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New RP-HPLC Method of Miglitol in Tablet Dosage form Including Forced Degradation Studies and Estimation in Spiked Rabbit Plasma

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ABSTRACT

A simple, accurate, precise, economic, and specific stability indicating the RP-HPLC method has been developed for the estimation of Miglitol in tablet dosage form and spiked rabbit plasma. Chromatographic separation was achieved using Lachrom HPLC with Lichrospher, ODS, (250x 4.6) mm, ϕ 5 column at ambient temperature with 0.05 M ammonium acetate as a mobile phase at a flow rate of 0.5 ml/min and 216 nm was selected as a wave length for detection of a method. The retention time was 6.45 min. The standard curve was found to be linear (r^2= 0.996) in the concentration range 800-1200 µg/ml using 1000 µg/ml as the test concentration. Apparent recovery was 98.338-101.704 % with RSD 0.942-0.964 for two brands. The method was repeatable with RSD 0.736. The intraday and interday precisions were RSD 0.957 and 1.019, respectively. LOD and LOQ of the method were 20 and 70 µg/ml, respectively. The presented study separates degradents and peaks appeared after spiking drug with plasma with a resolution of more than 1.5.

Key words: Forced degradation, Miglitol, RP-HPLC, stability indicating

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INTRODUCTION

Miglitol, (2*R*, 3*R*, 4*R*, 5*S*)-1-(2-hydroxyethyl)-2-(hydroxymethyl) piperidine-3,4,5-triol [Figure 1], an oral anti-diabetic drug acts by inhibiting the ability of the patient to breakdown complex carbohydrates into glucose and slows the digestion of sugars so our body has time to store extra sugar.[1]

The development of analytical method and validation followed by stability testing forms an important part of the process of drug product development.[2,3] The assay of drug product in stability test sample needs to be determined using a stability-indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines.[4-7] The literature survey reveals that there are methods for the estimation of Miglitol in bulk and dosage forms including capillary electrophoresis,[8] tendom mass liquid chromatography,[9,10] and complexometric method,[11] but no RP-HPLC method was found with UV detection. The aim of this study is to develop a precise, accurate, economic, and simplest stability indicating the RP-HPLC method for the estimation of Miglitol (MGL) in tablet dosage forms and spiked plasma.

MATERIALS AND METHODS

Apparatus and chemicals

The chromatographic system consists of an L-7110

solvent delivery system (Merk Hitachi), L-7400 double-beam UV detector (Merk Hitachi), L-7500 integrator, and a rheodyne injector valve bracket fitted with a 20 µl sample loop. Chromatographic separation was performed on a stainless steel Lichrocart ODS Column, (250×4.6 mm) packed with 5 µ particle diameter, Lichrocart HPLC guard cartridge system, and a winchrom software. All of the chemicals and reagents were of analytical grade. Solvents of HPLC grade purchased from Merck Ltd, Mumbai. Reference standard Miglitol was kindly gifted by Glenmark Pharmaceuticals, Mumbai. Misobit and Mignar tablet of Lupin Pharma and Glenmark Pharmaceuticals, respectively, were purchased from the local market.

Chromatographic conditions

The mobile phase consisting of a 0.05 M ammonium acetate solution prepared by 3.85 g ammonium acetate was accurately weighed and dissolved in 1000 ml water. The mobile phase was degassed and filtered by passing through a 0.45 µ millipore filter prior to use. The elution was carried out under isocratic condition at a flow rate of 0.5 ml/min., with UV detection at 216 nm at ambient temperature. The chromatogram of finally optimized chromatographic condition has been shown in Figure 2.

Preparation of standard solution and calibration curve

500 mg pure Miglitol was weighed and dissolved in water in 50 ml volumetric flask and diluted up to the mark with water to get a 10 mg/ml solution. Suitable aliquots of standard stock solution of Miglitol were taken in the concentration range and diluted with the mobile phase to obtain working standard solutions of suitable concentrations. Triplicate 20 µl injections were made for each concentration and are chromatographed under the above-mentioned conditions. The average peak area of the three replicates was plotted against the corresponding concentration to obtain the calibration curve [Figure 3], regression equation was found to be (y =21185x +3E+06) with a 0.9996 correlation coefficient.

