# Validated Analytical Methods of Repaglinide in Bulk and Tablet Formulations

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Four validated spectrophotometric methods for the estimation of repaglinide in bulk drug and its tablet formulations have been developed. Method I is extractive spectrophotometric method based on the formation of a yellow coloured ion-pair of repaglinide with bromothymol blue. Method II is a simple UV spectrophotometric method, and methods III and IV are based on derivative and difference spectroscopy respectively. Quantitative recoveries were obtained from bulk and from marketed tablet formulations. Various validation parameters were also studied.

Repaglinide is a hypoglycaemic agent used for the treatment of non-insulin-dependent diabetes mellitus[1]. Chemically, repaglinide (RPL) is (+)-2-ethoxy-α-{[(S)-isobutyl-o-piperidinobenzyl]carbamoyl}-p-toluic acid[2]. Repaglinide is not official in any pharmacopoeia. Literature survey revealed that there is only one HPLC[3] method for its determination. The present work describes four simple and inexpensive spectrophotometric methods for the estimation of RPL in bulk and tablet formulations. A Hitachi U-2000 UV/Vis recording spectrophotometer with 1 cm matched quartz tubes was used for spectral measurements. All the reagents used were of ANALAR grade. Aqueous solutions of 0.1% bromothymol blue (BTB), acid phthalate buffer of pH 2.4, 0.1N hydrochloric acid and 0.1N sodium hydroxide solutions were prepared as per IP procedure. Standard drug solution was prepared (100 μg/ml) in methanol.

Two brands of tablets (Eurepa of Torrent Pharmaceuticals, Mehsana, and Rapillin of Sun Pharmaceuticals, Ankleshwar) were analyzed by the proposed methods. Tablet powder equivalent to 10 mg of repaglinide was suspended in 15 ml of methanol and stirred using a magnetic stirrer for 15 min. The solution was filtered and diluted while rinsing the residue to 25 ml with methanol. Suitable aliquots from this stock solution were diluted further to get working samples.

Aliquots of 0.1 to 3 ml from standard drug solution were transferred to a 60 ml separatory funnel in method I. To this, 4 ml of acid phthalate buffer of pH 2.4 and 3 ml of 0.1% BTB solution were added. The yellow coloured ion pair was extracted with 5 ml of chloroform and diluted to

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10 ml with chloroform. Absorbance of these solutions were measured against a reagent blank at 438 nm. The calibration curve was linear in the concentration range of 5-25 μg/ml.

For method II, aliquots of 0.1 to 3 ml from standard drug solution were diluted to 10 ml with methanol. The absorbance of these solutions was measured at 281.2 nm. The calibration curve was linear in the concentration range of 1-200 μg/ml. First derivative curve of these methanolic solutions (method III) were obtained, which showed maxima and minima at 293 and 273 nm respectively. The amplitude was linear to the concentration in the range of 2-35 μg/ml.

A series of standard solutions were prepared in 0.1N NaOH and 0.1N HCl for method IV. The difference spectra were obtained after measuring the absorbance of acidic solutions against basic solutions of the same concentration. The amplitude was measured between 302 and 269 nm maxima and minima respectively of the difference spectra. The calibration curve was linear in the concentration range of 5-50 μg/ml.

Repaglinide has a secondary amino group in its molecular structure, which makes it possible to form the ion pair with various acidic dyes. Bromothymol blue has been selected to form the ion pair, which was found to be stable for more than 6 h. The Job's plot showed the drug-to-dye ratio in ion pair as 1:2.

The methanolic solution of repaglinide showed a well-defined peak at 281.2 nm and so a simple UV spectrophotometric method could be developed. The derivative and difference methods provide a specific tool to analyze drug with improved sensitivity and selectivity and so first derivative plot and difference spectrum were also obtained. The optical parameters and other constants for the methods are listed in Table 1.

The four methods were applied to analyze RPL in its marketed formulations. The recovery studies were also carried out after spiking the sample solutions at two levels. These results are summarised in Table 2.

Repaglinide is easily hydrolyzed by acids and bases. The drug is also affected by strong oxidizing agents. The applicability of the four methods to analyze repaglinide in presence of its degradation products was also studied. These degradation products were prepared as per ICH guidelines. None of the methods could be used to analyze repaglinide in presence of its acid or base hydrolyzed products.

The proposed methods I, II, III and IV were validated by studying several parameters such as accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ) and robustness. The accuracy of the methods was greater than 98%, and RSD did not exceed 2%. Method I was found to be dependent upon pH, whereas the nature and concentration of acid affects the

**TABLE 2: ANALYSIS OF MARKETED FORMULATIONS**

| Method | Amount taken (mg) | | Amount found (mg) | | | |
|---|---|---|---|---|---|---|
| | T 1 | T 2 | T 1 | % recovery | T 2 | % recovery |
| I | 2 | 2 | 1.974 | 99.85 | 1.98 | 99.97 |
| II | 2 | 2 | 2.015 | 100.23 | 1.99 | 100.02 |
| III | 2 | 2 | 1.96 | 100.24 | 1.97 | 100.32 |
| IV | 2 | 2 | 1.99 | 100.21 | 1.97 | 100.23 |

% recovery was determined by spiking the sample with standard solution at two levels. T1 and T 2 were the Eurepa (Torrent Pharmaceuticals) and Rapillin (Sun Pharma) tablets.

**TABLE 1: OPTICAL AND VALIDATION PARAMETERS**

| Data | Results | | | |
|---|---|---|---|---|
| | Method I | Method II | Method III | Method IV |
| λmax(nm) | 438 | 281.2 | 293 | 302 |
| λmin(nm) | - | - | 273 | 269 |
| Beer's law range (μg/ml) | 5 - 25 | 1 - 200 | 2 - 35 | 5 - 50 |
| Molar extinction coefficient L.mol $^{-1}$.cm $^{-1}$ | 9.63 *$10^3$ | 2.85 *$10^3$ | 5.65 *$10^3$ | 4.65 *$10^3$ |
| Sandell's sensitivity(μg/cm$^2$/.001 absorbance units) | .047 | .158 | .08 | .097 |
| Regression equation | | | | |
| slope | 0.0113 | 0.0063 | 0.0124 | 0.010 |
| Intercept | 0.1057 | 0.0000 | 0.0008 | 0.0008 |
| Correlation coefficient | 0.9969 | 0.9995 | 0.9996 | 0.9997 |
| Precision | 1.183 | 0.788 | 0.4644 | 1.3915 |
| LOD (μg/ml) | 0.97 | 0.26 | 0.42 | 1.16 |
| LOQ (μg/ml) | 3.27 | 0.87 | 1.39 | 3.86 |

Precision was evaluated as Inter day and Intra day . RSD levels for both were less than 1%, All values are the mean values of three readings.

difference spectrum. All the four methods are simple and precise and can be used for the estimation of repaglinide in bulk and in tablet formulation.

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