Review Article

Thai Journal of Pharmaceutical Sciences

Development and validation of novel stability-indicating high-performance liquid chromatography method for the determination of miglitol in pharmaceuticals

Kanakapura Basavaiah[1], Nagaraju Rajendraprasad[2]

[1]Department of Chemistry, University of Mysore, Manasagangothri, Mysuru, Karnataka, India, [2]PG Department of Chemistry, JSS College of Arts, Commerce & Science, Mysuru, Karnataka, India

Corresponding Author:
Kanakapura Basavaiah,
Department of Chemistry,
University of Mysore,
Manasagangothri,
Mysuru - 570 006, Karnataka,
India.
E-mai: Kanakapurabasavaiah
@gmailcom

Received: Jan 06, 2017
Accepted: Jan 16, 2017
Published: Feb 23, 2017

Keywords:
Determination,
miglitol,
pharmaceuticals,
reversed-phase high
performance liquid
chromatography,
stability indicating

ABSTRACT

A reversed-phase high-performance liquid chromatographic method for the determination of miglitol (MGL) in pharmaceuticals is presented. The assay was performed on a Phenomenex Luna C18 (150 mm × 4.6 mm; 5 μm particle size) column using phosphate buffer (pH 3.7)-methanol (40:60 v/v) as mobile phase at a flow rate of 1 ml/min and UV detection at 270 nm**.** The column temperature was maintained at 30°C and the retention time of the analyte was 5.58 min. Mean peak area versus concentration plot was found to be linear over 1–750 μg/ml (r = 0.9999), and the calculated limits of detection and quantification were 0.05 and 0.15 μg/ml, respectively. Intraday and interday precisions, expressed as percent relative standard deviation, were ≤0.07% and ≤0.69%, respectively; and the corresponding accuracies, expressed as % relative error, were better than 1%. Robustness and ruggedness of the method were checked. The method was also validated for selectivity by placebo blank and synthetic mixture analyses. The method was applied to determine active ingredient in tablets and results agreed well. The results of stress studies showed that the drug was prone to slight degradation under acid- and oxidant-induced stress conditions but inert to other conditions.

INTRODUCTION

Miglitol (MGL) is chemically called 1,5-Dideoxy-1,5-[(2-hydroxyethyl)imino]-D-glucitol [Figure 1].[1] MGL is an α-glucosidase inhibitor (AGI) and used as an antihyperglycemic agent in the treatment of non-insulin-dependent diabetes mellitus (NIDDM). MGL delays the digestion of ingested carbohydrate, thereby resulting in a smaller blood glucose concentration. The antihyperglycemic action of MGL results from a reversible inhibition of membrane-bound intestinal α-glycosidase which hydrolyses oligosaccharides and disaccharides to glucose and other monosaccharaides in the brush border of small intestine. In diabetic patients, this enzyme inhibition results in delayed glucose absorption and lowering of postprandial hyperglycemia. Current consensus supports the use of AGI as monotherapy or adjunct therapy for poorly controlled NIDDM.[2-7]

On account of its medicinal use, many methods using high-performance liquid chromatography (HPLC),[8] ultra performance liquid chromatography–mass spectrometry (MS),[9] and liquid chromatography–MS/MS[10-14] techniques have been applied to the determination of MGL in body fluids for clinical and pharmacokinetic studies.

Literature reveals that several HPLC methods have been reported for the determination of MGL in pharmaceuticals. A rapid and sensitive method for the determination of MGL in tablets was developed by Chungath *et al.*[15] using a C_{18} column. The mobile phase consisted of NaH_2PO_4 buffer-acetonitrile (85:15) at a flow rate of 1 mL/min with UV detection at 232 nm. Linearity was found in the range 1–11 μg/mL. Using a Zorbax TMS column (250 mm × 4.6 mm; 5 μm) as stationary phase, and KH_2PO_4 and 1-decanesulfonic acid sodium salt buffer, pH 5.5 - acetonitrile (85:15) as mobile phase, Wu and Qin[16] developed a sensitive

method for tablets. The detection wavelength was set at 210 nm. The calibration curve was linear in the range 20–500 pg/mL. Jia and Sun[17] used aminopropyl-bonded silica gel column (250 mm × 4.6 mm; 5 μm) as stationary phase with 0.025 M KH_2PO_4-acetonitrile (30:70) as mobile phase for the determination of MGL in tablets in the concentration range 0.2–0.6 mg/mL. The flow rate was 1 mL/min and the UV detector was set at 210 nm. The method is claimed to be specific and accurate.