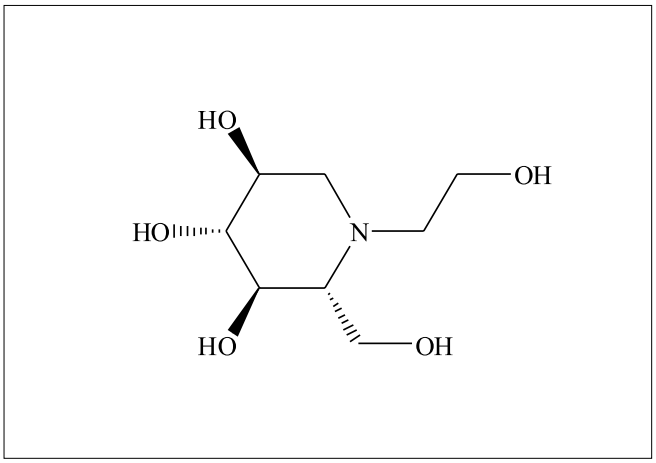

Figure 1: Structure of Miglitol

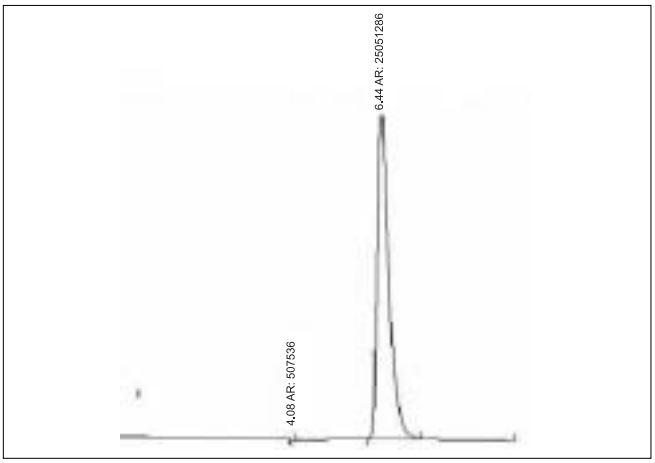

Figure 2: Chromatogram of optimized condition of the proposed method

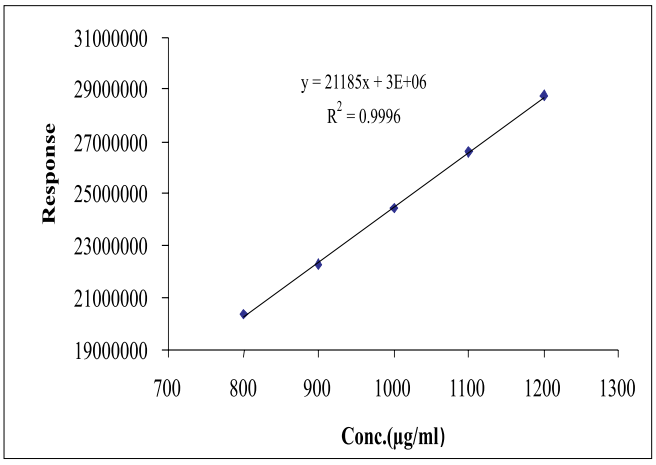

Figure 3: Calibration curve of Miglitol

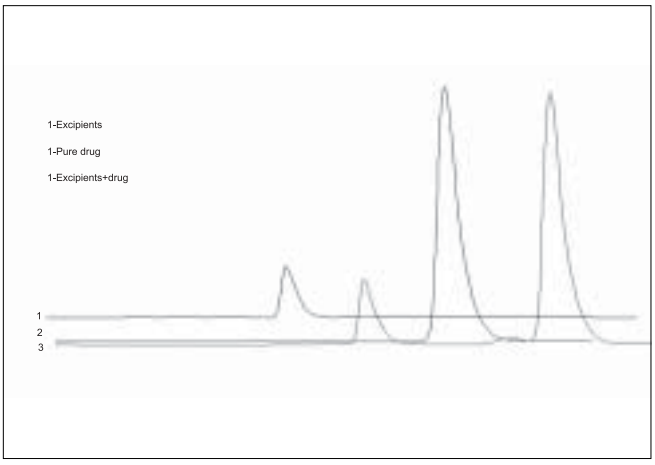

Figure 4: Overlay chromatogram of excipients, pure drug, and excipients-drug mixture

Validation of the proposed method

The developed method was validated for specificity, linearity, precision, accuracy, LOD, LOQ, and system suitability testing.

