Research Article

Drug Testing and Analysis

Received: 25 July 2010 Revised: 8 October 2010 Accepted: 8 October 2010 Published online in Wiley Online Library: 29 December 2010

(www.drugtestinganalysis.com) DOI 10.1002/dta.226

Ultra-performance liquid chromatography electrospray ionization–tandem mass spectrometry method for the estimation of miglitol in human plasma using metformin as the internal standard

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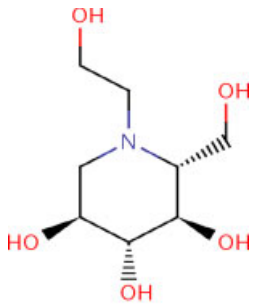
A rapid and sensitive method for the determination of miglitol in human plasma was developed using ultra-performance liquid chromatographic separation with tandem mass spectrometry detection. The preparation of samples required a deproteinization step with acetonitrile. Chromatography was performed on a 5 μ (50 mm × 4.6 mm, ID.) C18 inertsil column, with the mobile phase consisting of acetonitrile 2 mM and ammonium acetate (pH 3.5) with formic acid. Detection was performed using an Applied Biosystems Sciex API 2000 mass spectrometer set at unit resolution in the multiple reaction monitoring mode. Electrospray ionization was used for ion production. The mean recovery of miglitol was 88.9%, with the lower limit of quantification set at 150 ng/mL. Linearity was established for concentrations in the range of 150–4000 ng/mL, with a coefficient of determination (r^2) of 0.9981. This assay method makes use of the increased sensitivity and selectivity of tandem mass spectrometric detection, resulting in high-throughput analysis of miglitol for bioequivalence studies.

Keywords: miglitol; LC-MS/MS; human plasma; UPLC; metformin.

Introduction

Miglitol [A] is an oral alpha-glucosidase inhibitor, used in the management of noninsulin-dependent diabetes mellitus. It is a desoxynojirimycin derivative and is chemically known as 3,4,5-piperdinetriol, 1-(2-hydroxyethyl)-2-(hydroxymethyl)-[2R-(2α, 3β, 4α, 5β)].[1] It is a white to pale-yellow powder with a molecular weight of 207.2. Miglitol is soluble in water and has a pK of 5.9. Its empirical formula is $C_8H_{17}NO_5$.[2] It delays the digestion of ingested carbohydrates, resulting in a smaller rise in the blood glucose concentration following meals. As a consequence of plasma glucose reduction, miglitol reduces the levels of glycosylated hemoglobin in patients with type II (non insulin-dependent) diabetes mellitus. Systemic nonenzymatic protein-glycosylated hemoglobin is a function of average blood glucose concentration over time.

Few methods have been reported in the literature for monitoring the plasma levels of miglitol. The technique used in these includes mass spectrometric detection, with atmospheric pressure chemical ionisation APCI as the source of ionization.[3] Liquid chromatography tandem mass spectrometry (LC-MS/MS) with liquid–liquid extraction has also been reported. However, the liquid–liquid extraction technique was found to be a time-consuming and less-sensitive procedure. LC-MS/MS with electrospray ionization (ESI) methods have also been reported.[4] Although these assays are sufficiently sensitive, the limitation with them is that the method requires a sensitive instrument for detection. Therefore it was necessary to develop a simple, specific, rapid and sensitive, analytical method for the quantification of miglitol in human plasma.



[A]

This paper describes the development and validation that can be used for the determination of maglitol in human plasma using an ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method. The method used API2000 as a detection mode, with UPLC as the solvent delivery system. The limit of quantification (LOQ) for this method was 150 ng/mL, with a run time of 2.0 min, using metformin as an internal standard (IS).[5,6] The novelty of the method was that it used a less-sensitive instrument than the one reported with a similar extraction technique,[3,4] but with a shorter run time.

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Materials and Methods

Chemicals and reagents

The chemicals and reagents used were acetonitrile and methanol (high-performance liquid chromatography [HPLC] grade; JT Baker, Germany). A MilliQ water (Millipore Co., Bedford, MA, USA) purification system was used to obtain purified water for the HPLC analysis. Miglitol (99.4%) and metformin HCl (99.6%) were obtained from Biocon (Bangalore, India) and Micro Lab (Bangalore, India), respectively. Ethylene diamine tetra acetic acid (EDTA) plasma of healthy volunteers was obtained from West Coast Blood Bank (Mumbai, India).

Instrumentation

Chromatography was performed at ambient temperature, with the mobile phase consisting of acetonitrile and 2 mM ammonium acetate (pH 3.5, 80 : 20 V/V). An Inertsil ODS C18, 5 µ (50 mm × 4.6 mm, ID.) column obtained from GL Science (Tokyo, Japan) was used for the chromatographic separation at a flow rate of 0.3 mL/min. The mobile phase was delivered by the UPLC solvent delivery system and the sample was injected by a UPLC auto sampler (Waters® Corporation, Milford, MA, USA).

