Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2016, Volume 4, Issue 10
ISSN: 2321–3647(online)

RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride and Miglitol In Pharmaceutical Dosage Form

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ABSTRACT

RP-HPLC method has been developed for the simultaneous determination of Metformin hydrochloride and Miglitol in pharmaceutical dosage form. The method is simple, precise and accurate. RP-HPLC method is based on the separation eluted using a mobile phase mixture of methanol and phosphate buffer (adjusted to pH 4.5 using ortho phosphoric acid) in a ratio of 60:40 %v/v at a flow rate of 1.0ml/min. The detection was made at 218 nm. The retention times were 3.84 for MET and 5.07min for MIG. Calibration curve was linear over the concentration range of 50-150µg/ml for Metformin hydrochloride and 5-15 µg/ml for Miglitol. The accuracy of the method was assessed by recovery studies and was found to be 99.69% for Metformin hydrochloride and 99.80% for Miglitol. The developed method was validated as per the ICH guidelines parameters like Linearity, precision, accuracy, robustness, LOD and LOQ. The results were validated as per ICH Q2 R1 guideline and were satisfactory.

Keywords: Metformin hydrochloride, Miglitol, RP-HPLC.

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Received 08 September 2016, Accepted 21 September 2016

Please cite this article as: Patel DR *et al*., RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride and Miglitol In Pharmaceutical Dosage Form. American Journal of Pharmacy & Health Research 2016.

INTRODUCTION

Metformin hydrochloride (MET) is N,N-dimethylimidodicarbonimidic diamide. Metformin is a biguanide class of oral anti-diabetic drugs. (Figure I) It improves hyperglycemia primarily through its suppression of hepatic glucose production and activates AMP-activated protein kinase[1]. Miglitol (MIG) is (2R,3R,4R,5S)-1-(2-hydroxyethyl)-2-(hydroxymethyl)piperidine-3,4,5-triol. (Figure II) The antihyperglycemic action of Miglitol results from a reversible inhibition of membrane-bound intestinal alpha-glucoside hydrolase enzymes[2]. Metformin hydrochloride used in combination with Miglitol gives greater glycemic improvement than Metformin monotherapy.Thus, it is beneficial for the patients[3]. The review of literature reveals that there were analytical methods of two drugs individually in pharmaceutical dosage forms and even in biological samples[4-28]. As per our detailed literature survey as on date, there are very few reports using UV & RP-HPLC for the simultaneous quantitative estimation of Metformin and Migllitol in Bulk & Pharmaceutical dosage forms[29-31]. We here in reported a new, simple, sensitive, precise, accurate, linear and isocratic RP-HPLC method for the simultaneous quantitative estimation of Metformin and Migllitol in bulk & Formulation[32].

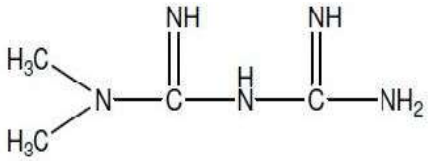

Figure I: Structure of Metformin hydrochloride

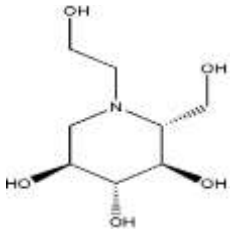

Figure II: Structure of Miglitol

MATERIALS AND METHOD

Apparatus

A gradient high performance liquid chromatograph from Shimadzu HPLC system, equipped with a Diode array detector and Shimadzu 20AT Spin chrome software was used. A reversed phase Varian C_{18} (250 X 4.6 mm i.d, 5 µm particle size) analytical column was used for the present analysis. Electronic Balance BL-220H Shimadzu Corporation Japan (1mg sensitivity), ultrasonic cleaner Model-D compact 1.5 litre (ElE Instruments pvt. Ltd.) and pH cal. Analab (Analab

Scientific Instruments Pvt. Ltd.) were used during the study.

Reagents and materials

MET and MIG were obtained from Astron research center, Ahmedabad. and Glenmark Pharmaceutical Limited, Mumbai respectively. All solvents were of HPLC grade and all reagents were of analytical grade. Methanol, Acetonitrile and were obtained from Merk (India). Ortho phosphoric acid, Potassium dihydrogen phosphate, Sodium hydroxide were obtained from S.D. fine-chem ltd (Mumbai). Triple distilled water was used throughout the experiment. All solvents and solutions were filtered through a membrane filter (ultipor N66 Nylon 6,6, 0.2µm pore size) and degassed using ultrasonic cleaner before use.

