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A novel method for estimation of Raloxifene Hydrochloride in bulk and pharmaceutical preparations by Visible Spectrophotometry

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ABSTRACT

A simple and sensitive visible spectrophotometric method is described for the determination of raloxifene hydrochloride in bulk and pharmaceutical preparations based on the formation of dark green colored molecular complex with sodium nitroprusside in presence of hydroxyl amine under alkaline conditions and exhibiting λ_{max} at 720 nm. The Regression analysis of Beer's Law plot showed good correlation in a general concentration range of 8-24 μ g/ml with correlation coefficient ($r= 0.999$). 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 raloxifene in tablet form without the interference of excipients.

Key words: Beer's Law, SERM, SNP, HA, Tablets, Spectrophotometry.

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-piperidinyl) ethoxy] phenyl]-, hydrochloride.

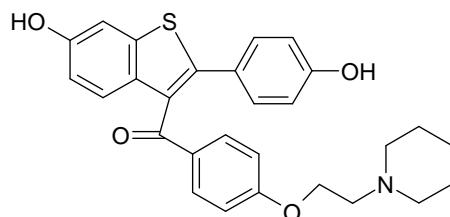


Fig.1 Showing Chemical structure of RLX

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 Index [1]. Some analytical methods which include HPLC [2-10], LC- MS-MS [11-12], Capillary electrophoresis [13], Resonance Rayleigh Scattering (RRS) [14], UV [15-16] and visible spectrophotometric [17-21] 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 have been found to be relatively complicated and tedious. The proposed method is based on the formation of molecular complex of drug with sodium nitroprusside in presence of hydroxylamine hydrochloride under alkaline conditions [22-23]. The method can be extended for the routine assay of RLX formulations.

MATERIALS AND METHODS

A Systronics UV/Visible spectrophotometer model -2203 with 10mm matched quartz cells was used for all spectral measurements. All the chemicals used were of analytical grade. SNP (Sd Fine, 0.4%, 1.34×10^{-2} M, solution prepared by dissolving 400mg of SNP in 100ml distilled water), Hydroxylamine hydrochloride (Loba, 0.4%, 5.75×10^{-2} M solution prepared by dissolving 400mg of hydroxylamine hydrochloride in 100ml of distilled water), sodium carbonate (Loba, 10%, 9.43×10^{-1} M solution prepared by dissolving 10g of sodium carbonate in 100ml of distilled water) were prepared.

Standard drug solution: Standard drug solution of RLX was prepared by dissolving 20mg of it in 4ml of 0.1M NaOH, followed by dilution to 100ml with distilled water to obtain 200 μ g/ml solution.

Sample solution: About 20 tablets were pulverized and the powder equivalent to 50mg of RLX was weighed and treated with 4x15ml portions of chloroform. The chloroform extract was diluted to 100ml with chloroform. Then 40ml of it was extracted with 3x5ml portions of 0.02M sodium hydroxide and the combined aqueous layers were taken in a 100ml volumetric flask and then diluted to 100ml with distilled water to get 200 μ g/ml.

Assay: Aliquots of standard RLX solution (1.0ml-3.0ml, 200 μ g/ml) were transferred into a series of 25ml calibrated tubes and the volume in each tube was brought to 5.0ml with distilled

eater. One ml each of ($1.324 \times 10^{-2} \text{M}$) SNP and ($5.75 \times 10^{-2} \text{M}$) hydroxylamine hydrochloride solutions were successively added to each test tube and shaken for 2 minutes.