Drug monitoring and quantification were performed using an API-2000 MDS Sciex triple quadropole mass spectrometer (Applied Biosystem, Foster City, CA, USA) equipped with an ESI source and run by Analyst 1.4.1 software. The mass spectrometer was operated in the positive ion mode with the turbo ionspray heater set at 350 °C. Sample analyses for miglitol were performed using transitions of m/z 208.1–146.1, with a dwell time of 200 ms. Mass transitions for the IS were m/z 130.1–71, with a dwell time of 150 ms. The spray voltage was set at +3 kV, the collision gas (nitrogen) was set at 30 V, the CAD gas was set at 4 V and the electron multiplier was set at 2900.

Preparation of standards and quality control samples

Stock solutions of miglitol and IS were prepared in methanol at a free base concentration of 1000 µg/mL. The secondary and working standard solutions were prepared from the above stock solutions by diluting with water and methanol in a ratio of 50 : 50 (v/v). These diluted working standards were used to prepare the calibration curve and the quality control (QC) samples.

Blank human plasma was screened prior to spiking to ensure that it was free of endogenous interference at the retention times of both miglitol and IS. The blank plasma was spiked with an appropriate amount of miglitol to prepare an 8-point standard curve of miglitol. The calibration curve ranged from 150 to 4000 ng/mL. QC samples for miglitol were prepared at three concentration levels of 400, 1800 and 3000 ng/mL as low-quality control (LQC), medium-quality control (MQC) and high-quality control (HQC), respectively, using procedures similar to those used for the preparation of standard solutions from the stock solutions.

Each standard in the calibration curve, blank plasma and QCs was purified using protein precipitation. One milliliter of acetonitrile was added to test tubes containing 0.5 mL of plasma and these were vortexed for 2 min each, followed by centrifugation at 15 000 rpm for 5 min. The resulting supernatant was transferred to a clean auto sampler vial. Ten microlitres of this supernatant was injected into the system.

Data treatment

The linearity of miglitol method determination in human plasma was tested for the concentration range 150–4000 ng/mL. The standard curves were calculated from the peak area ratio (p.a.r.) of miglitol/metformin using linear regression $y = ax + b$ with $1/x^2$ weighing. Miglitol concentrations (ng/mL) for QCs in a batch were calculated by interpolating the p.a.r. from the corresponding standard curves.

The measured p.a.r. of the QC samples were converted into concentrations using Equation 1:

$$\text{Miglitol concentration} = (\text{p.a.r. (miglitol/IS)} - b)/a \quad (1)$$

where a: slope of the corresponding standard curve; b: intercept of the corresponding standard curve.

The concentrations were reported in nanogram per milliliter plasma for miglitol.

Validation

The selectivity, sensitivity, linearity, precision, accuracy, recovery, stability and dilution integrity of the method have been validated according to the US Food and Drug Administration guidance for the validation of Bioanalytical methods.[7]

Validation of selectivity was performed by analyzing plasma samples from different sources (or donors) to test for interference at the retention times of both miglitol and the IS, metformin. The sensitivity was determined by analyzing five replicates of blank human plasma and plasma spiked with the analyte at the lowest level of the calibration curve. Replicate ($n = 4$) analyses of the QC samples at the LOQ extracted from the sample batch were performed to determine the inter-run and intra-run accuracies. The inter-run precision and accuracy of the calibration standards was assessed using the four calibration curves used for assay validation.

Accuracy, defined as the percent relative error (%RE), was calculated using the formula:

$$\%RE = (E - T)(100/T) \quad (2)$$

where, E is the experimentally determined concentration and T is the theoretical concentration.

Assay precision is calculated by using the formula:

$$\%RSD = (SD/M)(100) \quad (3)$$

where, M is the mean of the experimentally determined concentrations and SD is the standard deviation of M.

The extraction efficiencies of miglitol and metformin were determined by comparing the peak area of the extracted analytes with the peak area of the nonextracted standards. The extraction efficiency value can be calculated as follows:

$$\text{Extraction efficiency (\%)} = C/D \times 100 \quad (4)$$

where, C is the peak area of the extracted standard and D is the peak area of the unextracted standard.

Dilution integrity with acceptable precision and accuracy was performed to extend the upper concentration limits. Five replicates each at concentrations twice the highest concentration were prepared and diluted to two-fold and four-fold with blank plasma. These were then subject to processing.

