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Development, Validation, and Application for Simultaneous Assay of Metformin and Sitagliptin in Human Plasma by Liquid Chromatography–Tandem Mass Spectrometry

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ABSTRACT

Background: The objective of this study was to develop a liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for the determination of metformin and sitagliptin in human plasma and to apply this method for bioequivalence study.

Methods: An LC–MS/MS method was developed, validated, and applied to the quantification of metformin and sitagliptin in human plasma. Phenformin was used as an internal standard. LC–MS/MS with electrospray ionization (ESI) in positive ion mode, performed under the multiple reaction monitoring (MRM) mode, was used for analysis of the analytes.

Results: The method was developed and validated with respect to selectivity and carryover, as well as intra- and inter-day accuracy and precision, according to the United States Food and Drug Administration guidance. Analytes were extracted from human plasma by a protein precipitation technique using methanol after acidification with formic acid. The mean extraction recovery for the analytes was between 70% and 99.95%, and matrix effects had only a minor influence on precision.

Conclusion: The validated method was applied for a clinical bioequivalence study to evaluate the in vivo bioequivalence of a product containing 500 mg metformin hydrochloride and 50 mg sitagliptin and the Janumet XR® 50/500 mg commercial product. Fourteen healthy subjects participated in this randomized, two-period, two-treatment, open label, crossover-design study. Standard pharmacokinetic parameters were calculated to compare the test product to the commercially available Janumet XR® 50/500 mg reference product.

Keywords: bioequivalence; Human plasma; LC-MS/MS; Metformin; sitagliptin

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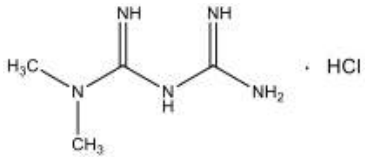
INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration (hyperglycemia) caused by insulin deficiency and it is often combined with insulin resistance. Non-insulin dependent diabetes mellitus patients form a heterogeneous group with a mild form of diabetes that occurs predominantly in adults. Drugs used to treat type 2 diabetes include sitagliptin (SG) and metformin hydrochloride (MH).

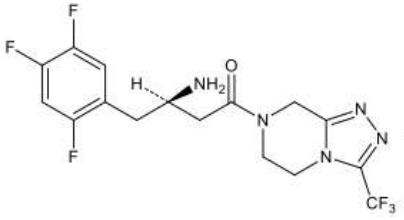
SG is a DPP-IV inhibitor used as an oral hypoglycemic agent,[1] while MH is typically used alone in the form of a biguanide anti-hyperglycemic agent for treating noninsulin-dependent diabetes mellitus.[2] The usual dosage of MH is 250–500 mg 3–4 times daily, up to a 2.5 g/day. The absolute bioavailability of MH is 50–60%, and it has a biological half-life of 6.2 hrs. A frequent dosing schedule can lead to unfavorable GI side effects; therefore, its high daily dose requirement makes its use uncommon. A need therefore exists for formulation of extended release MH tablets to prolong its duration of action and to reduce the total dose required.

The combination of a DPP-4 inhibitor like SG with MH allows a broad and complementary spectrum of anti-diabetic actions. This combination does not increase the risk of hypoglycemia, does not promote weight gain, and does not cause adverse effects caused by various other oral anti diabetic combinations. Both drugs have a complementary and possibly additive effect on glycemic control and reduced glycosylated hemoglobin.[3] This combination is available on the market as Janumet XR® 50/500 mg containing 500 mg MH and 50 mg SG. The chemical structures of MH and SG are shown in **Figure 1**.

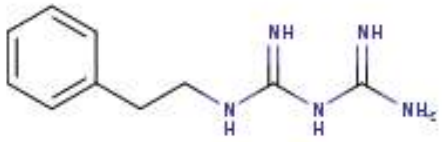
Only a few studies have reported using LC-MS/MS for the analysis of MH and SG in human plasma.[4-6] Our goal was to develop an easy and rapid LC-MS/MS method for the determination of MH and SG in human plasma and to apply this method to evaluate bioequivalence of a test product in comparison with Janumet XR® 50/500 mg reference product.