A specific method has been described by Chittora *et al.*[8] Chromatographic separation was achieved with Lichrospher ODS column (250 × 4.6 mm; 5 μm) at ambient temperature with 0.05M ammonium acetate as a mobile phase at a flow rate of 0.5 mL/min with UV-detection at 216 nm. The standard curve was linear in the concentration range 800–1200 μg/mL. Shrivastava *et al.*[18] have reported a stability-indicating RP-HPLC method to determine MGL in bulk drug and tablets. Separation and analysis were realized on a prepacked Inertsil amino C_{18} column (150 mm × 4.6 mm) using a mobile phase composed of acetonitrile-Na_2HPO_4 buffer; pH 7.5 (80:20) delivered at a flow rate of 1 mL/min. Detection was performed on an SPD-20A prominence UV-Vis detector at 220 nm. The drug was subjected to various stress conditions and found to undergo slight (<15%) degradation under acidic and oxidative stress conditions. An HPLC-UV method was developed by Dai *et al.*[19] for the determination of MGL in suspected counterfeit products but not applied to dosage forms. In addition, HPLC-UV method was also applied to the determination of MGL in combined dosage forms with metformin.[20-22]

Stress testing is defined as stability testing of drug substances and drug products under conditions exceeding those used for accelerated testing. This study is undertaken to elucidate the intrinsic stability of the drug substance. According to ICH guidelines Q1A (R2), stability testing of a drug substance should be carried under different stress conditions such as hydrolysis, oxidation, heat, and light to evaluate the stability-indicating supremacy of an analytical method used for assay.[23] The standard conditions for photostability testing are described in ICH guidelines Q1B.[24]

Some of the HPLC methods reported so far[15-22] suffer from poor sensitivity and narrow linear ranges of applicability, and except the method of Shrivastava *et al.*,[18] others are not stability indicating. Keeping in mind the advantage of HPLC, a sensitive- and stability-indicating method with a wide linear dynamic range of applicability was strongly felt. By introducing certain modifications in respect of column and mobile phase composition, an HPLC method, which does not require an internal standard was developed for the determination of MGL in pharmaceuticals. The method is applicable over a wide linear dynamic concentration range. The stability-indicating power of the method was established by comparing the chromatograms obtained under optimized conditions before forced degradation with those after degradation through acidic, basic, oxidative, thermal, and photolytic stress conditions.

EXPERIMENTAL

Instrument and Software

Chromatographic analysis was performed with a Waters HPLC system (Waters Corporation, Milford, USA) equipped with Alliances 2695 series low-pressure quaternary gradient pump, a programmable variable wavelength UV detector, and autosampler. Data were collected and processed using Waters Empower 2 software.

Materials and Reagents

Pure sample of MGL (99.8% assay) was kindly supplied by Torrent Pharmaceuticals, Ahmedabad, India, as a gift, and used as such. Two brands of tablets containing MGL, Euglitol-50 (Torrent Pharmaceuticals, Ahmedabad, India) and Miglit-50 (Biocon Ltd. Bangalore, India) were purchased from local commercial sources. HPLC grade methanol was purchased from Merck Ltd., India, potassium dihydrogen-orthophosphate, orthophosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide were purchased from Qualigens Pvt. Ltd., Mumbai, India. Water purified by the Milli-Q system (Millipore, Milford, Massachusetts, USA) was used for mobile phase preparation.

Hydrochloric acid (HCl, 1 M) and hydrogen peroxide (H_2O_2, 5% v/v) were prepared by appropriate dilution of concentrated acid (Specific gravity 1.18) and commercial sample (30%), respectively, with water. Sodium hydroxide (NaOH, 1 M) was prepared by dissolving the required quantity of chemical (S.D. Fine-Chem Ltd, Bengaluru, India) in water.

Mobile Phase Preparation

About 1 L of 20 mM KH_2PO_4 solution was prepared by dissolving 2.8 g of chemical in water, and pH adjusted to 3.7 with 0.1% H_3PO_4 using a pH meter. A 400 mL portion of buffer was mixed with 600 mL of methanol, shaken well and filtered using 0.22 μm nylon membrane filter.

Chromatographic Conditions

Chromatographic analysis was achieved on Phenomenex Luna C_{18} (150 mm × 4.6 mm, 5 μm particle size) column using phosphate buffer (pH 3.7)-methanol (40:60 v/v) as mobile phase. The flow rate was 1.0 mL/min, the detector wavelength was set at 270 nm, and the injection volume was 20 μL. The runtime was 8.0 min and the retention time of the analyte was about 5.58 min. The column temperature was maintained at 30°C.

Standard MGL Solution

A stock standard solution of MGL (1000 μg/mL) was prepared by dissolving an accurately weighed 100 mg of pure drug in mobile phase and diluted to the mark with same solvent in a100 mL volumetric flask.