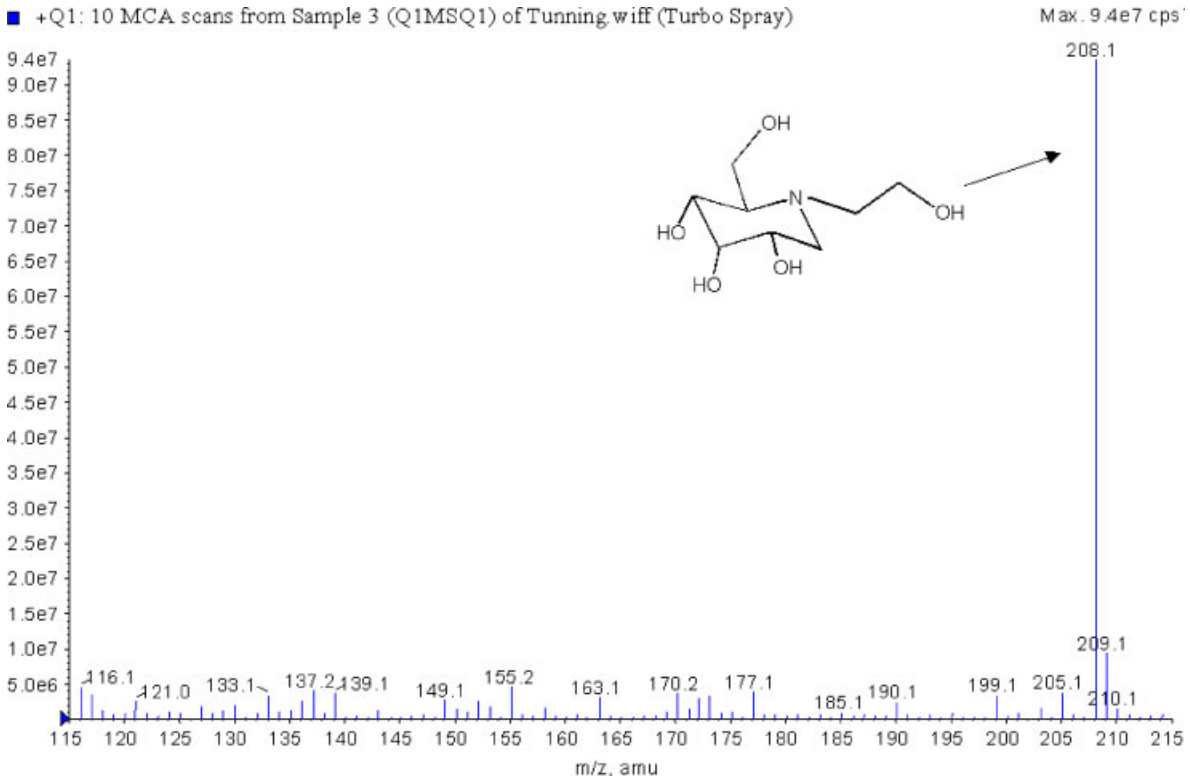

Figure 1. Full-scan spectra of miglitol m/z 208.1.

