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Development of validated RP-HPLC method for the estimation of Miglitol in tablet formulations

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ABSTRACT

A rapid and sensitive method for the determination of miglitol in tablet formulations using an internal standard has been developed and validated using C_8 column. The mobile phase consisted of sodium dihydrophosphate : acetonitrile in a ratio of 85:15 at a flow rate of 1ml/min. The detection was carried out at 232nm and the linearity was found to be in the range of 1-11 µg/ml. The retention time for drug and internal standard was found to be 3.40 and 7.30 min respectively. The method was validated for linearity, accuracy, specificity, limit of quantification, limit of detection and stability. The limit of quantification (LOQ) and limit of detection (LOD) for the estimation of miglitol was found to be 900ng/ml and 500ng/ml respectively. The method was validated as per ICH guidelines. Studies proved that about 99.52% of the drug could be recovered indicating high accuracy for the proposed method.

Keywords: Miglitol, RP-HPLC, Method Development and Validation

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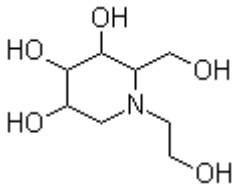
INTRODUCTION

Miglitol(1,5-dideoxy -1,5-[2-hydroxy ethyl] iminol)-D-glucitol is an α –glucicosidase inhibitor(AGI) used as an anti hyperglycemic agent in the treatment of non-insulin dependent diabetes mellitus(NIDDM). Miglitol delays the digestion of ingested carbohydrate, there by resulting in a smaller blood glucose concentration. The anti hyperglycemic action of miglitol results from a reversible inhibition of membrane bound intestinal α –glycosidase which hydrolyzes oligosaccharides and disaccharides to glucose and other monosaccharide in the brush border of small intestine. In diabetic patients the enzyme inhibition resulted in delayed glucose absorption and lowering of postprandial hyperglycemia. Current consensus supports the use of AGI's as monotherapy or adjunct therapy for poorly controlled NIDDM [1-6] .



Miglitol Structure

Miglitol derived from 1-deoxynojirimycin, is the first pseudo monosaccharide AGI, with a structure similar to that of glucose. Recently an assay of miglitol in plasma by LC/MS was reported which had a limitation of low sensitivity[7-8]. The literature survey revealed that no RP-HPLC method was developed for the quantification of miglitol in tablet formulations.

MATERIALS AND METHODS

Instruments

This analytical work performed on shimadzu, LC-10ADVP, series binary gradient pump with SPD-10AVP UV-detector, Phenomenex C8 – column (150×4.6 mm, 5µ particle size) as stationary phase. The samples were weighed on Shimadzu electronic analytical balance (AX-200).

Reagents and Chemicals

Miglitol and Voglibose were gift samples from Torrent Pharmaceutical Ltd. India. Acetonitrile was of HPLC grade and Sodium dihydrogen phosphate of AR grade 99.5 %, pure was purchased from Qualigens. HPLC water was prepared using Milli-Q water purification system procured from Millipore.

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Standard solutions

The stock solution of Miglitol was prepared by accurately weighing 25mg Miglitol of drug, transferring to 25 ml volumetric flask, dissolving in 5 ml of water and diluting it up to the mark with acetonitrile. Appropriate aliquot of this solution was further diluted to 10 ml with acetonitrile to obtain final standard solution of 100µg/ ml of Miglitol. Resultant solution was filtered through Whatman filter paper No.1.

The stock solution of Voglibose was prepared by accurately weighing 25mg voglibose of drug, transferring to 25 ml volumetric flask, dissolving in 5 ml of water and diluting it up to the mark with acetonitrile. Appropriate aliquot of this solution was further diluted to 10 ml with acetonitrile to obtain final standard solution of 50µg/ ml of Voglibose. Resultant solution was filtered through Whatman filter paper No.1.

HPLC Analysis

The liquid chromatographic system consisted of a model Shimadzu SPD 10AVP liquid Chromatography, using a variable wavelength programmable UV/VIS detector operating at 232nm,a LC-10 ADVP pump and a rheodyne injector with 20 µl fixed loop. Separations were performed with Phenomenex C8 reverse phase column with 150 x 4.6 mm i.d and 5µm particle size as stationary phase. Potassium dihydrogen phosphate was weighed (0.96 g) and dissolved in 1000 ml of water, from this solution 850 ml was taken, mixed with 150 ml of acetonitrile and the resultant solution was sonicated for 10 min and used as the mobile phase at a flow rate of 1.0 ml/min.

