



INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

Available Online at: www.ijpar.com

[Research article]

Development and Validation of RP-HPLC method for the simultaneous estimation of Miglitol and Metformin Hcl in Pharmaceutical formulations

Srinivas Ampati^{1*}, Sandeep Dyavarashetti¹, Kiran Gangarapu²

¹Department of Pharmaceutical Analysis, S.R.R College of Pharmaceutical Sciences, Karimnagar, India.

²Department of Pharmaceutical Analysis, Kakatiya Institute of Pharmaceutical Sciences, Pembarthu, Hasanparthy, Warangal, India-506 371.

ABSTRACT

A simple, accurate, precise and rapid reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the simultaneous estimation of Miglitol (MG) and Metformin Hydrochloride (MT) and in pure and tablet formulation. The proposed method is based on the separation of the two drugs in reversed-phase mode using zorabaxC₁₈ column (250×4.6 mm I.D., 5 μm particle size). The optimum mobile phase consists of Phosphate buffer of (pH 4.0): Methanol in the ratio of 80:20 v/v was selected as a mobile phase, flow rate is 1.0 ml/min and the α_{max} of UV detection was set at 251 nm. The retention times were observed at 3.045 and 4.460 min for Miglitol and Metformin Hydrochloride respectively. The method was validated according to ICH guidelines. The method could be accurate and reproducible. Linearity was obtained in the concentration range of 50-150% for Miglitol and 50-150% Metformin Hydrochloride. Mean percent recovery of samples at each level for both drugs were found in the range of 100%. The proposed method could be successfully applied in the quality control of bulk and pharmaceutical dosage forms.

Keywords: Miglitol and Metformin, RP-HPLC method, C₁₈ Column, Phosphate Buffer, Method development and Validation.

INTRODUCTION

Miglitol (MG) is an oral anti-diabetic drug in the alpha glucosidase inhibitor^[1, 2]. That acts by inhibiting the ability of the patient to breakdown complex carbohydrates into glucose^[3]. It is primarily used in diabetes mellitus Type 2 for establishing greater glycemic control by preventing the digestion of carbohydrates (such as

disaccharides, oligosaccharides, and poly saccharides) into mono saccharides which can be absorbed by the body. Chemically it is (2R,3R,4R,5S)-1-(2-hydroxyethyl)-2-(hydroxyl methyl) piperidine-3,4,5-triol^[4].

Metformin (MT) is an oral antidiabetic drug in the biguanide class^[5]. It is the first-line drug of choice for the treatment of Type-2 diabetes, in particular, in overweight and obese people and

*Corresponding author: Ampati Srinivas
E-mail address: drampaty@gmail.com

those with normal kidney function(2). Its use in gestational diabetes has been limited by safety

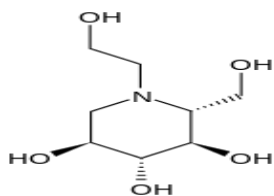


Fig 1: Structure of Miglitol

In literature, HPLC-UV-MS^[7] Force degradation study^[8] and LC-MS^[9] methods for estimation of MG have been reported. Many analytical methods like Spectrophotometric method^[10] available for estimation of MT individually and determination of MT in human plasma by HPLC with spectrophotometric detection^[11] and HPLC methods in combination with Glipazide^[12], Pioglitazone^[13], Rosiglitazone^[14], Repaglinide^[15], Potentiometry^[16], Spectrofluorimetry and UV-Visible Spectrophotometry, Stability indicating Capillary Electrophoresis^[17] are available in the literature. A simple, accurate, economical and reproducible UV spectrophotometric method for simultaneous estimation of Miglitol and Metformin in combined tablet dosage form has been reported. The developed method employs multi component spectroscopy using 300nm, 270nm, 240nm and 210nm as wavelengths for estimation^[18].

Various methods have been reported in literature to detect and quantify the individual drugs. So there is a need to develop a simple, accurate and precise HPLC method for the simultaneous determination of the Miglitol and Metformin drugs in formulation. To develop analytical method for the estimation of anti diabetic drugs preferably by chromatographic methods like HPLC. Validate the proposed method in accordance with ICH guidelines for the intended analytical application. Apply the proposed methods for the analysis of drugs in their bulk and pharmaceutical dosage forms.