Optimization of wavelength maxima

Solutions of MET and MIG were scanned between 200 and 400nm. UV spectra of both drugs show absorbance at 218nm.(Figure III)

Chromatographic conditions

The samples were chromatographed on a reversed phase C_{18} (250 X 4.6 mm i.d, 5 µm particle size) column with a flow rate of 1.0 ml/min. All analyses were carried out at isocratic conditions. The mobile phase consisted of a mixture of Methanol: Phosphate buffer (60:40% v/v) pH 4.5 adjusted with 1%ortho phosphoric acid. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. The volume of injection was 10µl and the detection was made at 218nm.

Preparation of solutions

Phosphate buffer

Accurately weighed 2.72gm Potassium dihydrogen phosphate was transferred in 1000ml water. Around pH 4.7 was achieved. The pH was adjusted with 1% orthophosphoric acid.

MET standard stock solution

Accurately weighed MET (100mg) was transferred in 100ml volumetric flask. The drug was dissolved in water with sonication for 5min and final volume was adjusted with mobile phase upto mark to prepare a 1000µg/ml stock solution.

MET working standard solution

From the stock solution (1000µg/ml), an accurately measured 0.5, 0.75, 1.0, 1.25, and 1.5ml was transfer into separate 10ml volumetric flask and final volume was adjusted with mobile phase upto mark to prepare 50-150µg/ml solutions.

MIG standard stock solution

Accurately weighed MIG (10mg) was transferred in 100ml volumetric flask. The drug was

dissolved in water with sonication for 5min and final volume was adjusted with mobile phase upto mark to prepare a 100µg/ml stock solution.

MIG working standard solution

From the stock solution (100µg/ml), an accurately measured 0.5, 0.75, 1.0, 1.25, and 1.5ml transfer into separate 10ml volumetric flask and final volume was adjusted with mobile phase upto mark to prepare 5-15µg/ml solutions.

Sample solution

Twenty tablets were weighed accurately and finely powdered. Powder exactly equivalent to 100mg of MET and 10mg of MIG was transferred to a 100ml volumetric flask. The powder was dissolved in 60ml of methanol with sonication for 20 minutes and volume was made up with mobile phase. Filtered through Whatman filter paper No.41.Resulting solution gave conc. 1000µg/ml and 100µg/ml for MET and MIG respectively. An aliquot of 1ml of the solution was transferred into 10ml volumetric flask and diluted upto mark with mobile phase. This solution was injected for HPLC determination.

Optimization of the solvent system

Mobile phase was selected based on the review of literature. Mixture of methanol: water, ACN: water, methanol: ACN with various pH, and different volumes at 1mL/min flow rate were tried. The mixture of Methanol: Phosphate buffer (60:40% v/v) pH 4.5 adjusted with 1%ortho phosphoric acid at 1mL/min flow rate, proved to be better than the other mixtures in terms of resolution and peak shape.

Method Validation

Linearity

The linearity was evaluated by linear regression analysis. The calibration curve was obtained with concentrations of pure MET and MIG solution ranging from 50-150µg/ml and 5-15µg/ml respectively for the chromatographic method. (Figure IV & V)

Precision

The precision of the procedure was determined by repeatability (intraday). Intraday precision was evaluated by assaying same concentration and during the same day. Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of sample. Another replicate determination on three different days to estimate interday precision.

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To a pre-analyzed sample solution, a definite concentration of standard drug was added and recovery was

studied. A 80%,100% and 120% of pure drug solutions were added to the pre-analyzed samples.

Limit of detection and limit of quantification

For HPLC method, the limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the response and the slope by using calibration curves.

Robustness

Robustness was determined by the analysis of the samples under a variety of conditions making small changes in the buffer pH, in the ratio of mobile phase, and in the flow rate.

System suitability

System suitability parameter is established to ensure that the validity of the analytical method is maintained whenever used. Typical variations are the stability of analytical solution, different equipment, and different analyzer. In case of liquid chromatography typical variations are the pH of the mobile phase, the mobile phase composition, different lots or supplier of columns, the temperature and flow rate.

Analysis of Laboratory mixture

The response of sample solution was measured at 218nm using HPLC. The amount of MET and MIG were determined by regression equation.

