

# RP-HPLC Estimation of Raloxifene Hydrochloride in Tablets

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**A simple, efficient, and reproducible method for the determination of raloxifene hydrochloride in tablets has been developed using reverse phase high performance liquid chromatographic method. The elution was done using a mobile phase consisting of methanol and water (50:50 v/v) on Water's Symmetry C<sub>18</sub> 4.6×150 mm analytical column with flow rate of 1 ml/min with detection at 230 nm. An external standard calibration method was employed for quantitation. The elution time was 4.1 min. The linearity range was 10-60 µg/ml for raloxifene hydrochloride.**

Raloxifene hydrochloride (RLH), [6-hydroxy-2-(4-hydroxy phenyl) benzo[b]thien-3-yl]-[4-[2-(1-piperinyl) ethoxy]-phenyl] methanone, is an antiosteoporotic. It is a nonsteroidal benzothiophene, which is the first selective oestrogen receptor modulator (SERM) to be approved for

the prevention and treatment of osteoporosis in postmenopausal women. It is listed in Merck Index<sup>1</sup>. A survey of literature revealed a spectrophotometric<sup>2</sup>, capillary electrophoresis<sup>3</sup> and a few chromatographic methods for its determination in bulk drug<sup>4</sup> and in plasma<sup>5</sup>. No method has been so far reported for the estimation of RLH from pharmaceutical dosage forms. The present paper aims at reporting an isocratic RP-HPLC

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**TABLE 1: DETERMINATION OF RALOXIFENE HYDROCHLORIDE IN TABLETS**

| Drug RLH | Label claim/<br>tablet (mg) | Amount found*<br>(mg) | Recovery studies        |                             |                            |
|----------|-----------------------------|-----------------------|-------------------------|-----------------------------|----------------------------|
|          |                             |                       | Amount added<br>(mg/ml) | Amount recovered<br>(mg/ml) | Percentage recovery<br>(%) |
| Tablet A | 60                          | 59.57±0.22            | 10                      | 9.96                        | 99.6                       |
|          |                             |                       | 20                      | 19.85                       | 99.2                       |
| Tablet B | 60                          | 59.68±0.34            | 50                      | 50.12                       | 100.8                      |

\*Mean of three determinations; Tablet A is Fiona (Dr. Reddy's Laboratories) and Tablet B is Bonebay (Novartis, Mumbai)

method for the determination of RLH in tablets.

The apparatus used was Water's HPLC SPD chromatograph equipped with dual-wavelength detector and model 7725i Rheodyne injector with 20 µl external loop. The column used was Water's Symmetry C<sub>18</sub>, 4.6×150 mm analytical column; the elution was carried out isocratically at the flow rate of 1 ml/min using methanol and water in the ratio 50:50 v/v as mobile phase. The detector was set at a wavelength of 230 nm. Responses of peak areas were recorded and integrated using software. RLH was obtained from Dr. Reddy's Laboratories, Hyderabad. Methanol and water used were of HPLC grade obtained from S. D. Fine Chemicals Ltd., Mumbai.

Standard stock solutions of the drug were prepared by dissolving 25 mg of RLH in mobile phase and made up to 25 ml with the same (1000 µg/ml). Working standard solution was prepared by diluting 1 ml of the stock solution to 10 ml with mobile phase (100 µg/ml). The gradient dilutions were prepared by taking 1, 2, 3, 4, 5, and 6 ml of solutions and made up to 10 ml with the mobile phase. Twenty microlitres of the solution from each flask was injected two times. Calibration curve was constructed by plotting mean peak areas against the corresponding drug concentrations. The detector response was found to be linear in the concentration range of 10-60 µg/ml.

For the estimation of drugs from commercial formulations, 20 tablets of two brands – Fiona (Dr. Reddy's Laboratories, Hyderabad) and Bonebay (Novartis India Ltd., Mumbai) – each containing 60 mg of raloxifene hydrochloride were powdered finely. A quantity equivalent to 25 mg was transferred into a 25 ml volumetric flask and dissolved in 10 ml of methanol. The volume was then diluted with the mobile phase and filtered through Whatman No. 1 filter paper. One millilitre of the resulting solution was then diluted to 10 ml with mobile phase. From this, 1, 2, and 3 ml samples were taken and their volume was made up to 10 ml each. A

chromatogram of these solutions was obtained by injecting 20 µl of each sample into the chromatographic system. There was no interference from diluents and lubricants. The retention time of the drug was 4.1 min. Chromatographic parameters such as peak asymmetry (A<sub>s</sub>) and capacity factor (k) were found to be 1.12 and 0.927, respectively. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.23 and 4.04 µg/ml, respectively. Analytical recovery studies were carried out from a series of spiked concentrations added to the preanalysed dosage form (Table 1).

Analysing five replicates of fixed amount of RLH enabled checking the precision and accuracy of the proposed method. The precision of the method was calculated in terms of the relative standard deviation. Low values of relative standard deviation (0.56%) and percentage errors at 95% confidence limits (0.76) indicated high precision and accuracy of the proposed method. Hence the present method can be used for the routine analysis of RLH in formulation.

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