MATERIALS AND METHODS

Instruments

The chromatographic technique performed on a Shimadzu LC20-AT Liquid chromatography with SPD-20A prominence UV-visible detector and Spinchrom software, reversed phase C₁₈column (Thermo eletrole, ODS, 250 mm × 4.6 mm i.d, 5μm) as stationary phase. Thermo Electron

concerns. Chemically it is 1-carbamimidamido-N,N-dimethylmethanimidamide^[6].

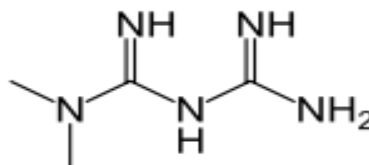


Fig 2: Structure of Metformin Hydrochloride

Corporation double beam UV-visible spectrophotometer (vision pro-software), Ultrasonic cleaner, Shimadzu analytical balance AY-220, Vacuum micro filtration unit with 0.45μ membrane filter was used in the study.

Reagent and Chemicals

Pharmaceutically pure samples of Miglitol and Metformin were obtained as gift sample from Rainbow Pharma Lab, Hyderabad, India. The purity of the drugs were evaluated by obtaining its melting point and ultraviolet (UV) and infrared (5) spectra. No impurities were found. The drugs were used without further purification. Acetonitrile, methanol, Water & Potassium dihydrogen phosphate (HPLC-grade, were from Merck). MIGNAR25-MF tablets containing 25mg of Miglitol and 500mg of Metformin hydrochloride was procured from the market.

Preparation of standard solution

Approximately 5 mg of Miglitol and 100 mg of Metformin hydrochloride were taken in 100 ml volumetric flask, added Methanol and sonicated until dissolved. Brought to the volume with same and mixed. From this 5 ml was transferred to 10 ml volumetric flask and make up the volume with water.

Preparation of sample solution

Precise test portions of powdered tablets equivalent to 25 mg of Miglitol and 500 mg of Metformin hydrochloride were transferred into 100 ml volumetric flask, added methanol and sonicated till dissolved with intermediate shaking for 20 min. Diluted up to volume with methanol and mixed. Filtered a portion of the resulted solution and discarded first few ml of the filtrate. 5.0ml of the above filtered solution was transferred into a 50 ml volumetric flask diluted to volume with water and mixed

Chromatographic conditions

The mobile phase consisting of Phosphate buffer (pH 4.0) & Methanol (80:20) was degassed and filtered by using Millipore vacuum filter system equipped with 0.45 μ membrane filter. Chromatography was performed at ambient temperature by pumping the mobile phase with a flow rate of 1.0 ml/min. The column effluent was monitored at 251nm

Assay Method

Estimation of Miglitol and Metformin hydrochloride in tablet dosage forms by the developed RP-HPLC method was carried out. The standard and sample solutions were prepared and the chromatograms were recorded. The assay procedure was performed and the assay percentage was calculated. The assay percentage of individual drugs and amount present per tablet were calculated.

$$\text{Assay \%} = \left[\frac{\text{AT}}{\text{AS}} \right] \times \left[\frac{\text{WS}}{\text{DS}} \right] \times \left[\frac{\text{DT}}{\text{WT}} \right] \times \left[\frac{\text{P}}{100} \right] \times \left[\frac{\text{AVG WT}}{\text{label claim}} \right] \times 100$$

Where: AT = Peak Area of obtained with test preparation.

AS = Peak Area of obtained with standard preparation.

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

METHOD VALIDATION

To develop a precise, accurate, economic and reproducible HPLC method for the estimation of MT and MG in bulk and tablet formulations^[19,20].

System Suitability

System suitability is performed by performing 5 injections on a sample mixture containing Miglitol and Metformin hydrochloride. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of Peak area, tailing factor, theoretical plates, Resolution and peak areas from five replicate injections.

