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A quantitative assay for Raloxifene hydrochloride in bulk and pharmaceutical preparations by visible spectrophotometry

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ABSTRACT

A simple, sensitive and cost effective visible spectrophotometric method has been developed for the determination of raloxifene hydrochloride from bulk and tablet dosage forms. The method is based on the formation of green colored coordination complex by the drug with cobalt thiocyanate which is quantitatively extractable into nitro benzene with an absorption maximum of 624.4 nm.The Regression analysis of Beer's Law plot showed good correlation in a general concentration range of 16-48µg/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, Cobalt thiocyanate, Extractive Spectrophotometry, Nitrobenzene, Raloxifene.

INTRODUCTION

Raloxifene hydrochloride (RLX) is a selective estrogen receptor modulator that belongs to the benzothiopene class of compounds. The chemical designation is methanone, [6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thien-3-yl]-[4-[2-(l-piperidinyl) ethoxy] phenyl]-, hydrochloride (Fig.1).

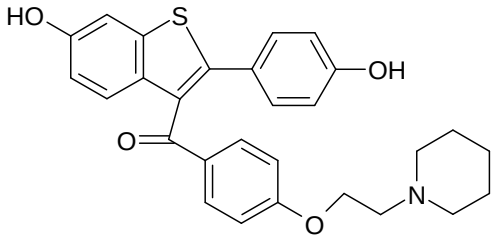


Fig.1 Showing Chemical structure of RLX

RLX is used in the treatment and prevention of osteoporosis in post-menopausal women and also effective in the treatment of breast cancer. 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, validated and inexpensive 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 expensive. So the authors have made some attempts in this direction and succeeded in developing a method based on the reaction between the drug and cobalt thiocyanate [22]. The method can be extended for the routine assay of RLX formulations.

EXPERIMENTAL SECTION

A Systronics UV/Visible spectrophotometer model -2203 with10mm matched quartz cells was used for all spectral measurements. A Systronics µ- pH meter model-362 was used for pH measurements. All the chemicals used were of analytical grade.CTC ($2.50x10^{-1}$M, solution prepared by dissolving 7.25 g of cobalt nitrate and 3.8 g of ammonium thiocyanate in 100ml distilled water), Citrate buffer pH(2.0) (prepared by mixing 306ml of 0.1M trisodium citrate with 694ml of 0.1M HCl and pH was adjusted to 2.0) were prepared.

Standard and sample drug solution: An accurately weighted quantity of RLX (pure or tablet powder) equivalent to 100mg was dissolved initially in 10ml of methanol and then followed by dilution to 100 ml with distilled water to get 1mg/ml stock solution. From this, 40ml stock solution was mixed with 5ml of 1M Na_2CO_3 solution and transferred into 125ml separating funnel. The freebase released was extracted with 3x15ml portions of chloroform. The total chloroform extract was evaporated to dryness and made up to 100ml with the same solvent to obtain 400µg/ml working standard solution.

Assay: Aliquots of standard RLX solution (1.0ml-3.0ml, 400µg/ml) were delivered into a series of 125ml separating funnels. Then 2.0ml of buffer solution (pH2.0) and 5.0 ml CTC solution were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0 ml with distilled water. To each separating funnel 10.0 ml of nitrobenzene was added and contents were shaken for 2 minutes. The two phases were allowed to separate and absorbance of nitrobenzene layer was measured at 624.4nm against a similar reagent blank (Fig-2 showing absorption spectra).The colored product was stable for 1 hour. The amount of RLX in the sample solution was computed from its calibration graph (Fig.3 showing Beer's Law plot).

