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# DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF MIGLITOL AND METFORMIN HYDROCHLORIDE IN TABLET DOSAGE FORM.

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Metformine Hydrochloride, Miglitol, RP-HPLC method

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**ABSTRACT:** A simple, rapid and precise stability indicating reverse phase high performance liquid chromatographic method has been developed for simultaneous estimation of Miglitol and Metformine Hydrochloride in their tablet dosage form. Chromatography was performed on a Phenomenex ODS C18 (250 X 4.6 mm) 5µm column using Water: Methanol (50:50, v/v) pH: 4 mixture as a mobile phase. The detection was carried out at 235 nm with a flow rate of 1.0 mL/min. The retention times were 4.807 and 3.273 minutes for Miglitol and Metformine Hydrochloride, respectively. The linearity of the method was excellent over a concentration range 2.5 to 7.5µg/mL for Miglitol and 25 to 75µg/mL for Metformine Hydrochloride. The correlation coefficient was 0.997 and 0.999 for Miglitol and Metformine Hydrochloride, respectively. The limit of detection was 0.6607µg/mL and 1.740µg/mL for Miglitol and Metformine Hydrochloride, respectively. The limit of quantitation was 2.0021µg/mL and 5.2736µg/mL for Miglitol and Metformine Hydrochloride, respectively. The relative standard deviation values for repeatability, intraday precision and interday precision studies were less than 2 % and % recovery was between 98 % to 102 % for both drugs.

**INTRODUCTION:** Miglitol (MIG) is chemically (2R, 3R, 4R, 5S)-1-(2-hydroxyethyl)-2-(hydroxy methyl) piperidine-3, 4, 5-triol an oral anti-diabetic drug. It reversibly inhibits membrane-bound intestinal alpha-glucosidehydrolyze enzyme which hydrolyzes oligosaccharides and disaccharides to glucose and other monosaccharides in the brush border of the small intestine. In diabetic patients, this enzyme inhibition results in delayed glucose absorption and lowering of postprandial. Metformin (MET) is chemically N, N-dimethylimidodicarbonimidicdiamide.

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It is abiguanide class of oral anti-diabetic drugs. It improves hyperglycemia primarily through its suppression of hepatic glucose production and activates AMP-activated protein kinase. It also increases insulin sensitivity, fatty acid oxidation, peripheral glucose uptake and decreases absorption of glucose from the gastrointestinal tract.

Literature review revealed that several spectroscopic and chromatographic methods have been reported for estimation of Miglitol alone and in combination with other drugs also for Metformin Hydrochloride alone.

However, no method is reported for simultaneous estimation of these two drugs by HPLC in combined dosage form

## MATERIALS AND METHODS:

### Chemicals and reagents:

Miglitol and Metformin in the form of gift samples were kindly supplied by Glenmark. Mumbai and zydus cadila Ahmedabad respectively. A combination of Miglitol (50 mg) and Metformin (500 mg) in tablet formulation was procured from local pharmacy (Mignar 50-MF Glenmark Ltd).

