New Spectrophotometric Determination of Raloxifene Hydrochloride in Tablets

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Three new simple and sensitive spectrophotometric methods in UV/Vis region have been developed for the determination of raloxifene hydrochloride in bulk drug and in tablet formulations. Raloxifene hydrochloride exhibited maximum absorbance at 289 nm in methanol (method A) with apparent molar absorptivity of 3.67×10^4 l/mol.cm and maximum absorbance at 303 nm in 0.1 M sodium hydroxide with apparent molar absorptivity of 3.60×10^4 l/mol.cm (method B). Third method is based on the formation of red coloured chromogen with ferric nitrate and 1,10-phenanthroline, which showed maximum absorbance at 511 nm with apparent molar absorptivity of 1.06×10^5 l/mol.cm. Beer's law was obeyed in the concentration range of 5-25 μg/ml for method A and B and in the range of 2-10 μg/ml for method C. Results of all methods were validated statistically and by recovery studies.

Raloxifene hydrochloride (RLH), [6-hydroxy-2-(4-hydroxy phenyl) benzo[b]thien-3-yl]-[4-[2-(1-piperinyl) ethoxy]-phenyl] methanone, is an antiosteoporotic[1]. It is a nonsteroidal benzothiophene that is the first selective estrogen receptor modulator to be approved for the prevention and treatment of osteoporosis in postmenopausal women. A survey of literature revealed a capillary electrophoresis method[2] and a few chromatographic methods for its determination in bulk drug[3] and in plasma[4]. No spectrophotometric method has been reported so far. Hence an attempt was made to develop simple and economical spectrophotometric methods with greater precision, accuracy, and sensitivity for the analysis of RLH in tablets.

This paper describes three simple spectrophotometric methods for RLH using methanol (method A) and using 0.1M sodium hydroxide (method B) and using ferric nitrate and 1,10-phenanthroline (method C). In the first method, RLH exhibits maximum absorbance at 289 nm, and in the second method, it exhibits maximum absorbance at 303 nm. In the third method, RLH reduces ferric nitrate to ferrous, which forms complex with 1,10-phenanthroline[5] to yield a coloured chromogen having maximum absorbance at 511 nm.

All chemicals used were of analytical grade. Aqueous solutions of sodium hydroxide (0.1M), ferric nitrate (0.033M), and 1,10-phenanthroline (0.1M Qualigens, Mumbai) were prepared in double-distilled water. Spectral and absorbance measurements were made on Shimadzu 1601 UV/Vis Spectrophotometer with 1 cm matched quartz cells. Standard stock solution of RLH (Dr. Reddy's Lab., Hyderabad) was prepared by dissolving 50 mg of RLH in 50 ml of methanol for all the three methods. Suitable working standard solutions were prepared with methanol from the standard stock solution for all the methods. Aliquots of stock solutions ranging from 1 to 5 ml (50 μg/ml) were then diluted with methanol to 10 ml in order to get final concentrations of 5, 10, 15, 20, and 25 μg/ml for method A, and with 0.1 M sodium hydroxide for method B. The absorbances were then measured at 289 nm and 303 nm for methods A and B, respectively. To aliquots of stock solutions ranging from 1 to 5 ml (20 μg/ml), 1.5 ml of ferric nitrate and 1.5 ml of 1,10-phenanthroline were

TABLE 1: OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY DATA

Parameters	Method A	Method B	Method C
λ_{max} (nm)	289	303	511
Beer's law limit (μg/ml)	5-25	5-25	2-10
Molar absorptivity (l/mol.cm)	3.67×10^4	3.6×10^4	1.6×10^5
Correlation coefficient (r^2)	0.9993	0.9997	0.9987
Sandell's sensitivity (μg/cm^2 absorbance unit/0.01)	0.140×10^{-1}	0.142×10^{-1}	4.81×10^{-1}
Regression equation (Y=bx+a)*			
Slope (b)	0.0772	0.0689	0.1401
Intercept (a)	0.036	0.0153	0.3798
Relative standard deviation (n=6), %	0.05	0.49	0.53

*With respect to Y=bx + a, where 'x' is the concentration of RLH in μg/ml

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TABLE 2: ANALYSIS OF RALOXIFENE HYDROCHLORIDE TABLETS

Formulation	Label claim (mg/tab)	Amount found (mg/tab)	% of label claim ± S.D	Standard error	% Recovery*
Method A					
Brand 1	60	59.58	99.3±0.05	0.020	99.6
Brand 2	60	59.62	99.4±0.03	0.012	98.6
Method B					
Brand 1	60	59.36	98.9±0.15	0.06	100.2
Brand 2	60	59.66	99.4±0.29	0.12	99.45
Method C					
Brand 1	60	60.03	100.1±0.26	0.104	99.1
Brand 2	60	60.42	100.7±0.32	0.129	98.4

Brand 1 – Bonebay, Novartis India Ltd., Mumbai. Brand 2 – Fiona, Dr. Reddy's Laboratories Ltd., Hyderabad. *Results of five replicates

added, heated on a boiling water bath for 15 min, cooled for 5 min, and the volume was made up to 10 ml with distilled water to get final concentrations of 2, 4, 6, 8, and 10 μg/ml. The absorbances of developed red coloured chromogen were measured at 511 nm. Calibration graphs were plotted for all the methods.

For the analysis of RLH in formulations, two different preparations of 60 mg each (Fiona, Dr. Reddy's; and Bonebay, Novartis India Ltd.) were taken. Ten tablets of each were weighed and powdered separately. The tablet powder equivalent to 50 mg of RLH was weighed and extracted with 50 ml of methanol. The solution was then filtered and appropriate aliquots of RLH within the Beer's law limit were taken and analysed by all the three methods using the procedure described earlier.

The applicability of the methods was also checked by analysing synthetic mixtures[6] of the drug containing the following amounts of excipients in mg. RLH (10), talc (80), starch (80), sucrose (40), lactose (40), gelatin (60), and magnesium stearate (90). A suitable amount of synthetic mixture was analysed using methods A, B, and C. Percent recovery of RLH using methods A, B, and C was found to be 99.9, 101.6, and 100.5, respectively, with RSD values less than 1.0 for six replicates.

The optical characteristics like molar absorptivity, Sandell's sensitivity, and linear regression equation of the above said methods are shown in Table 1. RLH exhibited maximum absorbance at 289 nm in methanol, 303 nm in 0.1 M sodium hydroxide, and 511 nm by forming a complex with ferric nitrate and 1,10-phenanthroline. The linear correlation was found between absorbances and concentration of RLH in the range of 5-25 μg/ml for methods A and B, and in the range of 2-10 μg/ml for method C. The results of analysis and recovery studies are presented in Table 2. The percentage recovery values close to 100% indicated that there is no interference of the excipients present in the formulation.

The extent of interference by commonly associated excipients such as magnesium stearate, starch, talc, gelatin, dextrose, lactose, and sucrose was determined by measuring absorbance of the solutions containing 2, 4, and 6 μg/ml of RLH. An error of ±2% in the absorbance readings was considered tolerable. The proposed methods were found to be free from interference by the excipients in the level found in dosage forms. This was quite clear from the data obtained on the analysis and synthetic mixtures. As no spectrophotometric method for analysis of RLH in pharmaceutical preparations is currently available, the proposed methods could not be compared for its validation. However, the data of analysis was supported by RSD values. Hence the developed methods were found to be sensitive, accurate, precise, repeatable, and reproducible and can be used for the routine analysis of RLH in bulk drug and in formulations.

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