Linearity

Linearity was studied by analyzing five sample solutions covering the range of 2.5-7.5 μ g/ml of MG & 50-150 μ g/ml of MT. From the primary stock solution which containing concentration of

1000 μ g/ml of MG & MT, from that 2.5ml, 3.75ml, 5ml, 6.25ml, 7.5ml of aliquots are pipette out into 10 ml volumetric flasks and made up to the mark with the mobile phase to obtain dilutions with concentrations 50%, 75%, 100%, 125%, 150% of MG and MT respectively. Overlay chromatogram of MG & MT is shown in Fig. 7. Calibration curve (Fig. 8) with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

Accuracy

The accuracy of the method was evaluated by determination of recovery of Miglitol and Metformin hydrochloride at three levels of concentrations. The sample solutions were spiked with standard solutions corresponding to 50, 100, and 150% of nominal analytical concentrations. Inject the above prepared sample into the system and calculate the % recovery.

$$\text{Recovery (\%)} = \frac{\text{Amount Found}}{\text{Amount added}} \times 100$$

Precision

For intra-day and inter-day precision values were estimated at three different concentration (10, 30 and 50 mg/ml) of Miglitol and Metformin hydrochloride three times on the same day and on three separate days to obtain the relative standard deviation (%RSD).

LOD & LOQ

Detection and quantification limit were calculated by the method based on the standard deviation (σ) and slope of the calibration plot, using the formula

$$\text{Limit of Detection} = \frac{\sigma \times 3.3}{S}$$

$$\text{Limit of Quantification} = \frac{\sigma \times 10}{S}$$

Robustness

For demonstrating the robustness of the developed method, experimental conditions were purposely altered and evaluated. The method must be robust enough to withstand such slight changes and allow routine analysis of the sample. Robustness of method was carried out with variation of flow rate (0.2 ml/min of set value i.e. 0.8 ml/min and 1.2 ml/min) and variation of temperature.

RESULTS & DISCUSSION

To develop a HPLC method, firstly, solubility profile and pKa of Miglitol and Metformin HCl was investigated. With the optimized chromatographic conditions, a steady baseline was recorded. The retention time of Miglitol and Metformin HCl was found to be 3.04 and 4.46 min respectively. A typical chromatogram of sample solution is given in figure-1. The developed method was described in Table 1.

Fig 1: Optimized method Chromatogram

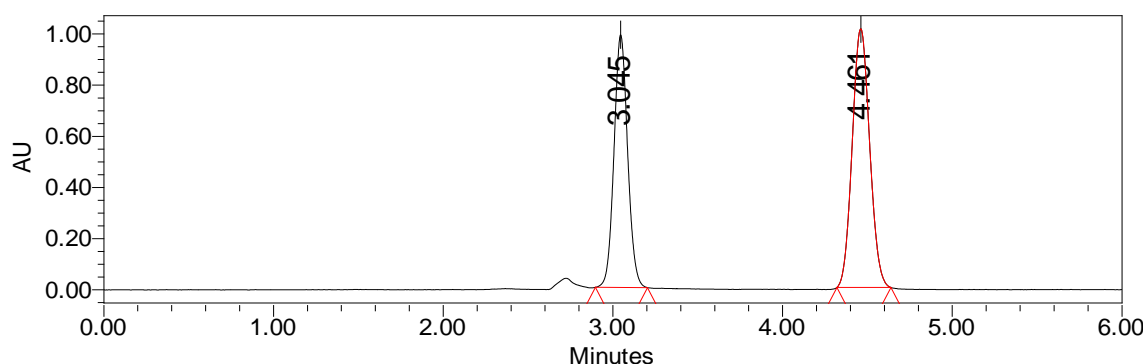


Table 1. HPLC method of Miglitol and Metformin HCl

Method	Buffer (0.02M KH ₂ PO ₄) and methanol in a ratio of 80:20 under isocratic condition
Column	C18, 5 μ m, 250 x 4.6 mm, Zorabax
Temperature	Ambient
Flow rate	1.0 mL/min.
Injection volume	10 μ L
Monitoring wave length	251 nm
Retention time	3.04 \pm 0.1 min and 4.46 \pm 0.1 min
Tailing factor (USP)	1.02
Peak purity	1.0000

System suitability

%RSD of system suitability parameters including retention time were 3.0434 \pm 0.001 min for MG and 4.4572 \pm 0.002 min for MT. Average theoretical plate 6786.6 \pm 33.862 for MG and 8775 \pm 56.171 for MT. Tailing factors were 1.0668 \pm 0.002 for MG and 1.0742 \pm 0.003 for MT. All

parameters were found well within acceptance limit set by FDA or ICH (% RSD of peak area \leq 2.0%; % RSD of retention time \leq 2.0%; theoretical plates $N \geq 1000$ plates; tailing factor $T \leq 2$). The data were shown in Table 2.