General Procedures

Procedure for bulk drug

Preparation of calibration curve

Working standard solutions containing 1–750 μg/mL MGL were prepared by appropriate dilution of the stock solution. Aliquots of 20 μL were injected (three injections) and eluted with the mobile phase under the stated chromatographic conditions. The average peak area versus concentration was plotted. Alternatively, the regression equation was derived using mean

peak area-concentration data and the concentration of the unknown was computed from the regression equation.

Procedure for tablets

Tablet powder equivalent to 50 mg MGL was transferred into a 100 mL calibrated flask containing 60 mL of the mobile phase. The mixture was sonicated for 20 min to achieve complete dissolution of MGL; the content was diluted to volume with the same solvent to yield a concentration of 500 μg/mLMGL and filtered through a 0.45 μm nylon membrane filter. The tablet extract was injected on to the HPLC column in five replicates.

Procedure for placebo blank and synthetic mixture

A matrix substance containing starch (15 mg), acacia (20 mg), sodium citrate (25 mg), hydroxyl cellulose (15 mg), magnesium stearate (20 mg), talc (15 mg), and sodium alginate (15 mg) was prepared by mixing all the components into a homogeneous mixture. A 50 mg of the placebo blank was treated with the mobile phase and its solution prepared as described under "procedure for tablets." Placebo blank solution so obtained was injected on to the column.

A synthetic mixture was prepared by adding an accurately weighed 50 mg of pure MGL to 50 mg of placebo mentioned above. The solution of the synthetic mixture equivalent to 500 μg/mL MGL was prepared as described under "procedure for tablets" and the resulting solution was assayed (n = 5) by the proposed method.

Procedure for stress study

All stress testing studies were performed at an initial drug concentration of 500 μg/mL in mobile phase. 5 mL of 1000 μg/mL MGL was placed in three separate 10 mL volumetric flasks and 2 mL of 1M HCl, 1M NaOH, or 5% H_2O_2 were added to the flasks and heated on a water bath at 80°C for 2 h. The flasks were cooled, acid or base neutralized with NaOH or HCl as the case may be. All the solutions were diluted to the mark with the mobile phase and chromatographed. For photolytic degradation, pure drug in solid state was exposed to cool white fluorescent light for 1.2 million lux hours in a photostability chamber. In addition, the drug powder was exposed to dry heat at 105°C for 3 h. Sample in each case was cooled and used to prepare 500 μg/mL solutions in the mobile phase and chromatographed.

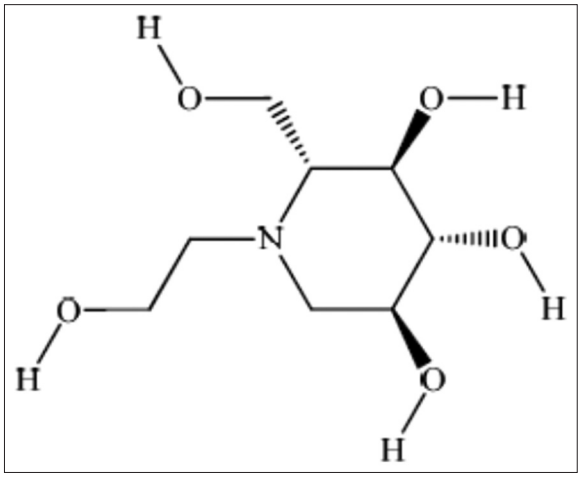

Figure 1: Structure of miglitol

RESULTS AND DISCUSSION

To obtain satisfactory performance characteristics, the method was developed and validated in accordance with the current ICH guidelines.[25] Figure 2 shows a chromatogram obtained under the optimized conditions.

Method Development

Chromatographic conditions were optimized so as to get a well-defined symmetrical peak with shorter retention time and satisfactory system suitability factors.

For performance investigations, five different columns Chromatopack (250 mm × 4.6 mm, 5 μm particle size); Hypersil BDS C_8 (250 mm × 4.0 mm, 5.0 μm particle size); Inertsil ODS 3V (250 mm × 4.0 mm, 5.0 μm particle size); Phenomenex Luna C_{18} (150 mm × 4.6 mm, 5.0 μm particle size); and Zorbax XDB (250 mm × 4.0 mm, 5.0 μm particle size) were tried. Phenomenex Luna C-18 column was chosen ultimately due to shorter retention time for analyte with high sensitivity. The UV detector response of MGL was studied and the best wavelength was found to be 270 nm showing the highest sensitivity. The elution of the analyte using mobile phase of different compositions was investigated. Change in the mobile phase composition with respect to the type of buffer