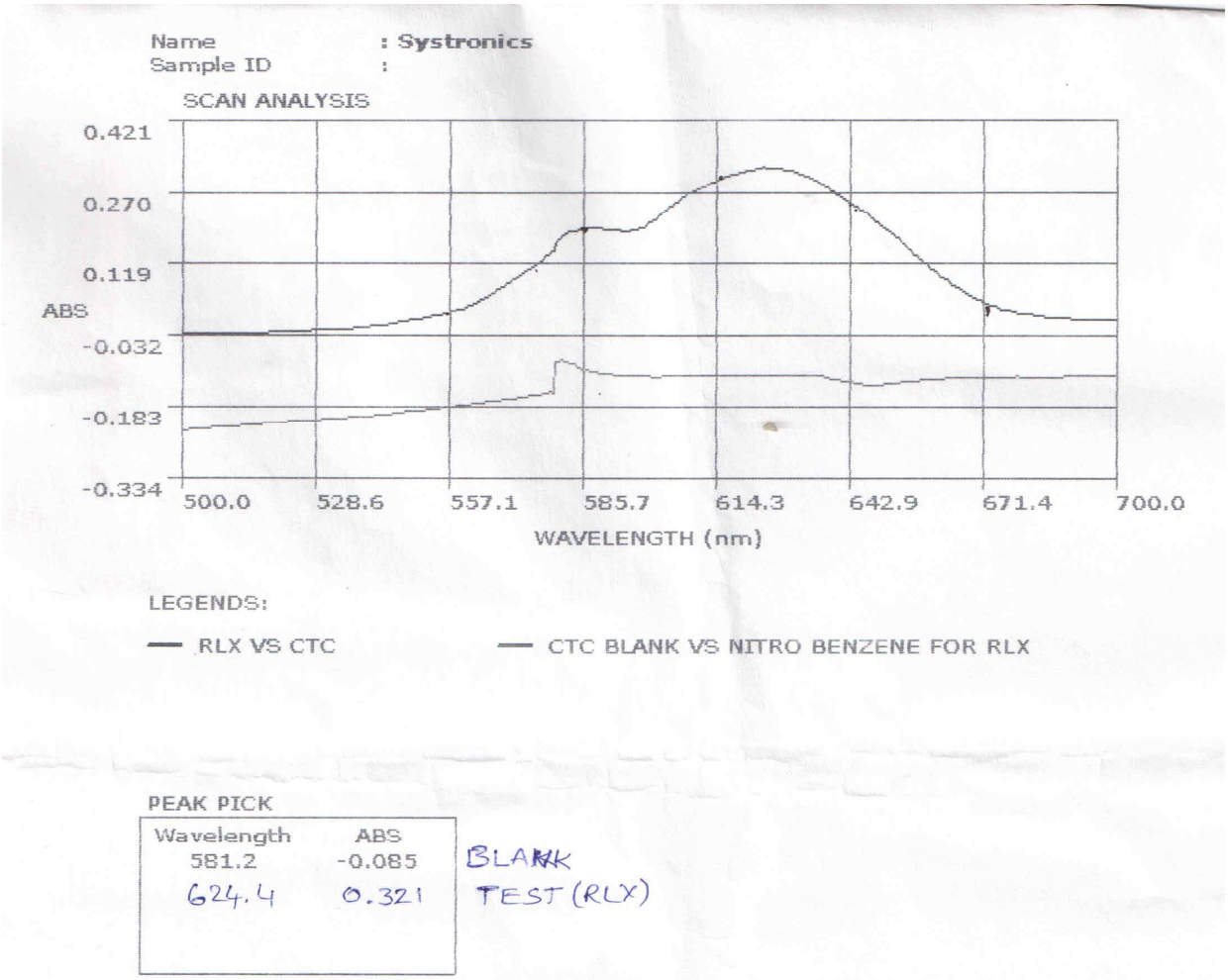


Fig.2.Showing Absorption Spectra of RLX vs. CTC

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, volume and strength of CTC reagent and pH buffer solution and solvent for final dilution of the colored species were studied and the optimum conditions were established. Among the various water immiscible organic solvents(C_6H_6, $CHCl_3$, di chloro methane, nitro benzene, chlorobenzene and CCl_4)tested for the extraction of colored

coordinate complex into organic layer, nitrobenzene was preferred for selective extraction of colored complex from organic phase.