MH



SG



Phenformin

Figure 1. Chemical structures of metformin hydrochloride (MH), sitagliptin (SG), and phenformin (Used as an internal standard)

METHODS

Chemicals and reagents

The following reference standard was obtained from the Institute of Drug Quality Control Ho Chi Minh City, Vietnam: metformin hydrochloride (MH) (92.20%). Phenformin, used as an internal standard (IS) (95.00%), and sitagliptin (SG) (95.00%) were purchased from Sigma Aldrich. LCMS-grade acetonitrile, HPLC-grade methanol were obtained from J.T. Baker (USA). LCMS-grade ammonium formate and methyl tert-butyl ether (MTBE) were supplied by Fisher Scientific. Formic acid was of analytical grade and obtained from Merck. Blank human plasma sources were supplied by Blood Transfusion Hematology Can Tho City, Vietnam and stored below -20°C prior to use.

Preparation of stock solutions, standards, and controls

Stock solutions of 2000 µg/mL (for MH and the IS) or 200 µg/mL (for SG) were prepared at 50× the required concentrations in individual glass vials. Nine calibration standards were prepared in blank human plasma at the following concentrations: 5, 10, 50, 200, 800, 1600, 2000, 2800, and 3200 ng/mL for MH and 1, 2, 10, 25, 100, 200, 400, 600, and 800 ng/mL for SG. The concentrations of the low, medium, and high-quality controls (QCs) in blank human plasma were 15, 1600, and 2400 ng/mL for MH and 3, 400, and 600 ng/mL for SG. The QCs were prepared separately from the calibration standards. Fresh calibration standards and QCs were prepared on each day of analysis during the validation and subject sample runs. A stock of the IS solution at 2000 ng/mL was prepared by dissolving an appropriate amount of phenformin in methanol. On each day of analysis, an aliquot of the IS stock solution was diluted in methanol to obtain the IS working solution (100 ng/mL). Stock solutions of the analytes and the IS were stored at -20 ± 2°C, while calibration standards and quality control samples in plasma were kept below -20°C.

Sample treatment

Prior to analysis, all frozen samples (subject samples, calibration standards, and QCs) were thawed and allowed to equilibrate at room temperature. A 1 mL volume of the spiked plasma sample was pipetted into a glass tube, and 100 µL IS working solution was added. The sample was acidified with 200 µL 0.1% formic acid (v/v), and combined with 800 µL methanol by vortexing for 45 seconds. After centrifugation of the sample at 6,000 x g for 10 min, the supernatant phase was filtered through a 0.22 µm membrane filter.

LC-MS/MS conditions

An Acquity H-Class System coupled to a Xevo triple quadrupole mass spectrometer was used for reverse-phase liquid chromatography. The system consisted of an autosampler, two pumps, a column oven, and a controller. The autosampler and oven temperature were maintained at 5°C and 40°C, respectively. Analytes and the internal standard were separated by high performance liquid chromatography (HPLC) on a Gemini® C18 column (250×4 mm; 5 µm). An isocratic mobile phase composed of 0.1% formic acid and methanol (45:55, v/v) was delivered at a flow rate of 1 mL/min. The sample injection volume was 10 µL. The total chromatographic run time was 4.0 min. The MS analysis was operated in positive ionization mode utilizing electrospray ionization (ESI). The interface voltage was set to 40 V, desolvation line temperature was 500°C, nebulizer gas flow rate was 1000 L/h, and dwell time per transition was 0.02 s. The multiple reaction monitoring (MRM) transitions for each analyte and IS, as well as their respective optimum MS parameters (including voltage potential [Q1, Q3] and collision energy [CE]), are summarized in **Table 1**. Peak areas were used for quantification. Data acquisition and processing were performed using a LabSolutions software for the LCMS-8040 system.

Table 1. Tandem mass-spectrometer main parameters.

Analytes	MRM transitions m/z	Q1 (V)	CE (kV)	Q3 (eV)	Acquisition time (min)
MH	130 → 71	40	3.5	20	1.20
SG	408 → 235	40	3.5	18	1.29
Phenformin	206 → 105	40	3.5	18	1.42

Method validation

The method was validated according to United States Food and Drug Administration (FDA) bioanalytical method validation guidelines.[7]

Selectivity: Six individual blank plasma samples were analyzed to check for interfering peaks at the retention time of each analyte. Selectivity was deemed acceptable if the blank response was less than 20% of the lower limit of quantitation (LLOQ) response for each analyte and less than 5% of the IS response.