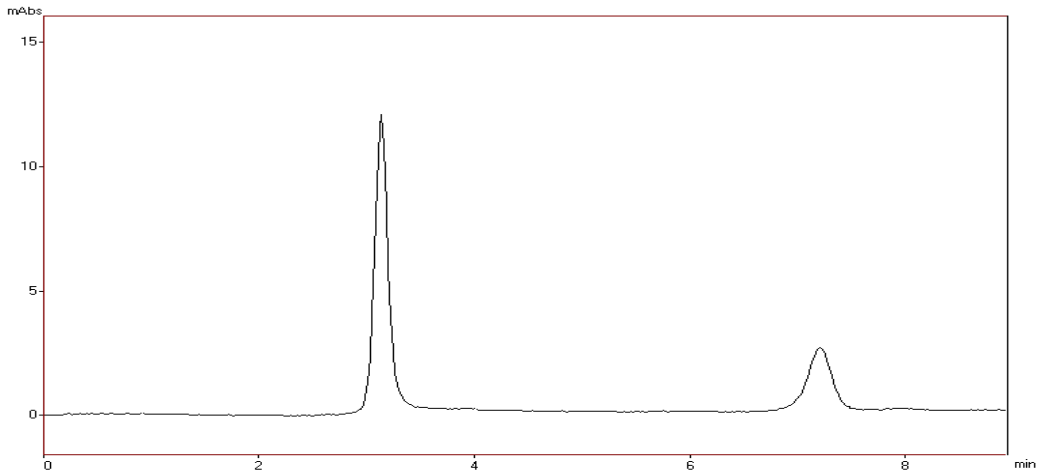

Fig 1. HPLC Chromatogram of Miglitol and voglibose in Mobile Phase

Calibration

Appropriate aliquots of standard Miglitol stock solution (100µg/ml) were taken in different 10 ml volumetric flasks, followed by addition of 1ml of standard Voglibose solution (50 µg/ml) and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 1, 3, 5, 7, 9 and 11 µg/ ml of Miglitol and 10 µg/ ml of Voglibose, respectively. These solutions were injected into chromatographic system and chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of Miglitol was constructed by plotting peak area ratio vs. applied concentration of Miglitol and regression equation was computed. Similarly the sample solution was chromatographed and concentration of Miglitol in tablet samples was found out using regression equation.

Tablet formulations

Twenty tablets were accurately weighed and finely powdered. Tablet powder equivalent to 10 mg of Miglitol was taken in 50 ml of volumetric flask containing acetonitrile (approximately 20 ml) and was shaken occasionally to dissolve the drug and filtered through Whatman filter paper No. 1. The filter paper was washed with more solvent collecting the filtrate. The filtrate volume was adjusted to the mark with the same solvent to obtain concentration of 200µg/ml. The resulting solution was filtered through Whatman filter paper No.1. Appropriate aliquots of this solution was taken in 10 ml of volumetric flask; 1ml of standard Voglibose stock solution was added and finally diluted to 10 ml with mobile phase to obtain final concentration of 1 µg/ml of miglitol and 10 µg/ml of Voglibose, respectively. The resulting solution was again filtered using Whatman filter paper No.1 and then it was sonicated for 10 min.

A reverse phase C8 column equilibrated with mobile phase sodium dihydrogen phosphate: acetonitrile (85:15v/v) was used. Mobile phase flow rate was maintained at 1.0 ml/min and effluents were monitored at 232 nm. The sample was injected using a 20 µl fixed loop, and the total run time was 10 min.

Assay Validation

The accuracy of the method was determined by calculating recovery of Miglitol using standard addition method. Known amount of Miglitol (0, 1, 4, 10 µg/ml) was added to a pre quantified sample solution, and the amount of Miglitol was estimated by measuring the peak area ratios and by plotting the calibration curve.

The intraday and inter day precision studies of Miglitol was carried out at 3 different concentrations (1, 5, 11 µg/ml) in triplicates. The linearity of the method was determined at six concentration levels ranging from 1-11 µg/ml for Miglitol.

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A calibration curve was prepared using concentrations in the range of 0.5-1.0 µg/ml (expected detection limit range). The standard deviation of y-intercept regression line was determined and kept in following equation for the determination of detection limit and quantitation limit. Detection limit = 3.3σ/s; Quantitation limit = 10σ/s, where σ is the standard deviation of y-intercept of regression lines and s is the slope of the calibration curve.