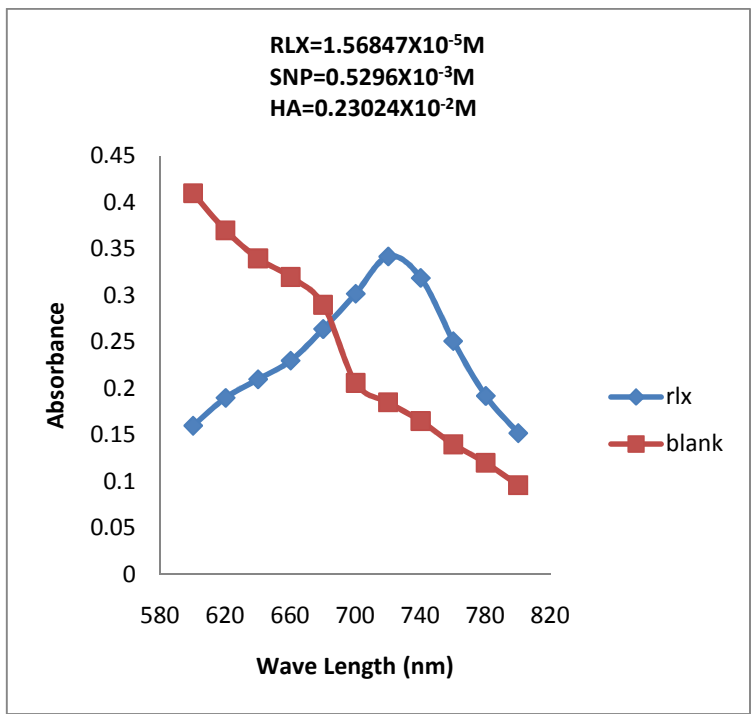


Fig.2. Showing Absorption Spectra of RLX –SNP-NH₂OH

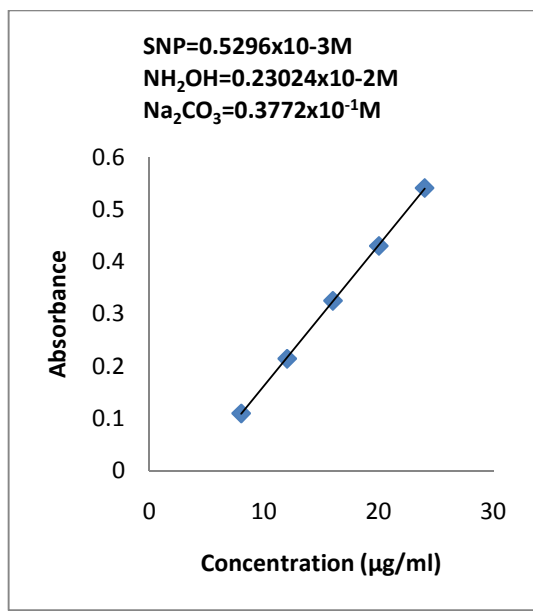


Fig.3. Showing Beer's Law Plot

Then 1.0ml of ($9.43 \times 10^{-1} \text{M}$) Na_2CO_3 solution was added and further shaken for 15 minutes. The contents were diluted to the mark with distilled water and the absorbances were measured at 720nm against a reagent blank within the stability period (immediate-120 min). (Fig-2 showing absorption spectra). The amount of RLX in the sample solution was computed from its calibration graph (Fig.3 showing Beer's Law plot).

RESULTS AND DISCUSSION

In developing this method, a systematic study 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, volume and strength of SNP, NH_2OH , Na_2CO_3 reagents, order of addition of reagents on color development and solvent for final dilution of the colored species were studied and the optimum conditions were established. Other water miscible solvents like methanol, ethanol, propan-2-ol and acetonitrile were found to provide no additional advantage. The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation (calculated from the six measurements containing $3/4^{\text{th}}$ 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 preanalyzed 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 the present investigation RLX functions as a donor due to the presence of cyclic tertiary nitrogen in piperidine portion. Sodium nitroprusside in the presence of hydroxylamine and alkali exists as aquoferrocyanide $[\text{Fe}(\text{CN})_5\text{H}_2\text{O}]^{3-}$. In a general way it may be expected that the electron transfer depends upon the extent of delocalization of the donor and acceptor metal orbitals of the intervening ligands. From this stand point, ligands such as water and ammonia, which contain single bonds, are expected to be much less effective in conducting electrons between metal ions than unsaturated ligands such as CN^- whose complexes are characterized by high degree of covalency and electron delocalization. Based on the analogy, the probable sequence of reactions is presented in scheme (Fig-4).

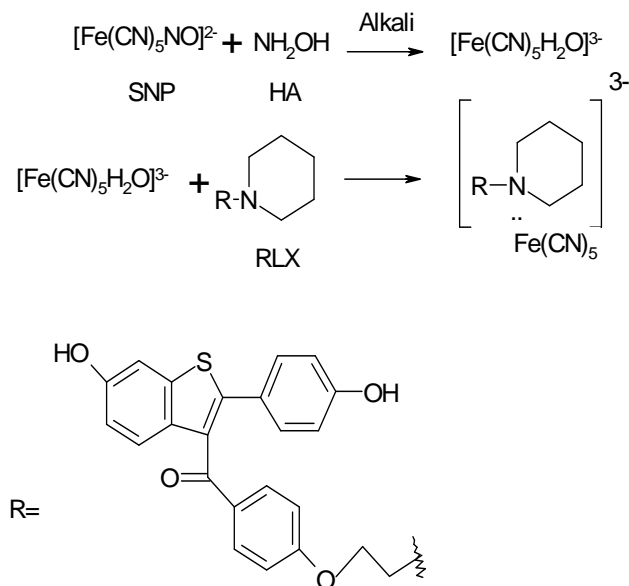


Fig.4 Showing the Scheme

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

Parameter	Values
λ_{max} (nm)	720nm
Beer's law limit($\mu\text{g/ml}$)	8-24
Sandell's sensitivity ($\mu\text{g/cm}^2/0.001$ abs. unit)	0.046404
Molar absorptivity (Litre/mole/cm)	10991.58
Regression equation (Y)*	
Intercept (a)	-0.107
Slope(b)	0.027
Correlation Coefficient (R^2)	0.999
%RSD	0.8648
% Range of errors(95% Confidence limits)	
0.05 significance level	0.9077
0.01 significance level	1.4235

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

Table-2 Analysis of Raloxifene Hydrochloride 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		
SNP-HA	Tablet-1	60	59.58 \pm 0.114	0.186	1.538	59.58 \pm 0.142	99.31 \pm 0.190
	Tablet-2	60	59.65 \pm 0.219	0.287	1.316	59.65 \pm 0.191	99.43 \pm 0.364

* 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 ($\lambda_{\text{max}} = 289\text{nm}$).

CONCLUSION

The reagents utilized in the proposed method are cheap 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 alternative method to the reported ones for the routine determination of RLX depending on the need and situation.

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