Carryover

Carryover into injected blank plasma, immediately following injection of the upper limit of quantification (ULOQ), was assessed. Carryover was deemed acceptable if it was less than 20% of the LLOQ for an analyte and less than 5% of the IS response.

Calibration standard curve

The plasma samples with a series of known concentrations, prepared as described above, were analyzed in three separate runs, and the data were subjected to linear regression analysis

for known concentrations of each analyte against the area ratios of the corresponding peaks and the IS peaks. The regression coefficient, slope, and *y*-intercept of the resulting linear calibration functions were determined.

Accuracy and precision

Three concentrations for each analyte (low, medium, and high QCs) were analyzed in five replicates per run. Three runs were carried out, and each run was conducted on a separate day. Intra-day (n = 5) and inter-day (n = 15) accuracy and precision were determined. Accuracy was deemed acceptable if the back-calculated concentration was within 15% of the nominal concentration for each QC. Precision was deemed acceptable if the coefficient of variation (CV) did not exceed 15% for each QC.

Matrix effect

Plasma samples from six individuals were tested at high and low QC concentrations. The matrix effects for each analyte and for the IS were determined separately in each sample by determining the ratio of the peak area for the post-extraction spiked plasma to the peak area for spiked methanol.

Recovery

Three series of six samples, with concentrations from high, middle, and low regions of the standard curve, were prepared separately and analyzed. The ratio of the recorded peak area to the peak area resulting from the direct injection of a methanol solution of the analyte at the same concentration was then determined as a percentage.

Stability

Freeze–thaw stability: Low and high QC plasma samples were kept at -20°C for 24 hours and thawed unassisted at room temperature. The cycles were repeated 3 times and the samples were then analyzed.

Short-term temperature stability: Low and high QC plasma samples were stored at room temperature for 6 hours before analysis.

Long-term stability: Low and high QC plasma samples were stored at -20°C and analyzed daily for 30 days.

Post-preparative stability: The autosampler steadiness was examined by repeated analysis of the extracted low and high QC samples maintained under the autosampler conditions (20°C) for 24 hours.

Application to human samples

Bioequivalence study design

An open label, randomized, two-treatment, two-period, two-sequence, single-dose, crossover design was used for the assessment of pharmacokinetics and the bioequivalence of a 500 mg/50 mg MH/SG test formulation (University preparation) and a reference formulation (commercial Janumet XR® 50/500 mg tablets, manufactured by Merck, Sharpe, and Dohme Corp., Whitehouse Station, NJ). The study was conducted in accordance with the Declaration of Helsinki and the rules of good clinical trial practice following Circulars No. 29/2018/TT-BYT on "Regulations for Clinical Trials" and No. 03/2012/TT-BYT on "Guidelines for Clinical Trials" of the Vietnam Ministry of Health. The study protocol was approved by the Ethics Committee of the National Institute of Drug Quality Control.

Fourteen healthy adult subjects were selected for this study, based on each subject's medical history, physical examination, vital signs (blood pressure, temperature, heart frequency), electrocardiogram, and standard laboratory test results (blood cell count, biochemical profile, hepatitis B and C, HIV, urinalysis); a quick urine pregnancy test was also give to female subjects. The demographic data of these subjects were age 20–28 years (mean 22.8±1.71) years, height 148–179 cm (mean 161.9±8.50 cm), weight 44–76 kg (mean 55.4±8.82 kg), and body mass index 18.1–26.6 kg/cm^2 (mean 21.0±2.12 kg/cm^2). Twenty subjects (55.6%) were male and sixteen subjects (44.4%) were female. The subjects had no history of drug or alcohol abuse or hypersensitivity to the study drugs, and none had taken any medication within 2 weeks before the study dosing. Alcohol, cigarettes, and tobacco, as well as beverages containing caffeine, were forbidden for 48 hours before and during the study. All subjects provided written informed consent prior to study enrolment, and all were allowed to terminate their participation in the study at any time, without restrictions. Standard meals were served to the subjects at four and ten hours after drug administration.