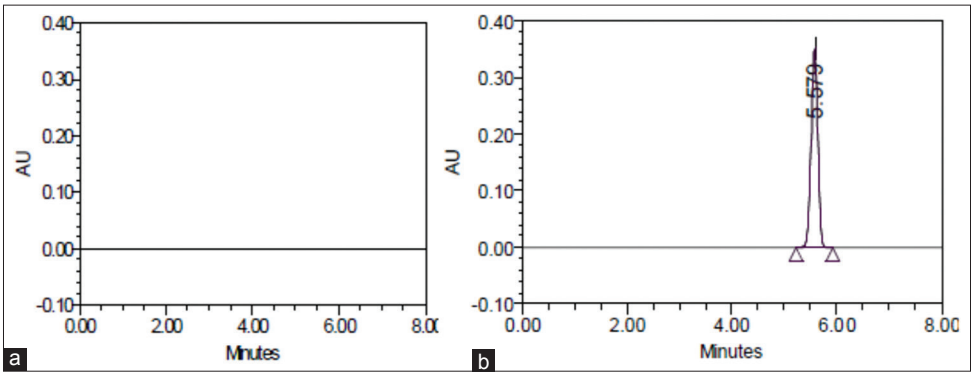

Figure 2: Chromatograms for: (a) Blank (mobile phase). (b) Pure miglitol solution (500 μg/mL)

and its pH, type of organic modifier and its proportion and strength of H_3PO_4, was effected. The effect of flow rate of mobile phase on the retention behavior of analyte was also studied. Phosphate buffer of pH 3.7 containing 0.1% H_3PO_4 was found ideal as the aqueous phase of the mobile phase, since it yielded symmetrical peak. Methanol, as the organic modifier of the mobile phase, gave elegant peak with more sensitivity, compared to acetonitrile. Buffer of pH 3.7 was most appropriate since buffers of higher and lower pH values were found to yield non-symmetrical peaks with lesser number of theoretical plates. A flow rate of 1 mL/min was chosen after several trials considering the symmetry of peak and the retention time. Optimization of these experimental variables is summarized in Table 1.

Method Validation

Linearity

Linearity was studied by recording the chromatograms of standard solutions of different concentrations from 1 to 750 μg/mL and preparing a plot of mean peak area versus concentration and also determining the linearity by least-square regression equation. The calibration plot was linear over the concentration range 1–750 μg/mL (n = 3) [Figure 3] and can be described by the equation:

y=mx+b

where, y is the mean peak area, x is the concentration of MGL in μg/mL, m slope, and b intercept. The slope (m), y-intercept (b), and their standard deviations were evaluated and are presented in Table 2. These results confirm the linear relationship between the peak area and concentration as well as the sensitivity of the method.

Precision and accuracy

Precision and accuracy were determined by replicate analysis during the same day and on 5 different days. Analyses were performed on three levels of analyte within the linear range. The percent relative error which is an indicator of accuracy is ≤1.24% and is reflective of high accuracy. The calculated percent relative standard deviation (%RSD) of <1% is highly satisfactory. The results of this study are compiled in Table 3.

Limits of Detection (LOD) and Quantification (LOQ)

LOD and LOQ were estimated from the signal-to-noise ratio method. Three different concentrations of MGL were subjected and for each the signal-to-noise ratios were calculated. The LOD defined as the lowest concentration that gave a peak area with signal-to-noise ratio between 2 and 3 was found to be 0.05 μg/mL. The lowest concentration that provided a peak area with a signal-to-noise ratio 9.38, which is called LOQ, was found to be 0.15 μg/mL.

Method Robustness

To determine the robustness of the method, small, but deliberate alterations in the chromatographic conditions such as detection wavelength, flow rate, mobile phase composition, and column temperature were made, and the results were compared with those obtained under optimized chromatographic conditions. Low values of %RSD, which are indicative of robustness of the method, are given in Table 4.

Method Ruggedness

The ruggedness of the method was assessed by performing the analysis using three columns of the same type, but of different batches, and also by three analysts with same column. These

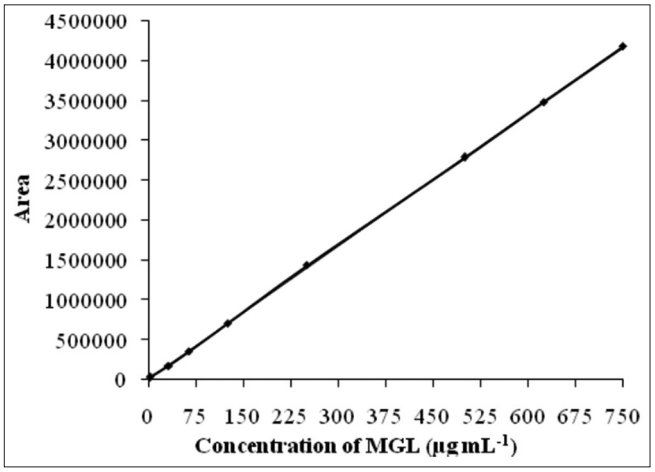

Figure 3: Calibration curve

Table 1: Effect of ratio of organic modifier, pH, and ionic strength of buffer on the number of theoretical plates

