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# Simultaneous Spectrophotometric Estimation of Metformin and Repaglinide in a synthetic mixture

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Patel, *et al.*: Simultaneous Estimation of Metformin and Repaglinide in Synthetic Mixture

Two simple, rapid, accurate and economical methods have been developed for the simultaneous estimation of metformin and repaglinide in the synthetic mixture. The linearity was observed in the concentration range of 4-24 µg/ml for the both metformin and repaglinide. First method is based on the simultaneous equations, absorbances of both the drugs were determined at 240 nm (λ max of metformin) and at 291.5 nm (λ max of repaglinide). Metformin does not show any absorbance at 291.5 nm, hence its absorptivity was taken zero in the calculation. The method was validated in terms of accuracy (99.24±0.99, 100.98±0.89) and precision (intra-day variations 0.58-1.21, 2.12-3.12 and inter-day variations 0.62-1.42, 2.20-3.08). Second method is based on Q-absorbance ratio; absorbances of both the drugs were determined at 240 nm (λ max of metformin) and at isoabsorptive point (254.8 nm). Q-absorption ratio method was validated in terms of accuracy (98.57±1.05, 98.62±1.2402) and precision (intra-day variations

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0.58-1.21, 1.01-3.53 and inter-day variations 0.62-1.42, 1.15-3.74). The proposed methods were found accurate, reproducible and economical for the routine analysis of both the drugs in the synthetic mixture.

Key words: Metformin, repaglinide, spectrophotometric analysis

Metformin Hydrochloride (MET) is a biguanide class of antidiabetic drug, chemically is N,N-dimethylimidodicarbonimidic diamide hydrochloride[1,3-12]. Repaglinide (REPA) is a meglitinide antidiabetic used for the treatment of type 2 diabetes mellitus, chemically is (+)-2-ethoxy-α-{[(*S*)-α-isobutyl-*o*-piperidinobenzyl]carbamoyl}-*p*-toluicacid[2,13-15].

Shimadzu model 1601 double beam UV/Vis spectrophotometer with a pair of 10 mm matched quartz cells was used to measure absorbance of the resulting solutions. Sartorius CP224S analytical balance, an ultrasonicater (Frontline FS 4). MET and REPA were obtained from Restech Pharmaceutical, Ahmedabad and absolute alcohol from S. D. Fine Chemicals, Mumbai.

Standard MET and REPA stock solution of 100µg/ml concentration was prepared in absolute alcohol. The synthetic mixture of MET and REPA was prepared in the ratio of 1:1. MET and REPA powder (5 mg each) was accurately weighed and transferred to 50 ml volumetric flask. The content was mixed with 40 ml alcohol. Common excipients, which are used in the tablet formulation, were added in this mixture and sonicated for 20 min. This solution was filtered through the Whatman filter paper No. 41 and the residue was washed thoroughly with alcohol. The filtrate and washings were combined and diluted to the 50 ml with alcohol to get solution having MET (100 µg/ml) and REPA (100 µg/ml).

The standard stock solutions of MET and REPA were scanned in the range of 200 nm to 400 nm against absolute alcohol as a blank. Maximum absorbance was obtained at 240 nm and 291.5 nm for MET and REPA, respectively. Iso-absorptive point was found at 254.8 nm. A calibration curve was plotted over a concentration range 4-24 µg/ml for both MET and REPA. Absorbance of each solution was measured at both the wavelength 240 nm and 291.5 nm. Calibration curves were constructed for MET and REPA by plotting absorbance versus concentrations at both wavelengths. Each reading was average of three determinations. Absorbance of each solution was measured at the three wavelengths 240 nm, 291.5 nm and 254.8 nm. Calibration curves were constructed for MET and REPA by plotting absorbance versus concentrations at three wavelengths. Each reading was average of three determinations.

Accuracy was determined in term of percent recovery. The proposed method was applied to determine MET and REPA in the synthetic mixture. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the synthetic mixture with three different concentrations of standards. Precision was determined in term of intra-day and inter-day precision. The absorbance of final sample solution was measured against absolute alcohol as a blank at 240, 291.5 and 254.8 nm. The amount of MET and REPA were calculated using simultaneous equations as well as Q-absorbance ratio method.