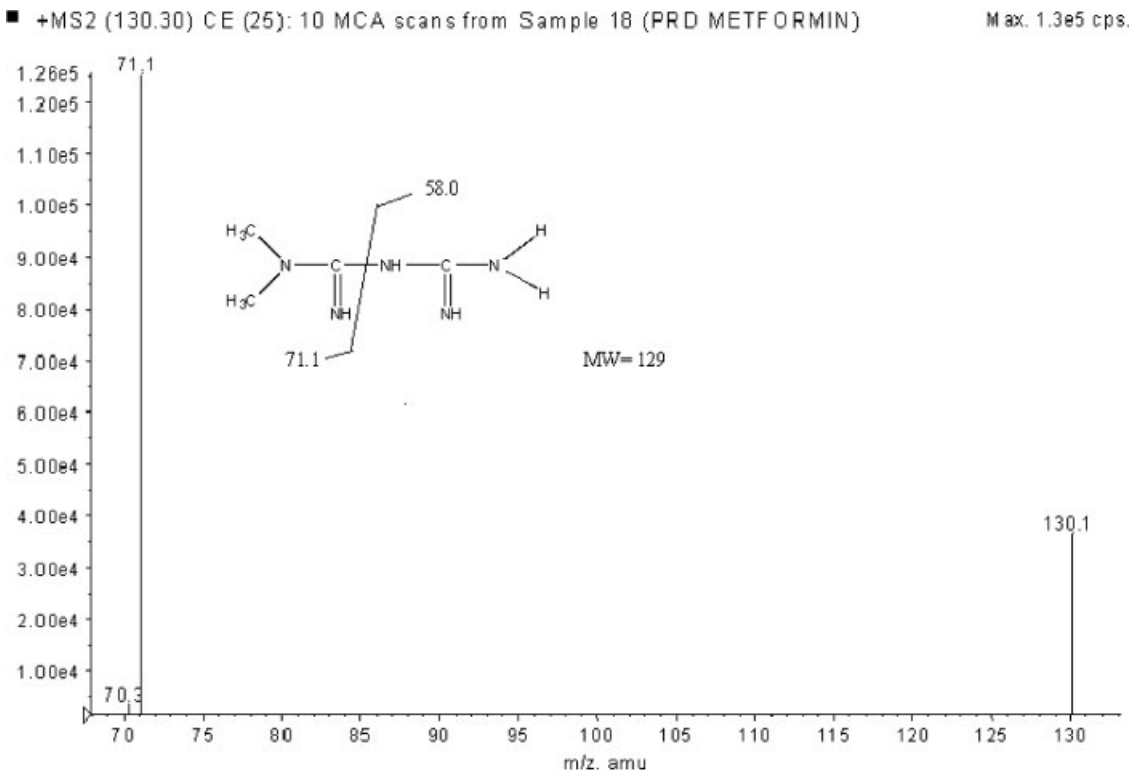

Figure 2. Product ion spectra of metformin.

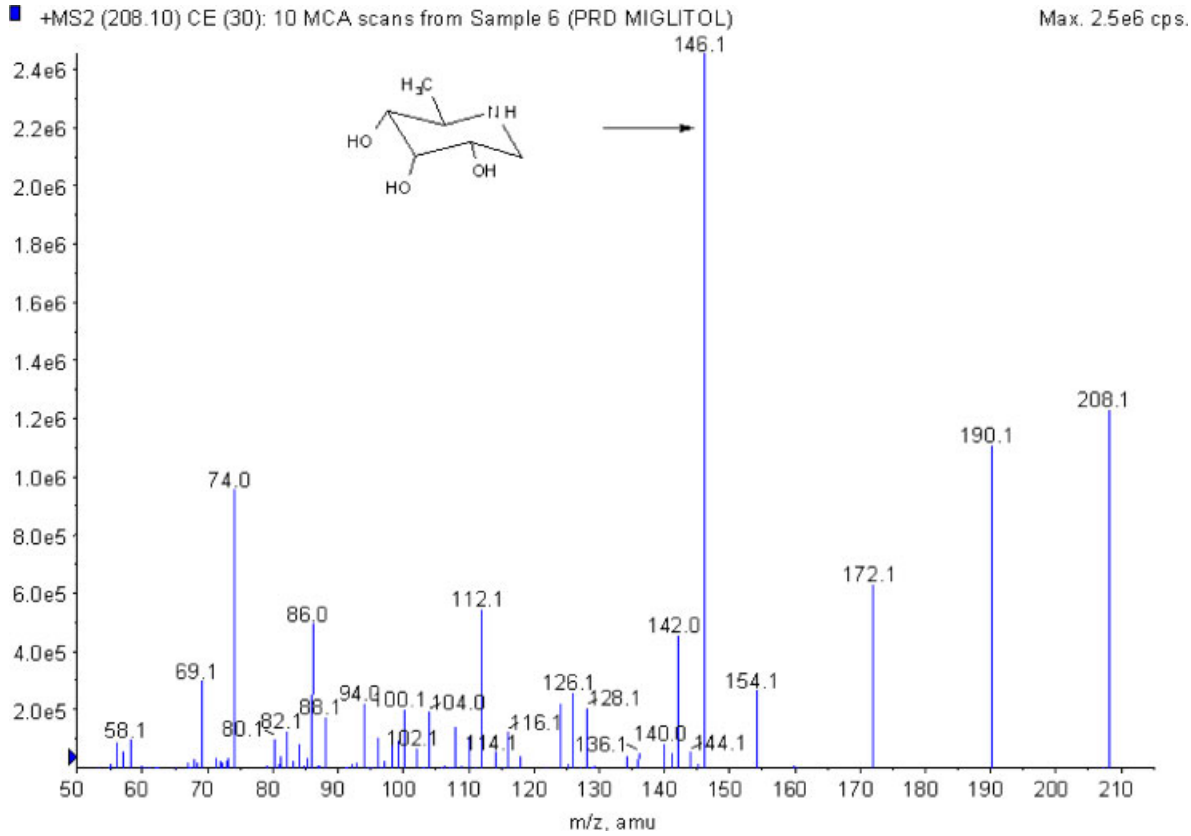

Figure 3. Product ion spectra of miglitol.