Ratio (A/B)[a]	Number of theoretical plates (N)	pH of the medium	Number of theoretical plates (N)	Flow rate, mL/min	Number of theoretical plates (N)
50/50	7584	2.5	5789	0.50	8075
40/60	8963	3.0	5891	0.60	7645
45/55	6692	3.2	6672	0.70	7761
30/70	6263	3.5	7254	0.80	8769
25/75	5718	3.7	8829	0.90	8863
20/80	5536	4.0	8031	1.00	9027
15/85	5018	4.5	7721	1.20	7975

[a]A - phosphate buffer and B - methanol

alterations in the operational conditions did not affect the system suitability parameters as shown by low values of %RSD [Table 5].

Selectivity

Selectivity of the method was evaluated by injecting the mobile phase, placebo blank, pure drug solution, and tablet extract. No peaks were observed for mobile phase and placebo blank and no extra peaks were observed for tablet extracts [Figure 4]. Synthetic mixture when analyzed at 500 μg/mLconcentration level yielded percent recoveries of 98.4% with standard deviation of 1.3% indicating the absence of interference from the tablet excipients.

Table 2: Linearity and regression parameters

Parameter	Value
Linear range (μg/mL)	1–750
Limits of detection (LOD) (μg/mL)	0.05
Limits of quantification (LOQ) (μg/mL)	0.15
Regression equation (y*)	5568.2
Slope (m)	9846.7
Intercept (b)	69.2
Standard deviation of intercept (S_b)	27.9
Standard deviation of slope (S_m)	0.9999
Correlation coefficient (r)	

*y=mx+b, where y is the mean peak area, x concentration in μg/mL, b intercept, m slope

Solution Stability

The drug solution stored at 25°C ± 2°C was injected at different time intervals of 0, 12, and 24 h, and chromatograms were recorded. At the specified time interval, %RSD for the peak area obtained from drug solution was within 0.33% [Table 6]. This shows no significant change in the elution of the peak and its system suitability criteria (retention time, tailing factor, and theoretical plates). The results also confirmed that the standard solution of drug was stable at least for 24 h during the assay performance. Pooled %RSD values for peak area, retention time, theoretical plates, and tailing factor were ≤1%, which are reflective of stability of the drug solution during the study period.

Application to Tablets

The developed method was applied to the determination of MGL in two brands of tablets. The same tablet powder was assayed by published method[15] for comparison. The published

Table 3: Results of accuracy and precision study (n=5)

MGL injected (μg/mL)	Intraday				Interday			
	MGL found (μg/mL)	% RE[a]	% RSD[b]	% RSD[c]	MGL found (μg/mL)	% RE[a]	% RSD[b]	% RSD[c]
250	253.1	1.24	0.63	0.23	251.9	0.76	0.41	0.34
500	496.8	0.64	0.48	0.31	505.7	1.14	0.36	0.69
750	753.5	0.47	0.59	0.67	754.6	0.61	0.54	0.46

[a]Relative error; [b]Relative standard deviation based on peak area; [c]Relative standard deviation based on retention time. MGL: Miglitol

Table 4: Results of method robustness

Condition altered	Modification	Mean Peak area±SD*	% RSD	Mean Rt±SD*	% RSD	Mean Theoretical plates±SD*	% RSD	Mean Tailing factor±SD*	% RSD
Actual	-	2788956±9817	0.35	5.579±0.003	0.054	8969±5.436	0.061	1.119±0.004	0.36
Column temperature	30±2 °C	2769345±8981	0.32	5.572±0.004	0.072	9025±6.192	0.069	1.122±0.003	0.27
Mobile phase composition	(Buffer: methanol)	2794992±8414	0.30	5.581±0.002	0.036	8936±5.112	0.057	1.117±0.005	0.45
Flow rate	1.0 mL/min	2811129±8196	0.29	5.575±0.003	0.054	9019±5.775	0.064	1.115±0.003	0.27
Wavelength	270±1 nm	2782237±9312	0.33	5.583±0.004	0.072	8954±5.861	0.065	1.121±0.004	0.36

*Mean value of three determinations at MGL concentration of 500 μg/mL. MGL: Miglitol, RSD: Relative standard deviation, SD: Standard deviation

Table 5: Results of method ruggedness (n=3)

Variable	Mean Peak area±SD*	%RSD	Mean Rt±SD*	%RSD	Mean Theoretical plates±SD	%RSD	Mean Tailing factor±SD*	%RSD
Analysts (n=3)	2794982±8414	0.30	5.581±0.002	0.036	9019±5.775	0.064	1.122±0.003	0.27
Columns (n=3)	2795095±8454	0.36	5.575±0.003	0.059	9059±4.890	0.87	1.237±0.007	0.55

*Mean value of three determinations at MGL concentration of 500 μg/mL. MGL: Miglitol, RSD: Relative standard deviation, SD: Standard deviation

method consisted of HPLC analysis of the drug in NaH_2PO_4 buffer and acetonitrile (85:15 v/v) system at 232 nm. The results were compared statistically by applying the Student's test for accuracy and F-test for precision. As shown by the results compiled in Table 7, the proposed method and the reference method do not differ significantly with respect to accuracy and precision.