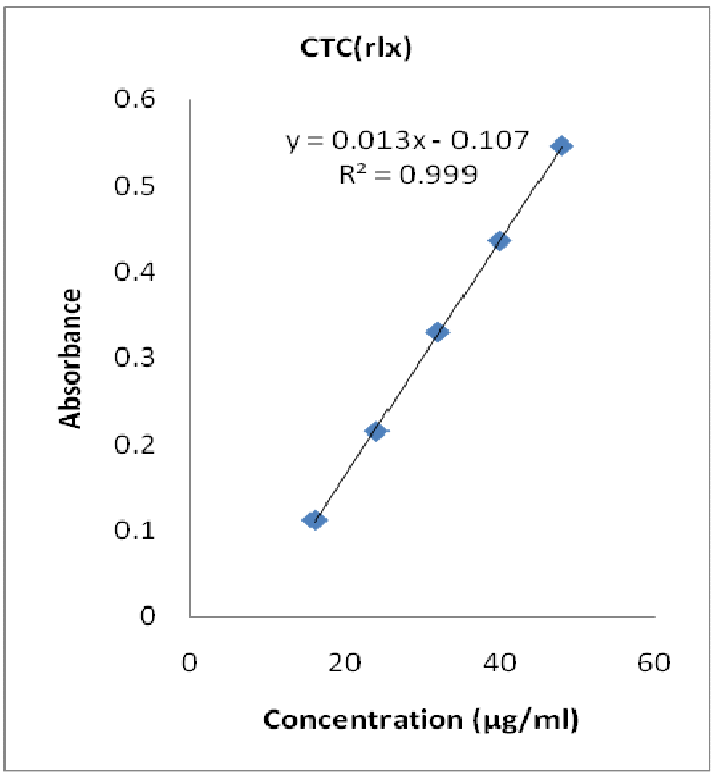
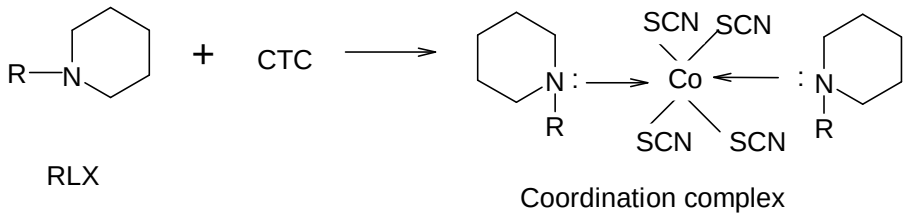


Fig.3. Showing Beer's Law Plot

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 (Sb), standard deviation of intercept (Sa), standard error of estimation (Se) 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 (50%, 75% and 100%). 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: The color species formed is the coordination complex of the drug (electron donor) and the central metal of cobalt thiocyanate, which is extractable into nitro benzene from aqueous solution. Formation of the green colored complex when RLX was treated with CTC is due to the presence of the cyclic tertiary amino group in it. It is based on the analogy of tertiary amine as given in the scheme (Fig-
4).



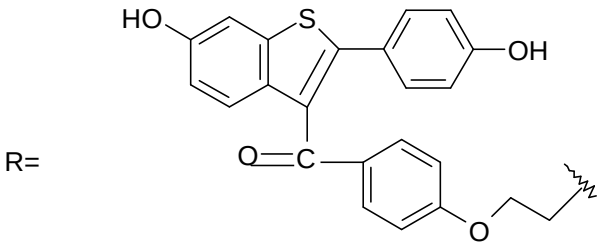


Fig.4 Showing the Scheme

Table 1: Optical characteristics, precision and accuracy of proposed analytical method

Parameter	**Values**
λ_{max} (nm)	624.4nm
Beer's law limit(µg/ml)	16 - 48
Sandell's sensitivity (µg/cm^2/0.001 abs. unit	0.09174319
Molar absorptivity (Litre/mole/cm)	5559.545
Regression equation (Y)*	
Intercept (a)	0.013
Slope(b)	-0.107
Correlation Coefficient (R^2)	0.999
%RSD	0.851
% Range of errors(95% Confidence limits)	
0.05 significance level	0.893
0.01 significance level	1.400

**Y = a+bx, where Y is the absorbance and x is the concentration of raloxifene in µg/ml*

Table-2 Analysis of raloxifene hydrochloride by proposed and reference methods

method	*formulations	labeled amount (mg)	found by proposed methods **amount found ± sd	t	f	found by reference method ± sd	#% recovery by proposed method ± sd
ctc	tablet-1	60	59.59 ± 0.11	0.326	1.645	59.58± 0.142	99.317± 0.184
	tablet-2	60	59.664 ± 0.214	0.407	1.264	59.65± 0.191	99.439± 0.357

** Tablet 1 and Tablet 2 from two different companies (Fiona from Dr. Reddy's and Ralista from Cipla)*
***Average ± 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 cheap, readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed extractive colorimetric 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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