Table 2: System suitability test parameter for MG and MT by the proposed RP-HPLC method (n=5)

S.No	Conc (mg/ml)	Retention time		Tailing Factor		Theoretical Plate	
		MG	MT	MG	MT	MG	MT
1	10	3.042	4.454	1.065	1.074	6822	8856
2	10	3.045	4.457	1.066	1.071	6801	8802
3	10	3.043	4.456	1.064	1.077	6811	8684
4	10	3.043	4.459	1.07	1.071	6771	8761
5	10	3.044	4.460	1.069	1.078	6728	8772
Mean		3.0434	4.4572	1.0668	1.0742	6786.6	8775
SD		0.001	0.002	0.002	0.003	33.862	56.171
%RSD		0.033508704	0.047909352	0.21701981	0.272365273	0.4989549	0.64012726

Specificity

No interference from impurity, excipients or additives found in tablets. No distinctive Peak was observed with the injection of blank.

and concentration MG and MT demonstrated that the calibration curves were linear in concentration range from 50-150%. The correlation coefficients were 0.9997 for MG and 0.9998 for MT. The results of the linearity experiment are listed in Table 3.

Linearity

Linear correlation was obtained between peak area

Table 3: Linear regression analysis of calibration curves

Drug	Linearity Range	Intercept	Slope	Correlation coefficient	LOD	LOQ
MG	50-150%	64150	54985	0.9998	0.14	0.468
MT	50-150%	91533	72545	0.9997	2.735	9.119

Accuracy (% Recovery)

The percentage recoveries for MG were found 100 and for MT were in the range of 99.68 to 99.79.

The values of the recovery (%) are shown in Table 4.

Table 4: Accuracy (%recovery) of the proposed method

Excess drug added to analyte %	Theoretical content (mg/ml)		Conc Found ($\mu\text{g/ml}$) (mean \pm SD)		%Recovery (mean \pm SD)	
	MG	MT	MG	MT	MG	MT
50	25.008	500.156	25.008 \pm 0.00	498.56 \pm 0.412	100	99.682
100	50.016	1000.312	50.016 \pm 0.00	998.003 \pm 0.306	100	99.769
150	75.023	1500.468	75.023 \pm 0.00	1496.332 \pm 3.81	100	99.724

Precision

The precision of the method was measured by the percentage relative standard deviation (%RSD). The % RSD is calculated and determined as 0.02 and 0.04 for Miglitol and metformin hydrochloride respectively. The results as demonstrated in Table 5 are satisfied and are in within the specified limits.

LOD and LOQ

The LODs for MG and MT were found to be 0.140 and 2.735 mg/ml, while the LOQs for MG and MT were 0.468 and 9.119 mg/ml respectively (Table 3).

Robustness

The deliberate changes in the method have not affected the peak tailing and the percentage assay

Of MG and MT by changing the flow rate and temperature. The results were presented in Table 5.

Table 5: Robustness study

Variation	Chromatographic parameters					
	Tailing Factor		Theoretical Plate		%Assay	
	MG	MT	MG	MT	MG	MT
Flow rate at 0.8 ml/min	1.092	1.071	8227	7739	100.12	101.09
Flow rate at 1.2 ml/min	1.077	1.078	5808	10202	99.75	101.89
Temperature 1	1.089	1.094	8636	9994	100.25	100.98
Temperature 2	1.064	1.071	5839	7695	101.52	100.58

Analysis of Marketed Formulations

The assay procedure was performed and the assay percentage was calculated. The assay percentage of individual drugs and amount present per tablet were calculated. The assay result should be between 97% to 103% of labeled amount of drug substance.

CONCLUSION

The proposed RP-HPLC method is accurate, precise, rapid, robust, sensitive and selective. The prescribed method is found as suitable as earlier reported methods and the method is economical and reliable that can be effectively applied for

routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies. The method utilizes easily available and cheap solvent for analysis, hence the method was also economic for estimation of Miglitol and Metformin HCl from Tablet. Hence, the method can be easily and conveniently adopted for routine analysis of Miglitol and Metformin HCl in bulk and pharmaceutical formulations.