Accuracy by Recovery Test

To further assess the accuracy and reliability of the method, recovery test through standard addition procedure was performed. To the preanalyzed tablet powder, pure MGL was added at three levels and the total was determined by the proposed method. Each test was triplicated. When the test was performed on 50 mg tablets, the percent recovery of pure MGL was in the range of 99.27–101.5 with standard deviation values of 0.67–1.14. The results indicated that the method is very accurate and the common excipients found in tablet preparations did not interfere. The results are compiled in Table 8.

Results of Forced Degradation Study

MGL was found to degrade slightly in acidic and oxidative stress conditions [Table 9]. The drug was found to be more stable under basic, thermal, and photolytic stress conditions. Figure 5 shows the chromatograms for sample of MGL after degradation.

CONCLUSIONS

A new HPLC method for the determination of MGL in pharmaceutical substances was developed and validated as per the ICH guidelines and this is the first stability-indicating method ever developed for MGL. Experimental conditions were optimized for satisfactory system suitability parameters. The method allows the determination of the drug over a wide concentration range (1–750 μg/mL) compared with any reported HPLC methods, thus widening the scope of the applicability. With an LOD value of 0.003 μg/mL (3 ng/mL), the method offers one of the sensitive means of assaying the drug in pharmaceuticals. The method has fulfilled the long felt need for a stability-indicating assay of MGL, which is now mandatory as per the ICH guidelines. High sensitivity, selectivity, and shorter run time make the method perfectly suitable for routine analysis.

ACKNOWLEDGMENT

Authors thank Torrent Pharmaceuticals, Ahmedabad, India, for gifting pure miglitol sample. The author (Basavaiah) is

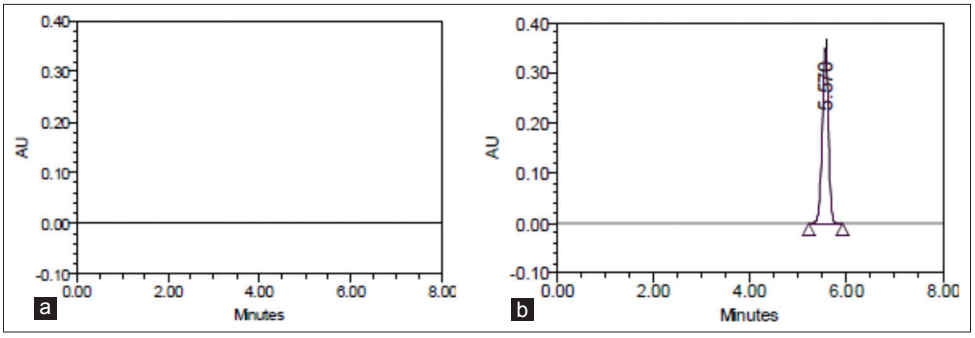

Figure 4: Chromatograms obtained for (a) placebo blank and (b) tablet extract (500 μg/mL miglitol)

Table 6: Results of solution stability

Time, h	Mean Peak area±SD*	Pooled %RSD	Mean Rt±SD*	Pooled %RSD	Mean Theoretical plates±SD	Pooled %RSD	Mean Tailing factor±SD*	Pooled %RSD
0	2784999±8319	0.38	5.575±0.003	0.081	9019±5.775	0.081	1.118±0.003	0.45
12	2792397±9118		5.572±0.004		9025±6.192		1.122±0.005	
24	2812795±8217		5.583±0.004		8954±5.861		1.121±0.004	

*Mean value of three determinations for MGL concentration of 500 µg/mL at each time interval. MGL: Miglitol, RSD: Relative standard deviation, SD: Standard deviation

Table 7: Results of determination of MGL in tablet and statistical comparison with the published method

Formulation brand name	Nominal amount (mg)	MGL found* (%)±SD		t value	F value
		Reference method	Proposed method		
Euglitol	50	99.72±1.04	100.5±0.63	1.47	2.73
Miglit	50	99.54±0.96	101.1±0.85	2.72	1.28

*Mean value of five determinations. Tabulated t-value at 95% confidence level is 2.77; Tabulated F-value at 95% confidence level is 6.39. MGL: Miglitol, SD: Standard deviation