In order to demonstrate the stability of both standard and sample solutions of Miglitol during analysis, both the solutions were analyzed over a period of 24 h at room temperature and then analyzed. UV overlain spectra of both Miglitol and Voglibose showed that both the drugs absorbs appreciably at 232 nm, so 232nm was selected as the detection wavelength(Fig-1).

RESULTS AND DISCUSSION

Optimization of mobile phase was performed based on resolution, asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolution and good symmetrical peaks were obtained with the mobile phase sodium dihydrogen phosphate: acetonitrile (85:15, v/v). The retention time of Miglitol was found to be 3.40 min and that of Voglibose was found to be 7.30 min, respectively. Resolution between Miglitol and Voglibose was found to be 3.8 which indicate good separation of both the compounds. The asymmetric factor for Miglitol was 1.4. The calibration curve for Miglitol was obtained by plotting the peak area ratio versus the concentration of Miglitol over the range of 1-11 µg/ml, and it was found to be linear with r^2= 0.999.The data of regression analysis of the calibration curves are shown in Table 1. Detection limit for Miglitol was 0.5 µg/ml and quantitation limit was 0.9µg/ml, which suggests that a nanogram quantity of both the compounds can be estimated accurately. The results for stability studies revealed that for the solutions, retention time and peak area of Miglitol and internal standard remained almost unchanged and no significant degradation was observed within the indicated period. The recovery of Miglitol was found to be in the range of 99.02.101.68%. The system suitability test parameters are shown in Table 2. The proposed liquid chromatographic method was applied for the determination of Miglitol in tablet formulations (A and B). The result for Miglitol tablet assay was comparable with the corresponding labeled amount (Table 3). The validation parameters are summarized in Table 4.

TABLE 1:Regression Analysis of the Calibration Curves for the Proposed Method

Parameters	Values
Calibration range (µg/ml)	:1- 11µg/ml
Slope	:0.036
Standard deviation of slope	:0.001137
Intercept	:0.00908
Standard deviation of intercept	:0.00572
Correlation coefficient (r)	:0.9990

TABLE 2: System Suitability Test Parameters for Miglitol by the Proposed Method

System suitability parameters	Values
Retention time (min)	: 2.50
Resolution	: 3.80
Tailing factor (asymmetric factor)	: 1.40

TABLE 3: Assay Results of Tablet Formulations Using Proposed Method

Formulations	Labelled Amount (Miglitol)	Amount obtained (Miglitol)[b]	% Recovery
A	25	24.98±0.026	99.49±0.76
B	25	25.02±0.015	101.1±1.15

[b]Mean value±standard deviation of five determinations;

Tablet A: Miglitol (Glenmark Pharmaceuticals Ltd.India)
Tablet B: Miglitol (Torrent Pharmaceuticals Ltd. India).

TABLE 4: Summary of Validation Parameters for the Proposed Method

Parameters	Values
Detection limit (ng/ml)	: 500
Quantitation limit (ng/ml)	: 900
Accuracy (%)	: 99.02-101.68
Precision (RSD[a],%)	
Intraday (n=3)	: 0.20-0.82
Interday (n=3)	: 0.18-1.50
Repeatability (RSD[a], n=3)	: 0.14-0.72

CONCLUSION

This study describes a new HPLC method for the estimation of Miglitol in tablet formulations. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore the proposed method can be used for routine analysis for estimating Miglitol in its tablet formulations.

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REFERENCES

[1] Scheen AJ. Diabet Metab 1998; 24: 311.
[2] Lebovitz HE. Drugs 1992; 44: 21.
[3] Heinz G, Komjati M, Korn A, Waldhausl W. Eur J Clin Pharmacol 1989; 37: 33.
[4] Campbell LK, White JR, Campbell RK. Ann Pharmacother 1996; 30: 1255.
[5] Johnston PS, Coniff RF, Hoogwerf BJ, Santiago JV, Pi-Sunyer FX, Krol A. Diabet Care 1994; 17: 20.
[6] Spengler M, Hansel G, Boehme K. Horm Metab Res Suppl 1992; 26: 50.
[7] Schnack C, Prager RJ, Winkler J, Klauser RM, Schneider BG, Schernthaner G. Diabet Care 1989; 12: 537.
[8] Nirogi RV, Kandikere VN, Shukla M, Mudigonda K, Maurya S, Boosi R, Yerramilli A. Arzneimittelforschung 2006; 56:328.