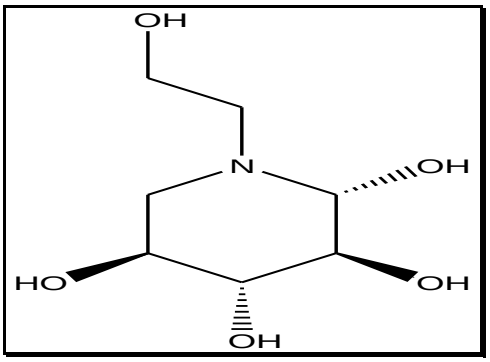


**FIGURE 1: STRUCTURE OF MIGLITOL**

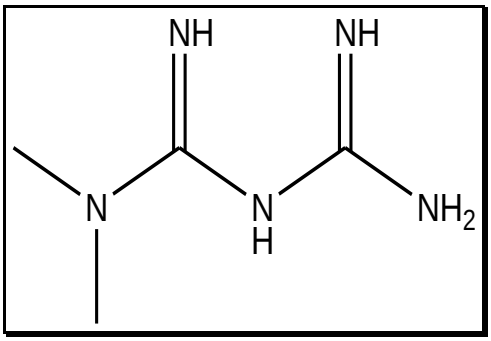


**FIGURE 2: STRUCTURE OF METORMIN HYDROCHLORIDE**

**Instrument and chromatographic condition:**
For the Chromatographic analysis, Shimadzu HPLC system was used that was equipped with a UV detector. The separation was achieved on RP C18 column {125 x 4.6 mm, 5 micron particle size} using KH2PO4 water: methanol (50:50 v/v) pH4 as mobile phase. The pH of mobile phase was adjusted to 5 with OPA. The flow rate was 1.0 ml/min.

**Preparation of Standard Stock Solution:**
Accurately weighed 50 mg of Metformin HCl and 5 mg of Miglitol were transferred into 100 ml volumetric flask, to which 50 mL of Methanol was added and sonicated for 15 min, dissolved completely and diluted up to the mark with Methanol to give a stock solution containing 500 µg/mL of Metformin HCl & 50 µg/mL of Miglitol.

**Preparation of Standard Working Solution:**
Working solution was prepared by taking 1 ml of above stock solution into 10 ml volumetric flask and diluting it up to the mark with Methanol to get the final working solution containing 50 µg/mL of Metformin HCl & 5 µg/mL of Miglitol.

**Method Validation:**
The proposed method was validated in compliance with ICH guidelines for system suitability, linearity, accuracy, precision, specificity, robustness parameters by the following procedures.

**System suitability:**
Six replicates of solution containing 50 µg/mL of Metformin Hydrochloride and 5 µg/mL of Miglitol were analysed. % RSD of Peak area was calculated.

**TABLE 1: SYSTEM SUITABILITY RESULTS FOR METFORMIN HYDROCHLORIDE AND MIGLITOL**

| Injection No. | Peak Area | |
|---|---|---|
| | MET | MIG |
| 1 | 2695.278 | 619.291 |
| 2 | 2681.583 | 620.531 |
| 3 | 2706.074 | 609.293 |
| 4 | 2711.476 | 623.023 |
| 5 | 2697.918 | 619.908 |
| 6 | 2703.337 | 621.173 |
| **Mean** | 2699.277 | 618.86 |
| **SD** | 10.4155 | 4.8638 |
| **% RSD** | 0.385 | 0.7 |

**Linearity:**
Linearity of developed RP-HPLC method was studied by obtaining calibration curves of MET and MIG, ranging from 25-75 µg/mL for MET and 2.5-7.5 µg/mL for MIG. **Table 2** shows the linearity data of MET and MIG. The linearity regression co-efficient ($r^2$) values were found to be 0.999 and 0.997 for MET and MIG. Each Linearity equation obtained for MET and MIG were y = 54.24x – 29.87, and y = 121.4x + 0.993 respectively. **Figure 3 and 4** shows calibration curves for MET and MIG respectively. High level of correlation coefficient indicates good linearity.

**TABLE 2: LINEARITY DATA OF METFORMIN HYDROCHLORIDE AND MIGLITOL**

| MET | | MIG | |
|---|---|---|---|
| **Con. µg/ml** | **Peak area** | **Con. µg/ml** | **Peak area** |
| 25 | 1340.464 | 2.5 | 307.931 |
| 37.5 | 1979.133 | 3.75 | 454.708 |
| 50 | 2706.05 | 5.0 | 621.797 |
| 62.5 | 3332.042 | 6.25 | 725.655 |
| 75 | 4054.368 | 7.5 | 931.652 |

