LCMS method for the simultaneous determination of Metformin and Miglitol in Human Plasma: Application to pharmacokinetic studies

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Objective: Adjunct therapy of metformin and miglitol is an ideal choice in the treatment of type 2 diabetes[1]. Thus, the quantification of these drugs in human plasma is equally important for the pharmacokinetic studies. The objective of the current study was to develop a rapid and sensitive method for the simultaneous estimation of these drugs in plasma.

Methodology: Conventional LC-MS method is developed to determine plasma concentration of metformin and miglitol using voglibose as internal standard. Chromatographic separation of active ingredients was achieved using a Zorbax eclipse C_{18} (150 mm×4.6 mm i.d, 5 μm) The mobile phase constitute 95% ammonium acetate (0.02 mM, pH 6.8) and 5% methanol was pumped at an isocratic flow rate of 0.5 mL/min. The data acquisition was carried out in positive ion mode by Single Ion Monitoring at 130.1 m/z for metformin, 208.1 m/z for miglitol and 268.4 m/z for IS. Liquid-liquid extraction and solid phase extractions are not good choice for the sample preparation due to hydrophilic nature of these drugs. Hence, simple protein precipitation using higher concentration of acetonitrile was utilized to remove the endogenous materials and avoid chromatographic peak interference during the LC-MS analysis. Developed method was validated by estimating the precision and accuracy for inter- and intraday analysis. Subsequently, the method was applied for determine the pharmacokinetic parameters such as Tmax, Cmax, AUC_{0-t}, $T_{1/2}$ and Kel for both the drugs in human plasma.

Results: The retention times were found to be 3.01, 4.0 and 3.45 min for miglitol, metformin and IS respectively(Fig.1). Further, the calibration curve was linear (>0.99) in the concentration range of 40 – 2000 ng/mL for metformin and 50-4000 ng/mL for miglitol. Average accuracy of assay of metformin and miglitol were found to be 98.18% and 99.13%, respectively. Relative standard deviations of both inter and intraday assays were less than 7%. Moreover, recovery of metformin and miglitol was more than 94.7% and 93.1%, respectively. Surprisingly, the pharmacokinetic profiles and parameters (Table 1) observed for both the drugs were comparable even though the dose administered were significantly

different. The developed LC-MS method is first of its kind and could be utilized for the simultaneous determination of metformin and miglitol in pharmacokinetic studies.

Conclusions: A simple, rapid, and accurate analytical method using LC-MS was established and validated for the simultaneous determination of MET and MIG in human plasma. Furthermore, the assay method showed high specificity because no plasma matrix peaks interfered with the MET, MIG, and IS peaks. The method also demonstrated the use of simple sample preparation procedures and a short assay time (5 min), along with excellent precision, recovery, and reproducibility. In addition, the analytes were stable at all storage and operational conditions. Hence, the method was effectively used to determine the pharmacokinetic parameters of MET and MIG in humans.