Specificity

Various generally used excipients such as lactose, talc, starch, and magnesium stearate were mixed in proportion approximately 90 mg, 28 mg, 30 mg, and 2 mg, respectively. They were mixed in 10 ml water in a volumetric flask, diluted up to the mark and filtered. 2 ml of this filtrate was diluted to 10 ml with mobile phase. 2 ml of the filtrate was mixed with a 2 ml standard stock solution in 10 ml volumetric flask and diluted up to the mark to produce 1000 μg/ml. One dilution of a 1000 μg/ml standard solution was also prepared. All the solutions were injected in the order of excipients mixture, 1000 μg/ml standard solution, and then excipients-drug mixture.

Overlay of excipients, pure drug, and excipients [Figure 4] with drug shows no peak of the excipients at the RT of the drug. Excipient peak shows a resolution of 2.5 with the drug peak, hence the method is specific.

Linearity

Linearity is accessed by visualizing the graph of the calibration curve. The points in the calibration curve distributed equally above and below the trend line show linearity.

Range

Linearity range: It is the interval in which the response is directly proportional to the concentration between the upper and lower levels (which is generally ± 5% of the intercept having slope equal to zero) and was 800-1200 μg/ml, respectively.

Working range: It begins from limit of quantification to the maximum concentration used for the development of the analytical method and in this case it is 70-1200.

Precision

Repeatability: Repeatability was accessed by six replicate injections of 1000 μg/ml solution of drug. % RSD found was found to be 0.736 showing that the method is repeatable.

Intraday and intraday precision: 0.8 ml, 1 ml, and 1.2 ml was taken out from the standard stock solution and diluted to 10 ml to make 800 μg/ml, 1000 μg/ml, and 1200 μg/ml, respectively. Three replicates of each dilution were injected three times a day for intraday and three replicates for 3 days for interday. % RSD found to be in this case were 0.957 and 1.019, respectively.

Accuracy

Recovery studies were performed with two brands Misobit (Lupin Ltd) and Mignar (Glenmark pharmaceuticals Ltd).

Powdered Misobit tablets (Lupin Ltd.) equivalent to 100 mg MGL was transferred to a 10 ml volumetric flask and ultrasonication was done for 20 min with approximately 5 ml water. Solution was then diluted up to the mark with water and filtered through 0.45 μ filter. 0.8 ml of this solution was spiked in three different 10 ml volumetric flasks with 0.1, 0.2, and 0.3 ml of previously analyzed standard stock solution. Finally, volume was made up to the mark with mobile phase and estimation of drug content was done by proposed method.

Same procedure was followed for Mignar tablets except ultrasonication time was kept for 15 min. The results of recovery studies are shown in Tables 1 and 2 for Misobit and Mignar, respectively.

Table 1: Recovery study of Misobit Tablet

Conc. (μg/ml)	Conc. found before spiking (μg/ml) C_1	Conc. of Std. added C_2	Conc. found after spiking (μg/ml) C_3	% Recovery $(C_3-C_1)*100/C_2$	Mean ± SD	RSD
800	810.945	103.938	915.542	100.634	99.786 ± 0.939	0.942
			913.653	98.8165		
			914.765	99.8864		
	812.501	207.875	1019.880	99.7595		
			1021.030	100.315		
			1020.650	100.134		
	814.065	311.813	1122.061	98.7743		
			1121.110	98.4706		
			1129.870	101.282		

