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Development and validation of RP-HPLC method for quantitative analysis of Miglitol in pure and Pharmaceutical formulations

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ABSTRACT

A reverse phase high performance liquid chromatographic method was performed by using Zodiac C_{18}column (250mmX4.6mmX5µ particle size) with UV detection at 216 nm. An isocratic mobile phase consisting of Acetonitrile:Methanol:Potassium dihydrogen Phosphate50:35:15(v/v/v) at a flow rate of 1ml/min.The retention time for Miglitol was 3.84 min. The method was linear in the concentration range of 40-100 µg/ml of Miglitol with the correlation coefficient of 0.999. The method was validated for linearity,accuracy,precision,limit of detection,limit of quantification,robustness and ruggedness.Recovey of Miglitol was found to be 99% to 102%.The developed reverse phase high performance liquid chromatographic method was simple, sensitive, precise and accurate and the method was found suitable for estimating in tablet dosage form.

Keywords: Miglitol, C_{18} column, Reverse phase, Validation

1. INTRODUCTION

Miglitol (MIG) is chemically 1-(2-hydroxyethyl)-2-(hydroxy methyl) - 3, 4, 5-trihydroxy piperidine an oral anti-diabetic drug[1]. Miglitol is an inhibitor of alpha glucosidases,this retards the digestion and absorption of carbohydrates in the small intestine and hence reduces the increase in blood glucose concentrations after a carbohydrate load [2-3] . In literature,a few methods have been described for the determination of Miglitol [4-10]. In the present study, a new RP-HPLC method was developed which shown high reproducibility and sensitivity. The developed method was validated as per ICH guidelines.

2. MATERIALS AND METHODS

2.1. Standards and chemicals used

Miglitol was provided by Lupin laboratories,Mumbai.All the chemicals Acetonitrile, Methanol, water were HPLC grade, Merck Specialties Private Limited, Mumbai, India. Commercial tablets of Miglitol were purchased from local market .

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2.2. Instrumentation

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Miglitol an isocratic PEAK HPLC instrument with Zodiac C_{18} column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

2.3. Preparation of the mobile phase

Into a 1000ml cleaned volumetric flask, HPLC grade methanol 350ml, acetonitrile 500ml and Potassium dihyogen phosphate 150ml (which are filtered through 0.25mm membrane filters by vacuum filtration) were slowly added , mixed well and sonicated upto 20min. Cool the above solution and pH was adjusted to 5.8 with ortho phosphoric acid.This solution is again sonicated to 10min. Cool the solution to room temperature and use for chromatography method.

2.4. Preparation of Standard drug solutions

100mg of Miglitol was accurately weighed and is dissolved in few ml of the mobile phase and sonicated for few min to dissolve the drug completely. Then it is filtered through 0.2µ pore filter paper and the volume is made up to 100ml with mobile phase to get a concentration of 1mg/ml stock solution. This solution is further diluted with same solvent to obtain required working standard concentrations.

2.5. Sample Preparation

20 commercial tablets of Miglitol (MISOBIT-50mg) were finely powdered and the powder equivalent to 10mg of Miglitol accurately weighed to 50ml volumetric flask and dissolved in few ml of mobile phase. The above solution was subjected to sonication for 15min. After getting clear solution it is filtered through 0.25µ membrane filters and the solution is made up to 50ml with mobile phase resulting in preparation of 10 mg/ml solution. This is further diluted so as to obtain required concentration of Miglitol pharmaceutical dosage form.

2.6. RP-HPLC Method development

Based on nature and solubility characteristics of Miglitol, reverse phase mode of HPLC was selected for chromatography. Among different RP-HPLC stationary phases tried, C_{18} column was found to be optimum.In order to get sharp peak with base line separation from interfering peaks carried out a number of experiments by varying the composition of solvents and mobile phase flow rate. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like methanol, water and acetonitrile with or without different buffers in different combinations were tested as mobile phase. A mixture of Methanol : Aceto nitile : Potassium dihydrogen phosphate (35:50:15) (v/v/v) was proved to be the most suitable of all the combinations, since the chromatographic peak obtained was better defined and resolved and almost free from tailing.The chromatographic conditions for the estimation of Miglitol were discussed in table1.

Table 1. Optimized chromatographic conditions for estimation of Miglitol

Parameter	Condition
Mobile phase	Acetonitrile: Methanol: potassium di hydrogen phosphate (50:35:15) (v/v/v)
Pump mode	Isocratic
pH	5.8
Diluents	Mobile phase
Column	Zodiac C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	216nm
Injection Volume	20 µl
Flow rate	1.0ml/min
Run time	7minutes

3.0 RESULTS AND DISCUSSION

3.1 Analysis of formulation

The sample solution was injected and a chromatogram was recorded. The injections were repeated six times and the peak areas were recorded. The amount of drug present in the pharmaceutical formulation was calculated using standard calibration curve (concentration in µg/ml was taken on X –axis and average peak area on Y –axis). A representative chromatogram has been given in Fig. 1.