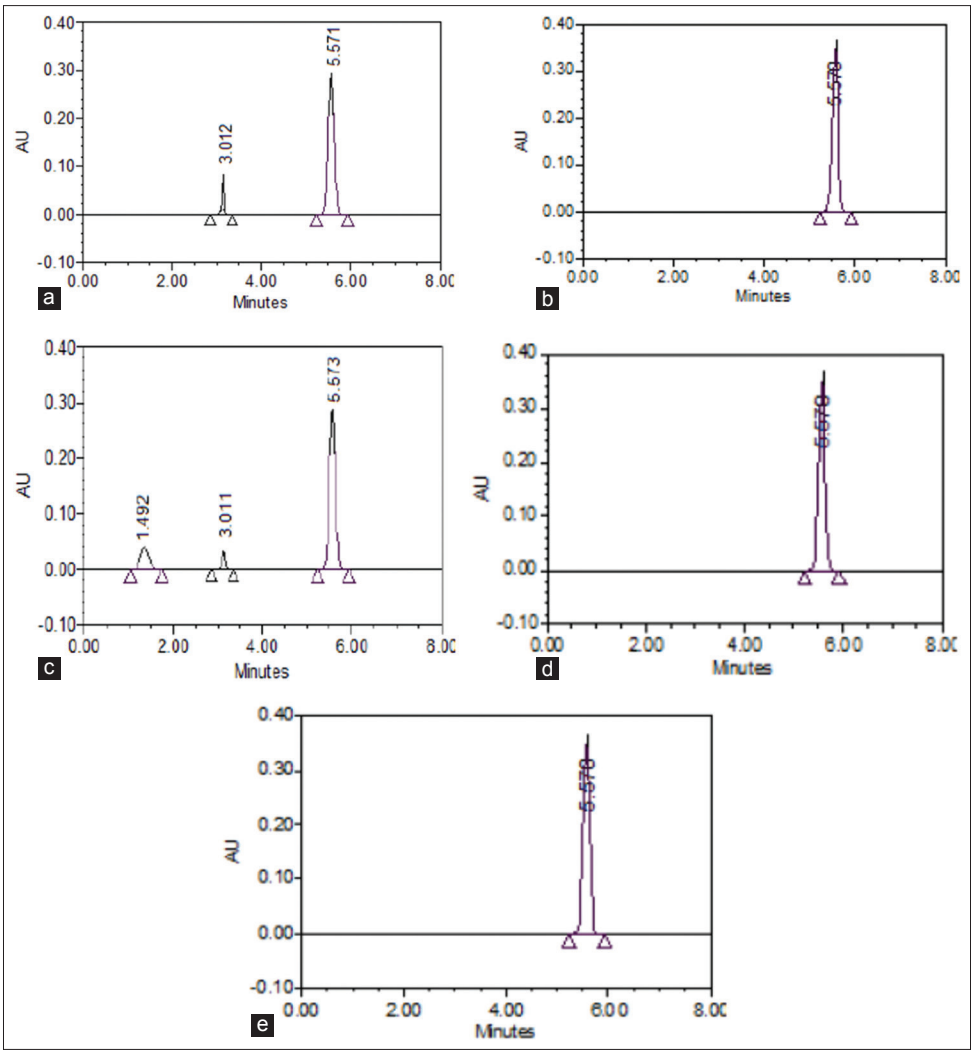

Figure 5: Chromatograms of miglitol (500 μg/mL) after forced degradation: (a) Acid degradation; (b) base degradation; (c) peroxide degradation; (d) photolytic degradation; and (e) thermal degradation

Table 8: Results of recovery study by standard addition method

Tablet studied	MGL in tablet (µg/mL)	Pure MGL added (µg/mL)	Total found (µg/mL)	Pure MGL recovered* (%NTG±SD)
Euglitol	201.0	100	303.4	100.8±1.14
	210.0	200	407.0	101.5±0.92
	201.0	300	497.3	99.27±1.06
Miglit	202.2	100	306.4	101.4±1.02
	202.2	200	404.2	100.5±0.81
	202.2	300	499.0	99.36±0.67

*Mean value of three determinations. MGL: Miglitol, SD: Standard deviation

Table 9: Results of degradation study

Degradation condition	% Degradation
Acid hydrolysis	18.3
Base hydrolysis	No degradation
Oxidation	19.5
Thermal (105°C, 3 h)	No degradation
Photolytic (1.2 million lux hours)	No degradation

indebted to UGC, New Delhi, India, for financial assistance in the form of BSR Faculty fellowship.

REFERENCES

1. Merck & Co., Inc., Whitehouse Station. The Merck Index. New Jersey, USA: Merck & Co., Inc., Whitehouse Station; 2003.
2. Scheen AJ. Clinical efficacy of acarbose in diabetes mellitus: A critical review of controlled trials. Diabetes Metab 1998;24:311-20.