Stability of the processed sample was evaluated by comparing the extracted plasma samples that were injected immediately (time 0) with the samples that were re-injected after keeping them in the auto sampler at 5 °C for 24.0 h. Stability of the spiked human plasma, stored at room temperature (bench-top stability), was evaluated for 6 h, and this was compared with the stability of the freshly prepared extracted samples. Freeze-thaw stability was evaluated by comparing the stability of the samples that had been frozen and thawed three times with the stability of freshly spiked QC samples. Long-term stability of the spiked human plasma stored at −20 °C was evaluated by analyzing the MQC and HQC samples that were stored at −20 °C for 60 days together with the freshly spiked calibration standards and QC samples. All stability evaluations were based on back-calculated concentrations. The analytes were considered stable if the deviation of the mean test responses were within 15% of the freshly prepared or comparison samples.

Results and Discussion

Method development

To develop a rapid, sensitive and simple assay method for the extraction and quantification of miglitol during method development, different options were evaluated to optimize detection and separation and selectivity.

Miglitol and the IS gave protonated precursor molecular ions (M+H) in the MS mode. The major ions observed were *m/z* 208.1 for miglitol (Figure 1) and *m/z* 130.1 for the IS (Figure 2). The most intense product ions observed in the MS-MS mode were *m/z* 146.1 for miglitol and *m/z* 71 for the IS. The corresponding product ion spectra of IS and miglitol are shown in Figures 2 and 3, respectively. ESI and atmospheric pressure chemical ionization were evaluated to obtain a better response from the analytes. It was found that the best signal was achieved with the ESI-positive ion mode.

Sample clean-up procedure for highly water-soluble compounds with a higher potential of matrix effect is a challenging task. Metformin was selected as the IS because it is commercially available and also because it has hydrophilic properties similar to miglitol. Both the drug and the IS, with acetonitrile precipitation, give the same extraction efficiency. Therefore, metformin was found to be a suitable IS to be compared with the product under investigation. Further optimization of the chromatography conditions increased the signal of the analytes with desired LOQ for pharmacokinetic determination. A mobile phase containing 2 mM ammonium acetate (pH 3.5) buffer in combination with acetonitrile resulted in an improved signal. Use of a short Inertsil ODS C18 (50 mm × 4.6 mm ID., 5 µ) column resulted in reduced flow rate and reduced run time, as low as 2.0 min. The resultant signal along with optimized chromatography using UPLC and detection parameters enabled the elimination of the matrix effect and higher flow rate without compromising the sensitivity, which further resulted in reducing the processing and analysis times.

Method validation

Selectivity

Mass spectrometric selectivity was enhanced by using predominant product ions for each compound. Mass transition ion pairs of 208.1 → 146.1 and 130.1 → 71 were selected for miglitol and metformin, respectively.[5,6] Predominant product ions of