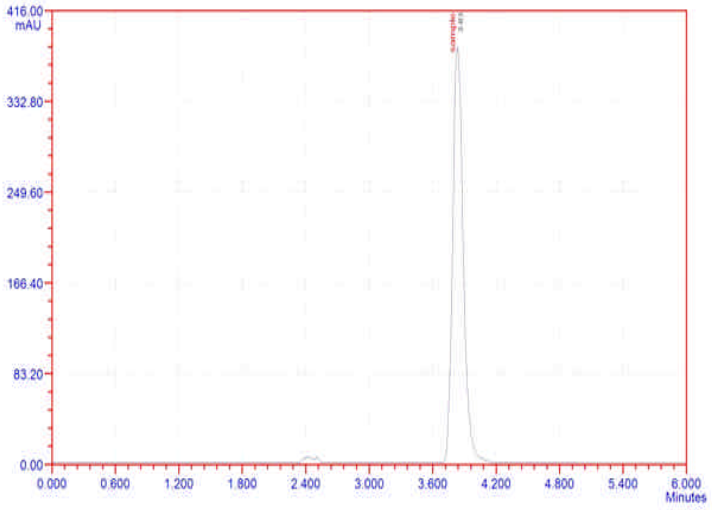

Fig. 1 chromatogram of Miglitol

3.2 VALIDATION OF THE PROPOSED METHOD

As an integral part of analytical method development is validation. The proposed method was validated as per ICH guidelines.

3.2.1. Linearity

It is the ability of the method to elicit test results directly proportional to analyte concentration with in a given range. Linearity was performed by preparing standard solutions of Miglitol at different concentration levels, twenty micro liters of each concentration was injected in into the HPLC system. The peak responses were read at 216nm and the corresponding chromatograms were recorded. Linearity plots of concentration over peak areas was constructed. Linearity results were obtained in the concentration range of 40-100µg/ml .The results were presented in Table 2.

Table- 2 Linearity results of Miglitol

Concentration of Miglitol in µg/ml	Peak area
40	243131
50	302308
60	354857
70	410902
80	462066
90	539611
100	585707

3.2.2. Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as intraday precision, Inter day precision.

Intraday precision

To study the intraday precision, six replicate standard solutions (60µg/ml) of Miglitol were injected. The percent relative standard

deviation (% RSD) was calculated and it was found to be 0.96 which are well with in the acceptable criteria of not more than 2.0.

Interday precision

To study the interday precision, six replicate standard solutions (60ppm) of Miglitol were injected on three consecutive days .The percent relative standard deviation (% RSD) was calculated and it was found to be 0.76 which are well with in the acceptable criteria of not more than 2.0.

32.3. Ruggedness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC(LC2010 A HT),Aglient HPLC .By different operators using different columns of similar type like Hypersil C_{18} Hichron C_{18} . It was observed that there were no marked changes in the chromatograms, which demon started that the RP-HPLC method developed, is ruggedness.

3.2.4. Limit of Detection and Limit of Quantification

A Calibration curve was prepared using concentrations in the range of 40-100 µg/ml (expected detection limit range). The standard deviation of Y-intercepts of regression line was determined and kept in following equation for the determination of Detection limit and Quantitation limit.The results were reported in table 3.

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

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

Where,

σ = the standard deviation of the response.

S = the slope of the calibration curve

Table 3. Limit of Detection and Limit of Quantification for Miglitol

Parameter	Values
Limit of Quantification	1.0µg/ml
Limit of Detection	0.28 µg/ml

3.2.5. Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed sample solution. The standard addition method was performed at 50%, 100% and 150% level of 40ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery was calculated and results are presented in Table 4. Satisfactory recoveries ranging from 99% to 102% were obtained by the proposed method. This indicates that the proposed method was accurate, there is no interference of additives.

Table 4 . Accuracy Recovery Results

Level	Target In µg/ml	Amount of Miglitol spiked (µg/ml)	Total in µg/ml	Amount of Miglitol recovered (µg/ml)	% Recovery
	40	20	60	60.4	100.76
50%	40	20	60	60.3	100.52
	40	20	60	60.7	101.2
	40	40	80	80.1	100.1
100%	40	40	80	79.5	99.4
	40	40	80	78.8	98.5
	40	60	100	1001	101.7
150%	40	60	100	100.1	101.9
	40	60	100	99	99.04

3.2.6. Robustness

The robustness study was performed by slight modification in flow rate of the mobile phase, pH of the buffer and composition of the mobile phase. . It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature.The robustneess results were mentioned in table 5.

Table 5. Robustness results

Condition	Mean area	% Difference
Unaltered	354857	-
Acetonitrile: Methanol : potassium di hydrogen phosphate (45:40:15) (v/v/v)	352867	0.55
Acetonitrile: Methanol : potassium di hydrogen phosphate (49:36:15)(v/v/v)	357829	0.68
Wl change-1 214nm	359242	1.23
Wl change-2 218nm	358092	0.91
pH-1 5.6	356526	0.47
pH-2 6.0	356184	0.37

3.2.7. System suitability parameters:

The system suitability parameters like Tailing factor ,Theoretical plates are discussed in the table 6.

Table 6. System suitability parameters

Drug	Retention time	Tail factor	Theoretical plates
Miglitol	3.84	1.28	4998

4. CONCLUSION

A convenient, rapid, accurate, precise RP-HPLC method has been developed for estimation of Miglitol.The proposed method followed the ICH guidelines.The proposed method can be used for the routine analysis of Miglitol in bulk preparations of the drug and in pharmaceutical dosage forms without interference of excipients.

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