3. Lebovitz HE. Oral antidiabetic agents. The emergence of alpha-glucosidase inhibitors. Drugs 1992;44 Suppl 3:21-8.
4. Heinz G, Komjati M, Korn A, Waldhausl W. Reduction of postprandial blood glucose by the alpha-glucosidase inhibitor Miglitol (BAY m 1099) in Type II diabetes. Eur J Clin Pharm 1989;37:33-6.
5. Campbell LK, White JR, Campbell RK. Acarbose: Its role in the treatment of diabetes mellitus. Ann Pharm 1996;30:1255-62.
6. Johnston PS, Coniff RF, Hoogwerf BJ, Santiago JV, Pi-Sunyer FX, Krol A, *et al.* Effects of the carbohydrase inhibitor miglitol in sulfonylurea-treated NIDDM patients. Diabetes Care 1994;17:20-9.
7. Spengler M, Hänsel G, Boehme K. Efficacy of 6 months monotherapy with glucosidase inhibitor acarbose versus Sulphonylurea Glibenclamide on metabolic control of dietary treated Type II diabetics (NIDDM). Horm Metab Res Suppl 1992;26:50-1.
8. Chittora NC, Shrivastava A, Jain A. New RP-HPLC method of miglitol in tablet dosage form including forced degradation studies and estimation in spiked rabbit plasma. J Young Pharm 2009;1:364-70.
9. Jain R, Lukram O, Dwivedi A. Ultra-performance liquid chromatography electrospray ionization-tandem mass spectrometry method for the estimation of miglitol in human plasma using metformin as the internal standard. Drug Test Anal 2011;3:255-62.
10. Li H, Zheng G, Luo J, Shentu J. Pharmacokinetics and relative bioavailability of miglitol split tablets in healthy volunteers. Her Med 2007;26:1299-300.
11. Li J, Ye Y, Zheng G, Shentu J, Huang Y. LC-MS/MS determination of miglitol in human plasma. Yaowu Fenxi Zazhi 2007;27:1193-6.
12. Nirogi RV, Kandikere VN, Shukla M, Mudigonda K, Maurya S, Boosi R, *et al.* Liquid chromatographic tandem mass spectrometry method for the quantification of miglitol in human plasma. Arzneimittelforschung 2006;56:328-36.
13. Ding CG, Zhou Z, Ge QH, Zhi XJ, Ma LL. Simultaneous determination of metformin and glipizide in human plasma by liquid chromatography-tandem mass spectrometry. Biomed Chromatogr 2007;21:132-8.
14. Li X, Wang Y, Wang J, Fawcett JP, Zhao L, Gu J, *et al.* Determination of miglitol in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2007;21:247-51.
15. Chungath TT, Reddy YP, Devanna N. Development of validated RP-HPLC method for the estimation of miglitol in tablet formulations. Res J Pharm Biol Chem Sci 2011;2:807-13.
16. Wu L, Qin J. Determination of the content of miglitol tablets by HPLC. Zhongguo Xiandai Ying Yong Yaoxue 2012;29:359-61.
17. Rong-Sheng J, Jian-He S. Determination of miglitol tablets by HPLC. J Pharm Res 2013;1:27-8.
18. Shrivastava B, Baghel US, Sahu M. Stability-indicating RP-HPLC method for estimation of miglitol in bulk and tablets. Indian J Pharm Sci 2010;72:781-4.
19. Dai XM, An N, Wu JM, Li HY, Zhang QM. Development and validation of HPLC-UV-MS method for the control of four anti-diabetic drugs in suspected counterfeit products. Yao Xue Xue Bao 2010;45:347-52.
20. Saritha S, Priya PV, Devi NA, Sharma JV. Simultaneous estimation and validation of miglitol and metformin by RP-HPLC method in pharmaceutical dosage form. Indo Am J Pharm Res 2013;3:9304-9.
21. Bhoomaiah B, Shree AJ. Development and validation of RP-HPLC method for simultaneous determination of metformin and miglitol in bulk and pharmaceutical formulation. Int J Pharm Pharm Sci 2014;6:135-41.
22. Patel SR, Kabra PV, Kimbahune RV, Markad R, Nargund LV. Development and validation of analytical method for quantitative estimation of miglitol and metformin in combined dosage form. J Appl Pharm Sci 2012;2:227-9.
23. ICH Harmonized Tripartite Guideline Q1A (R2). Stability Testing of New Drug Substances and Products. Geneva: ICH Harmonized Tripartite Guideline Q1A (R2); 2003.
24. International Conference on Hormonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Stability Testing: Photostability Testing of New Drug Substances and Products, Q1B; 1996.
25. International Conference on Hormonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1). London, UK: Complementary Guideline on Methodology dated 06 November 1996, Incorporated In November 2005.