RESULTS AND DISCUSSION

The mobile phase consisting of Methanol: Phosphate buffer (60:40% v/v) pH 4.5 adjusted with 1%ortho phosphoric acid at 1mL/min flow rate which gave sharp, well-resolved peak with minimum tailing factor for MET and MIG. The retention time for MET and MIG were 3.84 & 5.07 min respectively. (Figure VI)

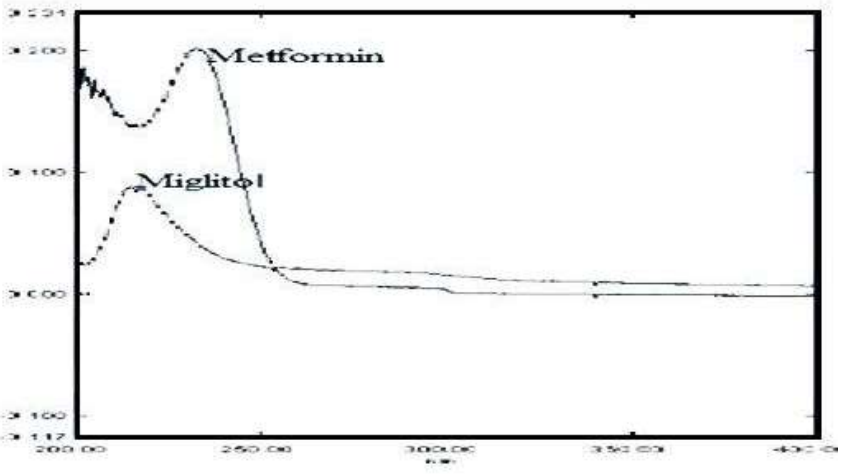

Figure III: Overlay UV spectra of MET and MIG

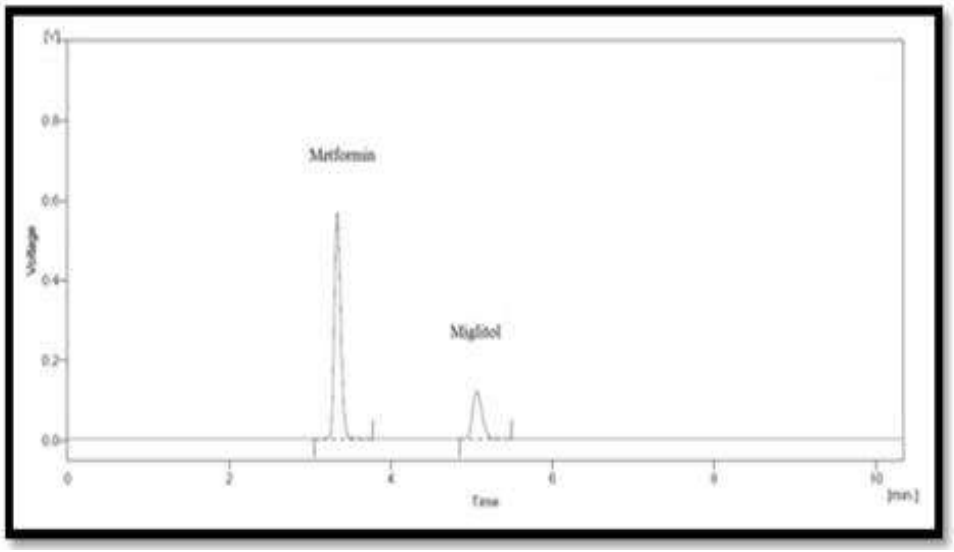

Figure VI: A typical chromatogram of met(100µg/ml) and mig(10µg/ml)