REFERENCES

- [1] Tripathi K. Insulin, oral hypoglycemic drugs and glucagon. Tripathi KD Essentials of medical pharmacology 6th ed New Delhi: Jaypee Brothers Medical Publishers (P) Ltd. 2008:254-74.
- [2] Scheen AJ, Lefebvre PJ. Oral antidiabetic agents. *Drugs*. 1998;55(2):225-36.
- [3] Jacob GS. Glycosylation inhibitors in biology and medicine. *Current opinion in structural biology*. 1995;5(5):605-11.
- [4] Reddy LA, Mylavarapu RK, Rodda S, Mala GC. *Chemistry & Biology Interface. Chemistry & Biology*. 2013;3(3):201-4.
- [5] Vigneri R, Goldfine ID. Role of metformin in treatment of diabetes mellitus. *Diabetes Care*. 1987;10(1):118-22.
- [6] Patil Sudarshan S, Bonde C. Development and Validation of analytical method for Simultaneous Estimation of Glibenclamide and Metformin HCl in Bulk and Tablets using UV-visible spectroscopy. *International Journal of ChemTech Research*. 2009;1(4):905-9.
- [7] DAI X-m, AN N, WU J-m, LI H-y, ZHANG Q-m. Development and validation of HPLC-UV-MS method for the control of four anti-diabetic drugs in suspected counterfeit products. *Acta Pharm Sin*. 2010;45:347-52.
- [8] Chittora N, Shrivastava A, Jain A. New RP-HPLC method of miglitol in tablet dosage form including forced degradation studies and estimation in spiked rabbit plasma. *Journal of Young Pharmacists*. 2009;1(4):364.
- [9] Li X, Wang Y, Wang J, Fawcett JP, Zhao L, Gu J. Determination of miglitol in human plasma by liquid chromatography/tandem mass spectrometry. *Rapid communications in mass spectrometry*. 2007;21(2):247-51.

- [10] Mubeen G, Noor K, Vimala M. Spectrophotometric Method for Estimation of Metformin Hydrochloride. *International Journal of ChemTech Research*. 2010;2(2).
- [11] Cheng C-L, Chou C-H. Determination of metformin in human plasma by high-performance liquid chromatography with spectrophotometric detection. *Journal of Chromatography B: Biomedical Sciences and Applications*. 2001;762(1):51-8.
- [12] Dhabale P, Seervi C. Simultaneous UV Spectrophotometric Method for Estimation of Gliclazide and Metformine Hydrochloride in Tablet Dosage Form. *International Journal of Chemical Technology Research*. 2010;2:813-7.
- [13] Sahoo P, Sharma R, Chaturvedi S. Simultaneous estimation of metformin hydrochloride and pioglitazone hydrochloride by RPHPLC method from combined tablet dosage form. *Indian journal of pharmaceutical sciences*. 2008;70(3):383.
- [14] Önal A. Spectrophotometric and HPLC determinations of anti-diabetic drugs, rosiglitazone maleate and metformin hydrochloride, in pure form and in pharmaceutical preparations. *European journal of medicinal chemistry*. 2009;44(12):4998-5005.
- [15] Patel J, Suhagia B, Patel B. Simultaneous spectrophotometric estimation of metformin and repaglinide in a synthetic mixture. *Indian journal of pharmaceutical sciences*. 2007;69(6):844.
- [16] Hassan SS, Mahmoud WH, Elmosallamy MA, Othman AHM. Determination of metformin in pharmaceutical preparations using potentiometry, spectrofluorimetry and UV-visible spectrophotometry. *Analytica chimica acta*. 1999;378(1):299-311.
- [17] Song J-Z, Chen H-F, Tian S-J, Sun Z-P. Determination of metformin in plasma by capillary electrophoresis using field-amplified sample stacking technique. *Journal of Chromatography B: Biomedical Sciences and Applications*. 1998;708(1):277-83.
- [18] Patel SR, Kabra PV, Kimbahune RV, Markad R, Nargund L. Development and Validation of Analytical Method for Quantitative Estimation of Miglitol and Metformin in Combined Dosage Form. *Journal of Applied Pharmaceutical Science*. 2012;2(06):227-9.
- [19] International Conference on Harmonization (ICH), Q2B: Validation of Analytical Procedures: Methodology, May 1997.
- [20] United States Pharmacopoeia 30 - National Formulary 25 (USP 30 - NF 25), Section <1225>, United States Pharmacopoeial Convention, Rockville, MD, 2007.