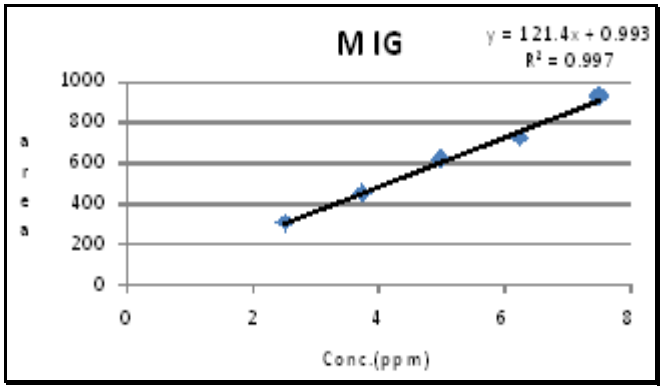


**FIGURE 4: CALIBRATION CURVES FOR MIG**

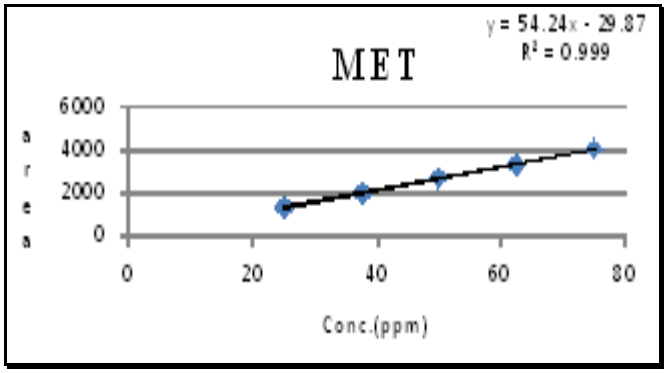


**FIGURE 3: CALIBRATION CURVES FOR MET**

## Accuracy:

The accuracy of the developed method was evaluated in triplicates by recovery studies at three different concentration levels of 80%, 100 %, and 120% for MET, MIG respectively. Known amounts of standard drug concentrations were added to the sample and peak area was determined.

The mean percentage recovery values are shown in **Table 3**. The mean recovery of the drugs was found to be in the range of 98- 102% indicating a high degree of accuracy for the developed method.

**TABLE 3: ACCURACY RESULTS**

| Drug | Level | Amt of Std taken (µg) | Amt of Std Recovered (µg) | Mean % Recovery |
|---|---|---|---|---|
| MET | 80 | 45 | 44.75 | 99.75 |
| | 100 | 50 | 49.80 | 99.70 |
| | 120 | 55 | 59.46 | 99.67 |
| MIG | 80 | 4.5 | 4.40 | 99.98 |
| | 100 | 5 | 4.90 | 99.83 |
| | 120 | 5.5 | 5.41 | 99.91 |

## Precision:

The precision was measured by intraday and interday. The % RSD of MET, MIG was calculated. The calculated values of % RSD for MET and MIG are mentioned in **Table 4 & 5**. The results indicated a high degree of repeatability.

**TABLE 4: INTRADAY PRECISION DATA FOR METFORMIN HYDROCHLORIDE AND MIGLITOL**

| Drug | Target conc. (µg/ml) | Peak Area | Average | SD | %RSD |
|---|---|---|---|---|---|
| MET | 25 | 131.506 | 1327.71 | 14.18 | 1.068161 |
| | 25 | 1333.83 | | | |
| | 25 | 1337.82 | | | |
| MIG | 2.5 | 305.778 | 612.97 | 0.964 | 0.315007 |
| | 2.5 | 305.545 | | | |
| | 2.5 | 307.32 | | | |
| MET | 50 | 2665.062 | 4026.22 | 18.67 | 0.695355 |
| | 50 | 2692.62 | | | |
| | 50 | 2700.682 | | | |
| MIG | 5 | 617.433 | 923.5223 | 10.54 | 1.720982 |
| | 5 | 600.926 | | | |
| | 5 | 620.559 | | | |
| MET | 75 | 3998.035 | 4026.226 | 25.15141 | 0.624689 |
| | 75 | 4034.278 | | | |
| | 75 | 4046.366 | | | |
| MIG | 7.5 | 925.121 | 923.5223 | 7.210172 | 0.780725 |
| | 7.5 | 915.647 | | | |
| | 7.5 | 929.799 | | | |