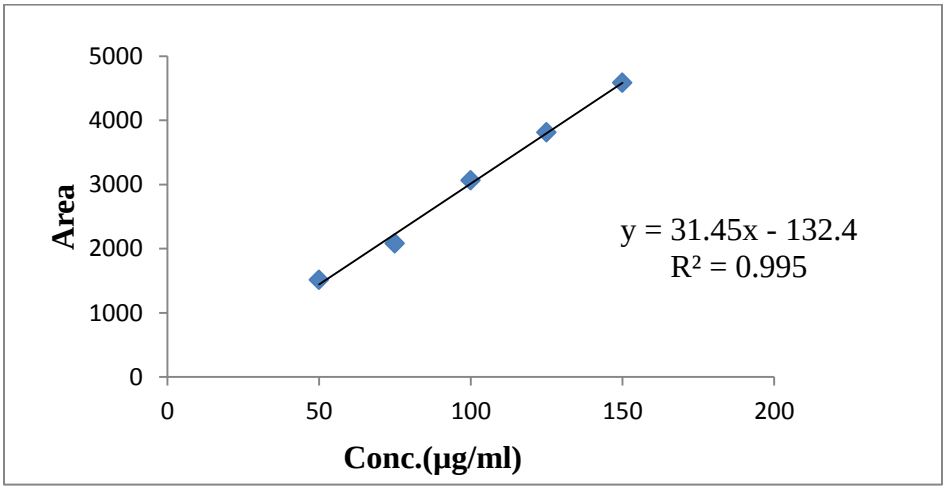

Figure IV: Calibration curve for MET

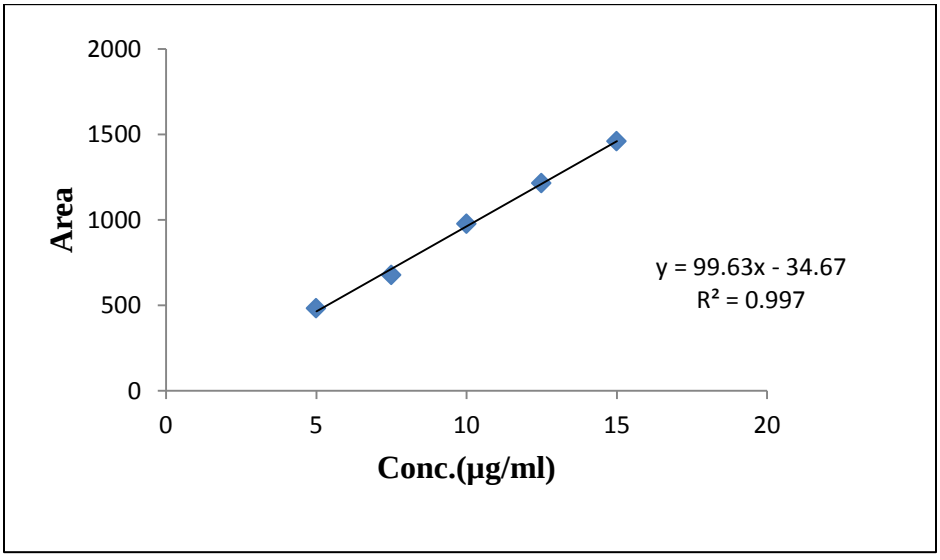

Figure V: Calibration curve for MIG

The calibration curve for MET & MIG were found to be linear over the range of 50-150µg/ml and 5-15µg/ml respectively. The data of regression analysis of the calibration curves is shown in Table I.

Table I: Linearity and Range data for MET and MIG

Drug	Linearity	Y=mx+c		r2*
		Slope*	Intercept*	
MET	50-150µg/ml	31.45	132.4	0.995
MIG	5-15µg/ml	99.63	34.67	0.997

*= Average result of six replicate samples

The LOD for MET and MIG were found to be 10.31µg/ml and 0.832µg/ml respectively, while LOQ were 31.25µg/ml and 2.52µg/ml respectively. The results for system suitability test parameters and recovery study are summarized in Table II &III.

Table II: System Suitability Parameters for MET and MIG by HPLC

Parameters	Data obtained		Standard limits
	MET	MIG	
Retention time (min)	3.84	5.07	-
Theoretical plates per meter	8212	7989	>2000
Symmetry/Tailing factor	1.286	1.367	≤2
Resolution	9.237		>2

Table III Recovery study of MET and MIG by HPLC

Drug	Conc. Of Form.(µg/ml)	Conc. of Std. added (µg/ml)	Conc. Recovered* (µg/ml)	% Recovery± SD*
MET	50	40	39.96	99.91±0.98
	50	50	49.75	99.51±0.55
	50	60	59.80	99.67±0.46
MIG	5	4	4.00	100.06±1.14
	5	5	4.98	99.63±0.64
	5	6	5.98	99.73±0.49

*= Average result of six replicate samples

The summary of validation parameters for analysis of MET & MIG were shown in Table IV.

Table IV: Summary of Validation Parameters of HPLC

Sr. No.	Parameters	MET	MIG
1	Linearity range	50-	5-
2	Correlation coefficient	0.995	0.997
3	Accuracy (%)	99.96	99.80
4	LOD (µg/mL)	10.31	31.25
5	LOQ (µg/mL)	0.832	2.52
6	Retention time (min)	3.84	5.07

7	Theoretical plates per	8212	7989
8	Symmetry/Tailing factor	1.286	1.367
9	Resolution	9.237	
10	Assay%	97.88	98.85
11	Robustness	Robust	Robust

CONCLUSION

The developed method was validated in terms of linearity, accuracy, and precision. The mobile phase is easy to prepare and the drugs are eluted within short run time. A good linear relationship was observed for MET and MIG. The results of recovery studies show that the method is free from interference of the excipients used in the formulation. The percentage RSD for precision is <2 which confirms that method is sufficiently precise. The proposed method is accurate, precise, simple, sensitive, and rapid and can be applied successfully for the estimation of MET and MIG in its bulk and pharmaceutical formulation without inference.

ACKNOWLEDGEMENT

The authors are thankful to Astron research center, Ahmedabad. and Glenmark Pharmaceutical Limited, Mumbai for providing gift sample of pure Metformin hydrochloride and Miglitol.

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