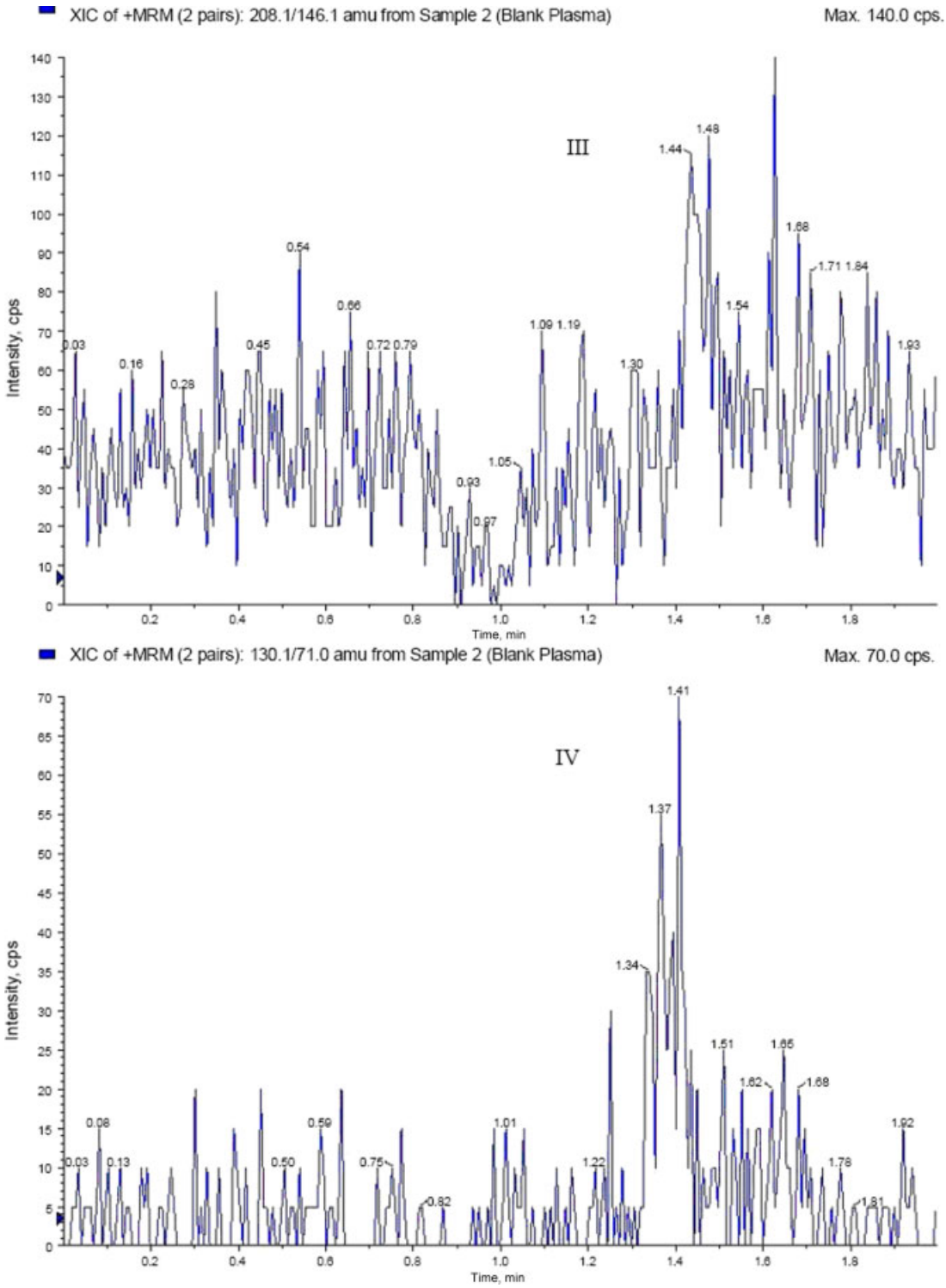

Figure 4. Representative chromatogram of the extracted blank plasma.

m/z 146.1 and *m/z* 71 were specific for miglitol and metformin, respectively.

Absence of endogenous interfering peaks in six different lots of extracted blank plasma at the retention times for miglitol and metformin established the chromatographic selectivity of the method. A representative chromatogram of the extracted blank plasma is presented in Figure 4.

Matrix effect

Matrix effect was determined at three concentration levels (LQC, MQC and HQC) using six plasma lots that passed the selectivity criteria. Samples were processed in triplicate to ensure that concentration was independent of variability in matrix due to its physiological nature. The results showed that the method was free from any matrix interferences. This was evaluated by

