteristics, Precision and Accuracy Of Proposed Analytical Method

Parameter	Values
λ_{max} (nm)	540.3nm
Beer's law limit($\mu\text{g/ml}$)	4 to12
Sandell's sensitivity ($\mu\text{g/cm}^2/0.001$ abs. unit)	0.022727273
Molar absorptivity (Litre/mole/cm)	22442.2
Regression equation (Y)*	
Intercept (a)	-0.124
Slope(b)	0.06
Correlation Coefficient (R^2)	0.998
%RSD	0.504
% Range of errors(95% Confidence limits)	
0.05 significance level	0.529
0.01 significance level	0.829

*Y = a+ b x, where Y is the absorbance and x is the concentration of RLX in $\mu\text{g/ml}$.

Table 2: Analysis of raloxifene hydrochloride in pharmaceutical formulations by proposed and reference methods

Method	*Formulations	Labeled Amount (mg)	Found by Proposed Methods			Found by Reference Method \pm SD	%# Recovery by Proposed Method \pm SD
			**Amount found \pm SD	t	f		
RLX-4-AP	Tablet-1	60	59.631 \pm 0.233	0.363	2.695	59.577 \pm 0.142	99.384 \pm 0.388
	Tablet-2	60	59.654 \pm 0.147	0.244	1.680	59.646 \pm 0.191	99.423 \pm 0.245

* Tablet 1 and Tablet 2 from two different companies

**Average \pm Standard deviation of six determinations, the t- and f-values refer to comparison of the proposed method with UV reference method. Theoretical values at 95% confidence limits t =2.57 and f = 5.05. # Recovery of 10mg added to the pre analyzed sample (average of three determinations)

Reference method (reported UV method) using methanol (λ_{\max} =289nm)

CONCLUSION

The reagents utilized in the proposed method are normal cost and readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed analytical method is validated as per ICH guide lines and possess reasonable precision, accuracy, simple, sensitive and can be used as a alternative method to the reported ones for the routine determination of RLX depending on the need and situation.

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