Table 2: Recovery study of Mignar

Conc. (µg/ml)	Conc. found before spiking (µg/ml) C1	Conc. of Std. added C_2	Conc. found after Spiking (µg/ml) C_3	% Recovery $(C_3-C_1)*100$ / C_2	Mean ± SD	RSD
800	820.487	103.938	925.581	101.112	100.361 ± 0.968	0.964
			924.787	100.348		
			922.698	98.338		
	817.978	207.875	1027.660	100.870		
			1026.230	100.182		
			1025.401	99.7806		
	818.294	311.813	1132.910	100.901		
			1130.160	100.018		
			1135.421	101.704		

Table 3: Summary of system suitability parameters

Parameters	Inference
Number of theoretical plates (N)	17500
Height equivalent to theoretical plate (HETP)	1.42 X 10-3
Retention time	6.44
Capacity factor	2.078
Tailing factor	0.87-0.90
Asymmetry factor	0.85-0.88

Table 4: Results of estimation of Miglitol in tablet dosage form

Formulation	Conc. found (mg)	Mean(mg) + SD	RSD
Misobit	49.756	49.879+0.148	0.297
	49.834		
	50.043		
Mignar	50.276	50.196+0.241	0.480
	50.387		
	49.925		

Limit of quantification (LOQ) and limit of detection (LOD)

Various dilutions of standard solution like 1200, 1000, 800, 600, 400, 200, 100, 50, 20, 15, and 10 µg/ml were prepared from the standard solution by taking (1.2, 1, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05, 0.02, 0.015, and 0.01 ml) in a 10 ml volumetric flask and diluting up to the mark with mobile phase. Six replicates of each dilution were injected and curve between RSD and concentration was plotted [Figure 5]. The concentration where RSD exceeds the acceptable value and not receiving any response was considered as LOQ (70 µg/ml) and LOD (20 µg/ml), respectively.

System suitability testing

For system suitability testing six replicates of 1000 µg/ml were injected and result obtained given in Table 3.

Estimation of MGL in tablet dosage form

Optimization of extraction time

Four dilutions of 1000 µg/ml of the formulation were prepared in the mobile phase and sonicated for 10, 15, 20, 25, and 30 min and 20 µl was injected immediately.

Procedure for estimation of MGL in tablet dosage form

Powdered Misobit tablet equivalent to 50 mg of MGL was taken in 50 ml volumetric flask and ultrasonication was done using approximately 25 ml water and diluted up to the mark with mobile phase. The content of drug in tablet was found by using equation. The same procedure was followed for Mignar tablets except ultrasonication time used is 20 min. The results of estimation of purity of Misobit and Mignar are given Table 4.

Forced degradation studies

Acidic degradation

5 ml of standard stock solution was transferred in 50 ml volumetric flask and 1 ml concentrated HCl was added to it and kept for 2 h at ambient temperature then diluted to 25 ml with water. The pH of the solution was adjusted to 6.5 with 1 M NaOH. The solution was finally diluted to 50 ml with mobile phase, and 20 µl volume was injected. The same procedure was repeated at 50 and 100 ºC, but no were degradents observed.

Alkali degradation

5 ml of the standard stock solution was transferred in a 50 ml volumetric flask and 1 ml 10 M NaOH was added to it and kept for 2 h then diluted to 25 ml with water. The pH of the solution was adjusted to 6.5 with 1 N HCl. The solution was finally diluted to 50 ml with mobile phase and 20 µl volume was injected. The same procedure was repeated at

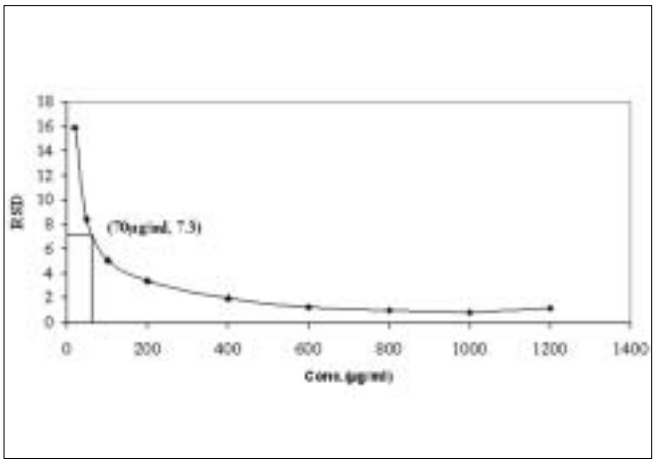

Figure 5: RSD V/S concentration (µg/ml) graph for the determination of LOQ and LOD of the proposed method