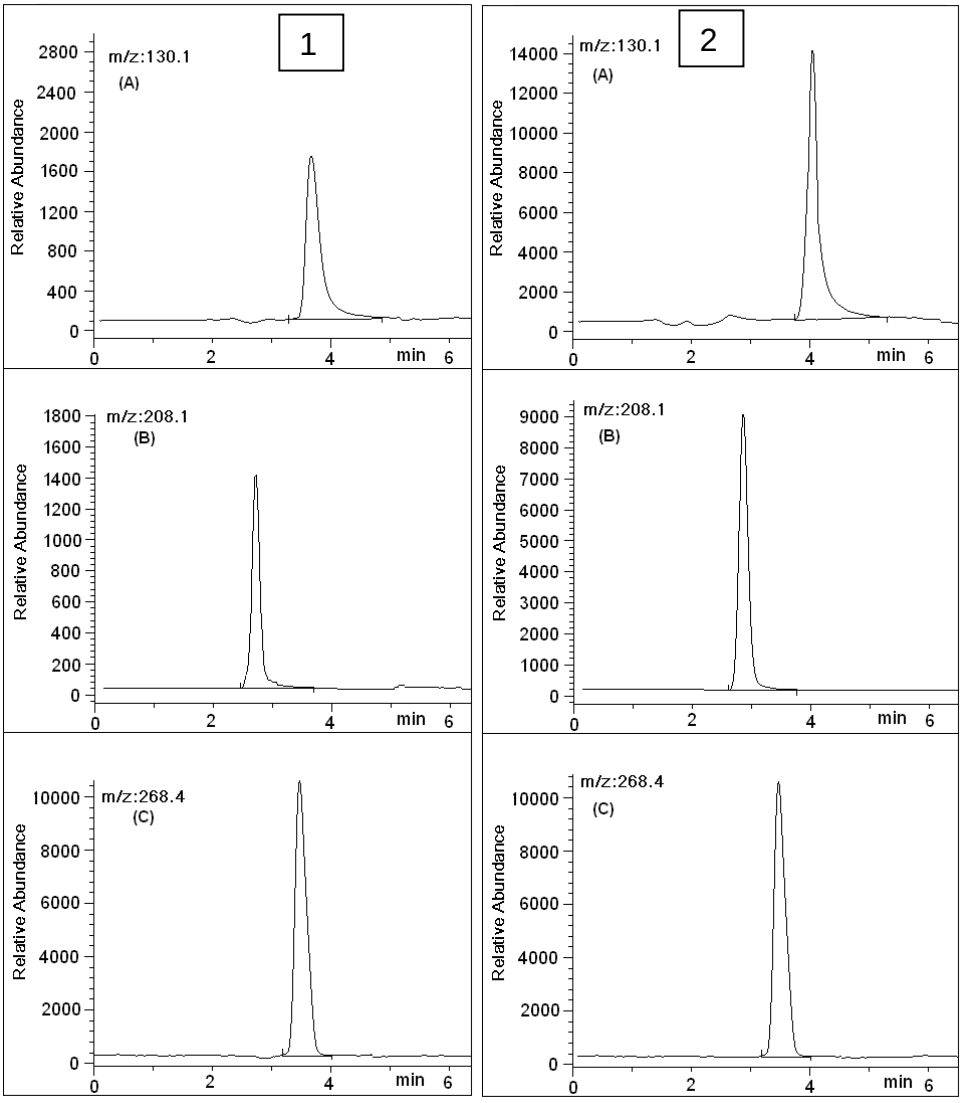

Figure 1. 1. Representative mass chromatograms obtained from a plasma sample spiked with 40 ng/mL of MET (A), 50 ng/mL of MIG (B), and 500 ng/mL of VOG (IS, C). 2. Representative mass chromatograms obtained form a volunteer plasma sample 3 h after the oral administration of a tablet containing MET (500 mg, A) and MIG (50 mg, B) and spiked with IS (C).

Table 1: Mean pharmacokinetic parameters observed in plasma following oral administration of single dose of MET (500mg) and MIG (50 mg).

Parameter	**Metformin**	**Miglitol**
T_{max} (h)	2.85	3.0
C_{max} (ng/ml)	1308.24 ± 174.10	1190.24 ± 122.47
$AUC_{0-\alpha}$ (min.ng/ml)	6364.76 ± 739.88	5678.12 ± 631.05
$t_{1/2}$ (h)	2.66 ± 0.42	2.34 ± 0.27
Kel	0.2602 ± 0.083	0.2839 ± 0.072

C_{max} indicates maximum concentration; T_{max}, time of maximum concentration; Kel, elimination rate constant; $AUC_{0-\alpha}$, area under the plasma concentration-time curve; $t_{1/2}$, elimination half-life.

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Reference : 1. Jean-Louis Chiasson, Lisa Naditch. The Synergistic Effect of Miglitol Plus Metformin Combination Therapy in the Treatment of Type 2 Diabetes. Diabetes Care, **24** (2001) 989-994.