**TABLE 5: INTERDAY PRECISION DATA FOR METFORMIN HYDROCHLORIDE AND MIGLITOL**

| Drug | Target conc. (µg/ml) | Peak Area | Average | SD | %RSD |
|---|---|---|---|---|---|
| | 25 | 1324.543 | | | |
| MET | 25 | 1335.167 | 1330.727 | 5.522386 | 0.41499 |
| | 25 | 1332.47 | | | |
| | 2.5 | 306.085 | | | |
| MIG | 2.5 | 302.256 | 304.81 | 2.21183 | 0.725642 |
| | 2.5 | 306.089 | | | |
| | 50 | 2669.724 | | | |
| MET | 50 | 2695.317 | 2684.974 | 13.48349 | 0.502183 |
| | 50 | 2689.88 | | | |
| | 5 | 618.052 | | | |
| MIG | 5 | 607.103 | 614.4103 | 6.328348 | 1.029987 |
| | 5 | 618.076 | | | |
| MET | 75 | 4006.444 | 4024.98 | 16.56048 | 0.411442 |
| | 75 | 4038.317 | | | |
| | 75 | 4030.18 | | | |
| | 7.5 | 926.048 | | | |
| MIG | 7.5 | 908.549 | 920.2257 | 10.1123 | 1.098894 |
| | 7.5 | 926.08 | | | |

## Specificity:

Chromatogram of only diluent was taken to check the interference of diluent with the peaks of MET and MIG at the retention time of respective drugs. There was no peak detected at retention time of

MET 3.273 min and MIG 4.807 min. so, proposed method is specific in nature

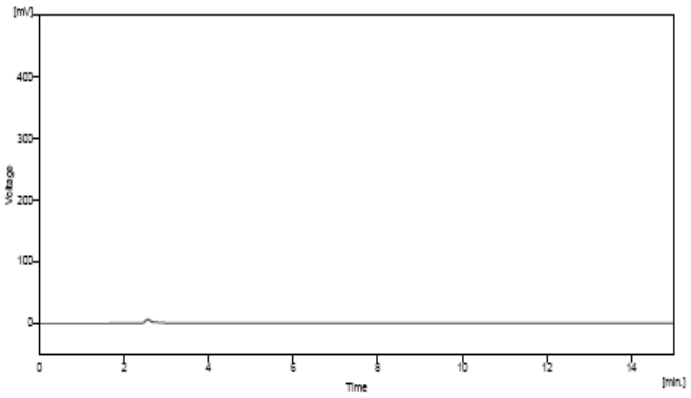


**FIGURE 5: CHROMATOGRAM OF DILUENT**

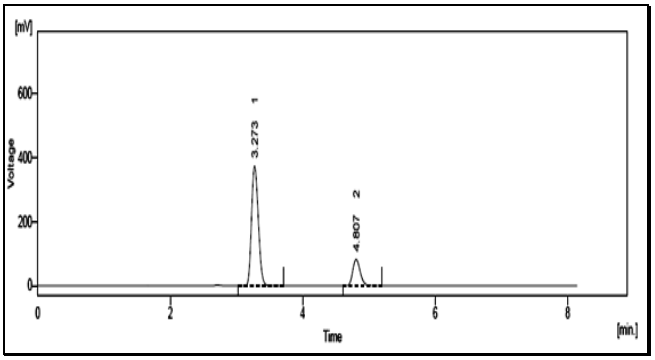


**FIGURE: 6 CHROMATOGRAM OF SAMPLE SOLUTION**

## LOD and LOQ:

The LOD and LOQ were calculated from following equation:

LOD = 3.3 × (SD/Slope)

LOQ = 10 × (SD/Slope)

Where, SD = Standard deviation of the Y-intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves. The results obtained are shown in Table 6.