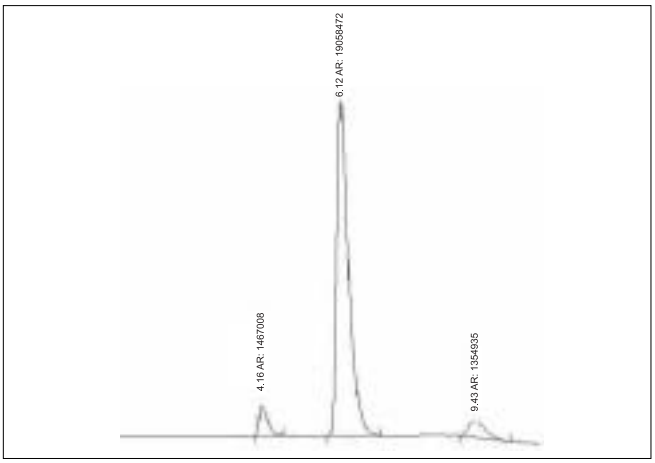

Figure 6: Chromatogram of wet heat degradation

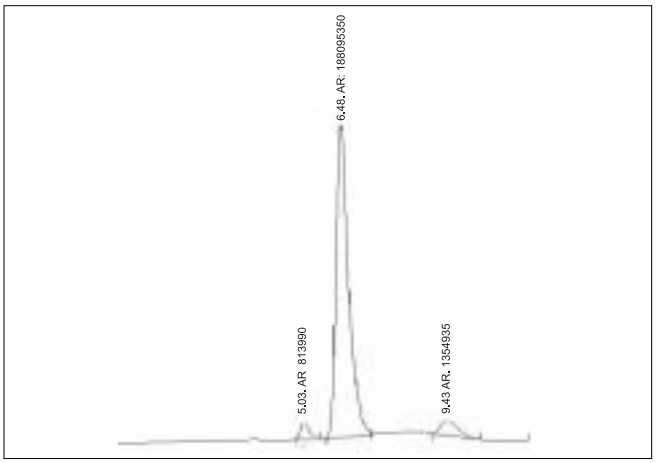

Figure 7: Chromatogram of dry heat degradation

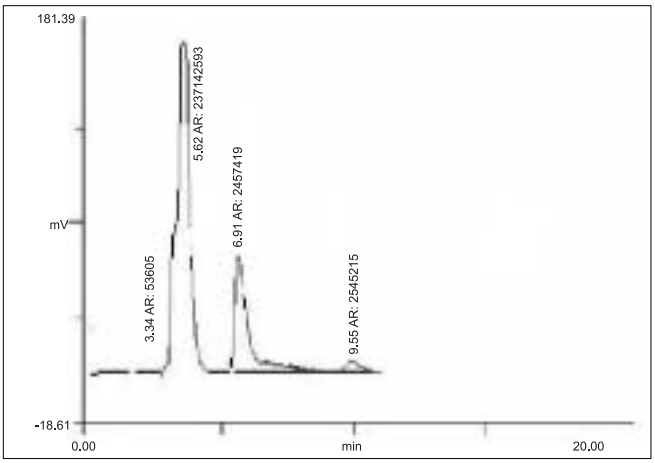

Figure 8: Chromatogram of MGL with spiked plasma

50 and 100 °C, but no degradents were observed.

Oxidative degradation

5 ml of the standard stock solution was transferred in a 50 ml volumetric flask.1 ml 30% H_2O_2 was added to it and kept for 2 h. It was diluted till 25 ml with water. The solution was finally diluted to 50 ml with mobile phase and 20 µl volume was injected. The same procedure was repeated at 50 °C and 100 °C, but no degradents were observed in the chromatogram.

Degradation by UV light

5 ml of the standard stock solution was transferred in a 50 ml volumetric flask in UV chamber for 24 h then diluted up to the mark with mobile phase to produce 1000 µg/ml and 20 µl was injected but again no degradents were observed.