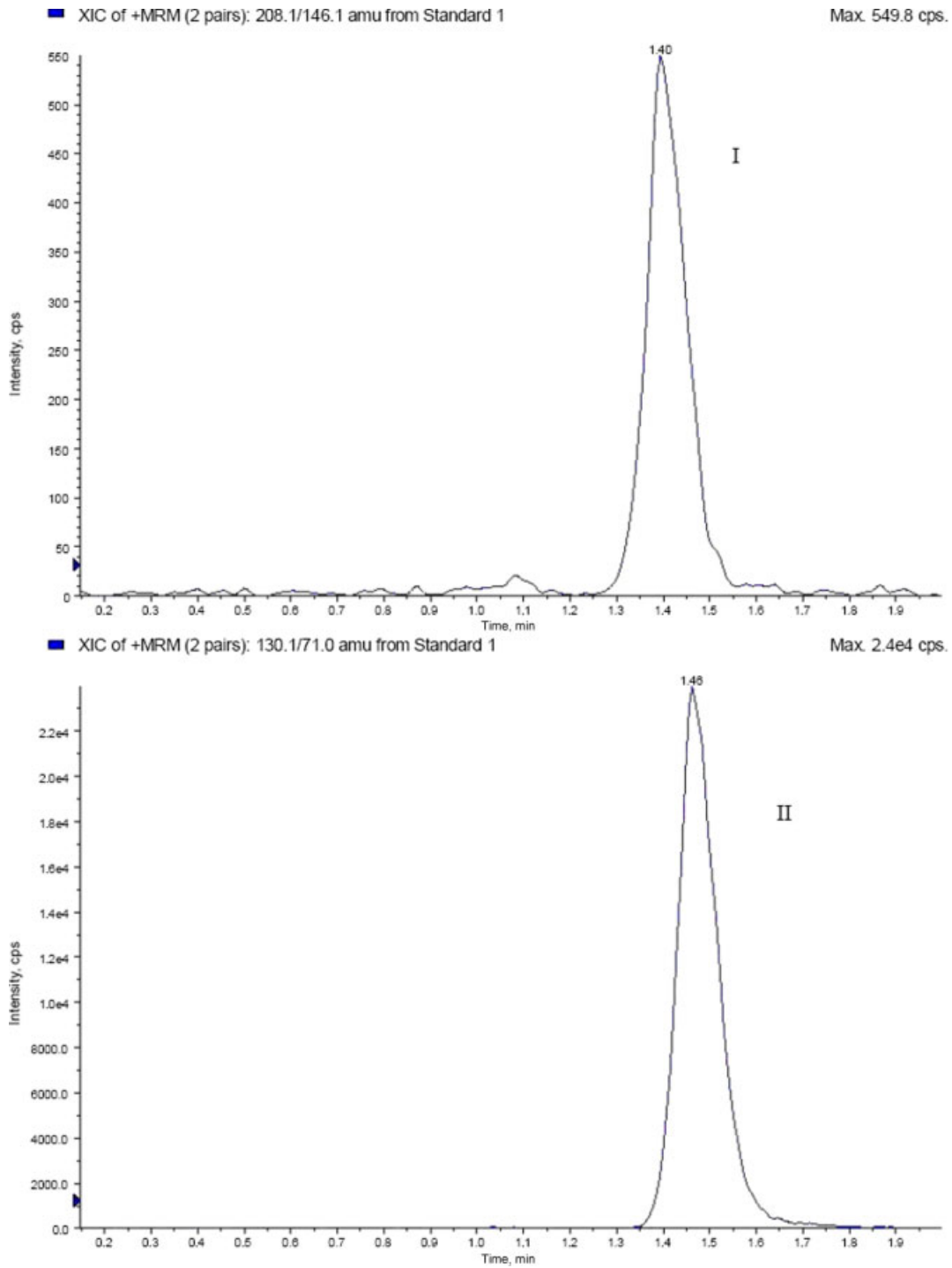

Figure 5. Representative chromatograms of the extracted standard 1 containing 150 ng/mL miglitol and the IS.

comparing with freshly prepared unextracted QC samples. The percentage coefficient of variance of all three compounds was within the acceptance acceptance range of ≤15%.

Linearity

For miglitol, the p.a.r. of the calibration standards were proportional to the concentration of the analytes in each assay over the nominal concentration range of 150–4000 ng/mL. Representative chromatograms of the extracted standard 1 containing 150 ng/mL miglitol and the IS are shown in Figure 5. The calibration curves appeared linear and were well described by least squares lines. The slopes, intercepts and correlation coefficients are presented in Table 1.

Sensitivity (LOQ)

LOQ is defined as the lowest concentration of the calibration standard yielding an accuracy of ±20% and a precision of 20%. The LOQ for miglitol was 150 ng/mL. These data are tabulated

Table 1. Summary of calibration curve parameters for Miglitol

Calibration curve	Slope	Intercept	Correlation coefficient
1	0.000343	−0.015	0.9991
2	0.000397	−0.024	0.9966
3	0.000613	−0.0124	0.9976
4	0.000574	−0.0145	0.9991

Table 2. Intra-run accuracy and precision ($n = 4$) of Miglitol in human plasma

Analyte	Concentration (ng/mL)	Mean (ng/mL)	%RSD	%RE
Miglitol	150	136.75	3.3	−8.83
	400	397.9	6.4	−0.53
	1800	1899.1	3.1	5.51
	3000	3058.9	8.6	1.96

in Table 2. The intra-run precision of the LOQ plasma samples containing miglitol was 3.3% whereas the mean intra-run accuracy of the LOQ plasma samples containing miglitol was 8.83%.

Precision and accuracy

Results of the inter-run precision and accuracy for miglitol plasma calibration standards are summarized in Table 3. The inter-run precision and accuracy for the calibration standards were 13.8% and 1.88%, respectively. The results for intra-run precision and accuracy for miglitol in the plasma QC samples are summarized in Table 2. The intra-run precision and accuracy were 8.6% and 8.83%, respectively. The results for inter-run ($n = 5$) precision and accuracy for miglitol plasma QC samples are summarized in Table 4. The inter-run precision for miglitol was 6.8% whereas the inter-run accuracy for miglitol was 6.33%.

Recovery

For recovery determination of miglitol, five replicates at the LQC, MQC and HQC concentrations were prepared. The mean recovery for miglitol was determined to be 88.9%, with a precision of 4.04%, while the mean recovery for metformin was 91.96%.

Dilution integrity

For miglitol, the upper concentration limits can be extended to 6000 ng/mL by a two-fold or four-fold dilution with human plasma blank, with a precision of 3.87% and an accuracy of 7.96%.