Calibration curves for MET and REPA over concentration range of 4-24 µg/ml were plotted and molar absorptivity for both the compounds were calculated at three wavelengths 240 nm (λ max of MET), 254.8 nm (Isoabsorptive point) and 291.5 nm (λ max of REPA). MET did not show any absorbance at 291.5 nm, hence absorptivity of MET was taken zero in the calculation. The linearity of the calibration graphs was validated by the high value of correlation coefficients of the regression (Table 1). The criteria for obtaining maximum precision[1], by simultaneous equations method, were calculated and found to be out side the range 0.1-2.0.

LOD for MET and REPA were found to be 0.38 µg/ml and 0.69 µg/ml, respectively while LOQ for MET and REPA were found to be 1.15 µg/ml and 2.08 µg/ml respectively by both the methods. These data show that both the methods are sensitive for the determination of MET and REPA.

The percent recoveries obtained were 99.24 to 101.23 and 100.98 to 101.08 for MET and REPA, respectively by simultaneous equation method; 98.05 to 99.05 and 98.62 to 99.12 for MET and REPA, respectively by Q-absorbance ratio method. The low value of SD

**TABLE 1: SUMMARY OF VALIDATION PARAMETERS FOR SIMULTANEOUS EQUATION AND Q-ABSORPTION RATIO METHODS**

| Parameters | 240 nm | | 291.5 nm | 254.8 nm |
|---|---|---|---|---|
| | REPA | MET | REPA | MET AND REPA |
| Beer's law limit (µg/ml) | 4-24 | 4-24 | 4-24 | 4-24 |
| Molar Absorptivity (lit/mole/cm) | 10249 | 9904 | 3220 | 1634 (MET) 5729 (REPA) |
| Sandell's sensitivity (µg/ml/cm$^2$/0.001) | 0.0126 | 0.0459 | 0.1408 | 0.0791 |
| LOD (µg/ml) | 0.378 | 0.686 | 1.206 | 0.869 |
| LOQ (µg/ml) | 1.146 | 2.081 | 3.98 | 2.695 |
| Regression equation (y= a+bc) | y= 0.0806x + 0.0085 | y= 0.0232x -0.0205 | y= 0.0066x + 0.0028 | y= 0.0132x -0.0076 |
| Correlation coefficient ($r^2$) | 0.9993 | 0.9980 | 0.9988 | 0.9986 |
| Precision | | | | |
| Intra-day (n=5) (%CV) | 0.58-1.21 | 1.00-3.01 | 2.12-3.12 | 1.01-3.53 |
| Inter-day (n=5) (%CV) | 0.62-1.42 | 1.12-3.23 | 2.20-3.08 | 1.15-3.74 |

indicates that both the methods are accurate. The low % CV values of intra-day and inter-day variations reveal that the proposed methods are robust (Table 1).

In the simultaneous equation method concentration of MET and REPA in the synthetic mixture were found out by solving following equations; $C_m = (A_2a_{r1} - A_1a_{r2})/(a_{m2}a_{r1} - a_{m1}a_{r2})$ and $C_r = (A_1a_{m2} - A_2a_{m1})/(a_{m2}a_{r1} - a_{m1}a_{r2})$, where; $C_m$, $C_r$= concentration of MET and REPA in the sample solution, $A_1$, $A_2$ = absorbances of the sample solution at 240 nm and 291.5 nm, respectively, $a_{m1}$ and $a_{m2}$= molar absorptivities of MET at 240 nm and 291.5 nm, respectively and $a_{r1}$ and $a_{r2}$= molar absorptivities of REPA at 240 nm and 291.5 nm, respectively

In the Q- absorbance ratio method concentration of MET and REPA in the sample solutions were calculated using equations $C_{m2} = (Q_o - Q_r/Q_m - Q_r) \times A_3/a_{m3}$ and $C_{p2} = A_3/a_{r3} - C_{m2}$, where $A_1$ and $A_3$ are absorbances of sample solution at 240 nm and 254.8 nm; and $a_{m3}$ and $a_{r3}$ are molar absorptivity of MET and REPA at 254.8 nm; $a_{m1}$ and $a_{r1}$ are molar absorptivity of MET and REPA at 240 nm. $Q_o = A_1/A_3$, $Q_m = a_{m1}/a_{m3}$ and $Q_r = a_{r1}/a_{r3}$.

The proposed validated methods were successfully applied to determine MET and REPA in the synthetic mixture. The % recoveries for MET and REPA obtained were 101.56±1.20, 101.00±1.53 by simultaneous equations method and 98.01±1.58, 98.15±1.63 by Q-absorption ratio method respectively. No interference of the excipients with the absorbance appeared; hence the proposed methods are applicable for the quantitative determination of MET and REPA in synthetic mixture.

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