**TABLE 6: LOD AND LOQ FOR METFORMIN HYDROCHLORIDE AND MIGLITOL**

| Drug | LOD | LOQ |
|---|---|---|
| **MET** | 1.7403 | 5.2736 |
| **MIG** | 0.6607 | 2.0021 |

## Robustness:

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized parameters were made in chromatographic conditions like of pH, flow rate and wavelength.

The effect of change in pH, flow rate and wavelength of detection on retention time and tailing factor were examined. The values obtained are mentioned in **Table 7**. The method was found to be unaffected by the small changes like ± pH, ± 0.1 ml/min in flow-rate of mobile phase and ± 1 nm in detection wavelength.

**TABLE 7: RESULTS OF ROBUSTNESS**

| Parameters varied | Drug name | System suitability parameters (n=3) | | |
|---|---|---|---|---|
| | | Mean Peak Area ± %RSD | Tailing factor± %RSD | Resolution ± %RSD |
| pH 4.1 | MET | 2582.92±0.61 | 1.290±0.132 | 7.167 ±0.211 |
| | MIG | 590.73±1.64 | 1.283±0.234 | |
| pH 3.9 | MET | 2773.54±0.91 | 1.266 ±0.687 | 7.153±0.168 |
| | MIG | 635.08±1.13 | 1.284±0.743 | |
| At Wavelength 336 | MET | 2634.70±0.93 | 1.308±0.624 | 7.113±0.168 |
| | MIG | 602.35±1.38 | 1.284±0.483 | |
| At Wavelength 334 | MET | 2767.80±0.75 | 1.277±0.458 | 7.167±0.239 |
| | MIG | 634.94±0.91 | 1.305±0.639 | |
| At flow rate 1.1ml/min | MET | 2635.75±1.09 | 1.290±0.172 | 7.134±0.327 |
| | MIG | 604.64±1.02 | 1.279±0.234 | |
| At flow rate 0.9ml/min | MET | 2797.68±0.88 | 1.286±0.216 | 7.140±0.571 |
| | MIG | 641.27±1.02 | 1.310±0.389 | |

## RESULT AND DISCUSSION:

### Optimized chromatography condition:

Chromatographic conditions were screened for mobile phase composition, mobile phase proportion, pH and flow rate Finally, mobile phase of Water: Methanol (pH 4) in the ratio of 50:50 v/v was optimized to give symmetric peak with short runtime at UV detection wavelength of 235 nm and flow rate at 1.0 mL/min was found to be appropriate with adequate separation between the two drugs. Chromatogram of MET, MIG at optimized chromatographic condition was recorded, the runtime was 4 min and the retention times of MET, MIG were found to be 3.273 and 4.807 min.

## Assay:

Twenty tablets were weighed and finely powdered. The powder equivalent to 50 mg of Metformin Hydrochloridee and 5 mg of Miglitol was weighed accurately and transferred to volumetric flask of 100 ml capacity and 10 ml of mobile phase was transferred to it and sonicated for 15 min. The flask was shaken and volume was made up to the mark

with mobile phase. The above solution was filtered through whatman filter paper (0.45µ). 1 ml of above solution was taken and transferred to 10 ml volumetric flask. Volume was made up to the mark with the mobile phase to give a solution containing 50µg/mL of Metformin Hydrochloride and 5µg/mL of Miglitol.

From the peak area obtained for MET, MIG, the amount of the drug in the sample was calculated and was found to be 101.9% for MET, and 95.9% for MIG.

**CONCLUSION:** The proposed HPLC method was found to be economical, simple, sensitive, accurate, precise, specific and robust and can be used for the routine quality control analysis of MET, MIG in bulk as well as in tablet formulation.

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