Thermal degradation

Wet heat: 5 ml of the standard stock solution was transferred to two different 50 ml volumetric flasks and kept on water bath for 3 and 6 h, respectively, at 80 °C. It was diluted up to the mark with mobile phase to produce 1000 µg/ml and 20 µl was injected. Figure 6 represents the chromatogram obtained due to wet heat.

Table 5: Summary of degradation studies

Degradation parameters	Degradants RT	Resolution	% Recovery
Acidic degradation	—	—	99.50
Alkali degradation	—	—	99.29
Oxidative degradation	—	—	99.80
UV light	—	—	99.90
Thermal			
Wet heat	4.16, 9.43	2.2, 3.45	88.0
Dry heat	5.03, 9.43	1.52, 2.96	86.0

Dry heat: 50 mg accurately weighed drug was taken in china dish and kept it in oven for 48 h at 100 °C. The drug was transferred to a 50 ml volumetric flask and diluted approximately 25 ml with water then volume was made up to the mark with mobile phase to produce1000 µg/ml and 20 µl volume was injected. Figure 7 represents the

Table 6: Recovery study of MGL in spiked plasma

Conc. (µg/ml)	Conc. found before Spiking (µg/ml) C1	Conc. of Std. added C_2	Conc. found after Spiking (µg/ml) C_3	% Recovery $(C_3-C_1)*100/C_2$	Mean ± SD	RSD
800	810.43	103.45	880.45	95.87	98.66 ± 3.068	3.110
			913.78	99.99		
			910.34	99.56		
	810.43	207.12	977.34	95.04		
			1012.24	99.34		
			1030.34	101.58		
	810.43	312.75	1085.34	95.33		
			1100.18	97.16		
			1156.39	104.09		

Table 7: Summary of validation parameters

Parameters	Results	
Specificity	Resolution of degradants and excipients with drug peak > 1.5, hence method is specific.	
Linearity	Method shows linearity between 800 and 1200 µg/ml	
Range	Linearity range	800-1200 µg/ml
	Target range	800, 1000, 1200 µg/ml
	Working range	70-1200 µg/ml
	Target concentration	1000 µg/ml
Accuracy (%recovery)	98.338-101.704	
Precision (RSD)	Intraday	0.957
	Interday	1.019
	Repeatability	0.736
LOQ and LOD	70 µg/ml and 20 µg/ml, respectively	

chromatogram obtained due to dry heat.

The overall summary of degradation studies is presented in Table 5.

Estimation of miglitol in spiked plasma

Preparation of sample

Approximately 1.5 ml blood was taken by cutting the marginal ear vein of rabbit and collected in eppendrof tubes with previously added EDTA. This was stored at −20 °C and centrifuged for 2 min at 8000 rpm for 10 min to separate plasma and again stored at 20 °C. 0.2 ml plasma was spiked with 0.1 ml of the standard stock solution and 0.6 ml mobile phase was added. Finally, the components were mixed thoroughly using cyclo mixture and then centrifuged for 5 min at 5000 rpm. The chromatogram obtained after injecting prepared sample is shown in Figure 8.

Recovery study of miglitol in spiked plasma

0.1 ml of 8000 µg/ml drug solution prepared by a standard stock solution and spiked with 0.2 ml plasma and volume was made 1 ml with mobile phase and analyzed. 0.1 ml of this solution was spiked with 0.1, 0.2, and 0.3 ml of 1000 µg/ml previously analyzed drug solution in three separate eppendrof tubes, and volume was made up to 1 ml with mobile phase. The results are shown in Table 6.

CONCLUSION

In the present work, a HPLC method for the estimation of Miglitol in tablet dosage form has been developed. The proposed method is simple, precise, and accurate and do not suffer from any interferences due to common excipients. The degradation studies and results obtained after spiking drug in rabbit plasma showed separation of drug peak and other peaks with a resolution of more than 1.5. The newly developed method can be used in pharmaceutical industry for routine analysis of Miglitol in tablet dosage form. The summary of validation parameters is given in Table 7. The presented study can be further proceeded for the development of the bioanalytical method for the estimation of Miglitol in biological fluids.

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