Stability

Results of the stability studies are presented in Table 5. Bench-top stability, process stability and freeze-thaw stability of miglitol in plasma were investigated by analyzing the QC samples in replicates ($n = 5$) at the LQC and HQC levels. Results for process stability indicated that the difference in the back-calculated concentration from time 0 h to time 24 h was $\leq$5.10%, which allowed us to conclude that the processed samples were stable for at least 24 h at 5 °C in the auto sampler. Results for bench-top stability allowed

Table 3. Inter-run accuracy and precision of plasma calibration standards ($n = 4$) for Miglitol

Spiked concentration (ng/mL)	Mean calculated concentration (ng/mL)	%RSD	%RE
150	148.8	13.8	−0.80
250	252.2	10.3	0.88
500	509.4	8.8	1.88
800	778.4	1.5	−2.70
1200	1232.1	5.3	2.67
1600	1571.2	4.2	−1.80
2000	1991.5	2.0	−0.43
4000	4016.6	1.4	0.41

Table 4. Inter-run accuracy and precision ($n = 5$) of Miglitol in human plasma

Analyte	n	Spiked concentration (ng/mL)	Mean calculated concentration (ng/mL)		%RE
Miglitol	5	150	140.5	2.9	−6.33
	5	400	398	4.8	−0.5
	5	1800	1854.8	6.8	3.04
	5	3000	2927	5.1	−2.43

us to conclude that miglitol is stable for at least 6 h at room temperature in plasma samples. Results of freeze-thaw stability indicated that the repeated freezing and thawing (three cycles) did not affect the stability of miglitol. Long-term stability analysis of miglitol in plasma at −20 °C was performed at the LQC and HQC levels, and it was found to be stable for at least 60 days at −20 °C.

Application of method

The validated method has been successfully used to analyze samples obtained after the administration of a single dose of 50 mg miglitol tablets to healthy volunteers participating in bioavailability studies. The plasma concentration of all the volunteers at times 0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 24.00 and 36.00 h of test and reference was determined after the analysis using UPLC-MS/MS The maximum mean concentrations of the test and reference products were 1182.7 ± 38.61 and 1283.3 ± 42.2 ng/mL, occurring at 2.25 ± 0.35 h and 2.11 ± 0.04 h. The mean elimination half-life was 2.12 ± 0.35 h and the mean area under the plasma concentration–time curve was 5365 ± 261 ng.h/mL and 5117 ± 128 ng.h/mL. Figure 6 shows the mean plasma concentration–time profile of 12 volunteers.

Conclusion

The procedure was successfully applied for the determination of miglitol in human plasma without any interference from additives and endogenous substances. It is a simple and accurate procedure, requiring inexpensive instruments, which can be used for rapid and reliable pharmacokinetic and bioavailability analyses in healthy volunteers.

Table 5. Stability sample results for Miglitol

Stability	*n*	Spiked concentration (ng/mL)	Mean calculated comparison sample concentration (ng/mL)	Mean calculated stability sample concentration (ng/mL)	Mean %changes
Process[a]	6	400	390.14	370.23	−5.10
	6	3000	3120.43	2820.91	−9.60
Bench-top[b]	6	400	386.1	365.7	−5.28
	6	3000	2890.31	2832.5	−2.00
Freeze-thaw[c]	6	400	459.12	483.2	5.24
	6	3000	3345.01	3744	11.93
Long-term[d]	6	400	369	399.1	8.16
	6	3000	3122.2	3460.1	10.82

Where, [a] : after 24 h in the auto sampler at 5 °C,
[b] : after 6 h at room temperature,
[c] : after three freeze-thaw cycles at −20 °C,
[d] : at −20 °C for 60 days.

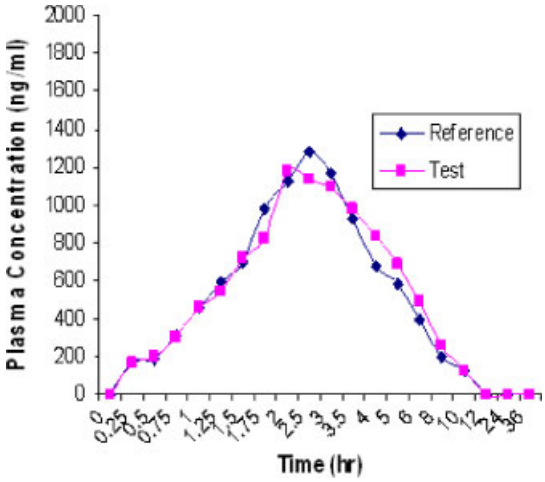

Figure 6. Mean plasma concentration–time profile from 12 volunteers receiving 50 mg miglitol as test and reference.

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