Subjects were randomized to one of the two treatment sequences (Test-Reference or Reference-Test formulations) prior to study drug administration. Each subject received both formulations using a balanced crossover design, according to a randomization schedule. Both test and reference formulations were administered as a single oral dose of 50 mg SG and 500 mg MH after an overnight fast of at least 10 hours. A washout period of 14 days was inserted between the two treatments. Each period was 7 days. During the study, subjects were confined to the test center in the evening before the start of the study procedure on day 1 and day 15. Subjects were confined for 24 hours in the test center after taking the study drugs. At the end of each period, the subjects returned home and understood that they were to avoid vigorous exercise throughout the duration of treatment.

Blood samples were collected into dipotassium EDTA tubes through a catheter inserted in the antecubital vein prior to dosing (time 0) and again at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 24, 36, and 48 h after the dose in each period. The samples were immediately centrifuged at 6,000 x g for 10 min. Plasma was separated into two aliquots (approximately 1.2 mL each), transferred to labelled 1.5 mL polypropylene tubes, and immediately frozen and stored at -20°C until analysis.

Pharmacokinetic analysis

The pharmacokinetic parameters for MH and SG were determined using Equiv Test PK software. The C_{max} and T_{max} were read directly from the experimental data. The elimination rate constant (K_e) was obtained from the least-squared fitted terminal log-linear portion of the plasma concentration-time profile. The elimination half-life ($t_{1/2}$) was calculated as $0.693/K_e$. The total area under the plasma concentration curve, from time zero to the last measurable concentration (AUC_{0-t}), was calculated using the trapezoi-dal rule-extrapolation method. The extrapolated parameter AUC_{0-inf} (AUC from time zero to infinity) was calculated by adding the value of C_t/K_e to the calculated AUC_{0-t}, where C_t is the last observed concentration higher than the LLOQ.

RESULTS AND DISCUSSION

Optimization of the LC-MS/MS conditions

During the method development, electrospray ionization was operated in the positive ionization mode. The Q1 MS full scan spectra for MH, SG, and the phenformin IS predominantly

contained protonated precursor $[M+H]^+$ ions at *m/z* 130.1, 407.9, and 206.0, respectively.

The most abundant product ion in Q3 MS spectra for MH, SG, and the IS was observed at 71.1; 235.1, and 105.1, respectively. **Figure 2** shows the Q3 MS spectra *m/z* of the analytes and IS. The MS/MS parameters were systematically optimized for each analyte and the IS to obtain a consistent and adequate response. A dwell time of 0.02 s was sufficient.

The chromatographic conditions were investigated by considering the column type, the mobile phase components, the pH and strength of the buffer, and the flow rate. We tested changes in these parameters to obtain a symmetric peak shape, a short run time, and minimum matrix interference and solvent consumption. Based on the outcome of the various trials, the Gemini® C18 (250 × 4 mm; 5 µm) column was selected. The mobile phase consisting of 0.1% formic acid and methanol (45:55, v/v) at a flow rate of 1.0 mL/min ensured separation of MH, SG, and the IS at retention times of 1.20 min, 1.29 min, and 1.42 min, respectively, for a total run time of 4.0 min. **Figure 3** shows the chromatograms for blank plasma and analytes at the LLOQ level with the IS. The blank plasma chromatogram was obviously clean and contained no endogenous interfering peaks at the retention times of the analytes or the IS.

Extraction procedure

Protein precipitation (PP) and liquid-liquid extraction (LLE) are routine sample pre-treatment strategies. The LLE method was carried out using chloroform and diethylamine as agents; but it failed to achieve a very clean extract and produced a high background noise with poor sensitivity. PP was initiated with MeOH and ACN, but ACN proved unsuitable as it gave low recoveries for all analytes. MeOH was adopted because of its good extraction recoveries.

Extraction under acidic conditions and an acidified solution (0.1% phosphoric acid and 0.1% formic acid) were also investigated. Formic acid generated higher recoveries than were achieved with phosphoric acid, so formic acid was chosen for further investigation at different added volumes. The results showed that addition of 200 µL 0.1% formic acid to the sample procedure gave satisfactory recoveries for all the analytes.

Based on these tests, 1,000 µL MeOH containing 200 µL 1% formic acid was used in subsequent experiments.

Method validation

The bioanalytical method described here met the validation criteria for the calibration curve, selectivity, carryover, accuracy, precision, matrix effect, stability, and dilution integrity, in accordance with the US-FDA guidelines. The results of our method validation are provided in **Table 2**.

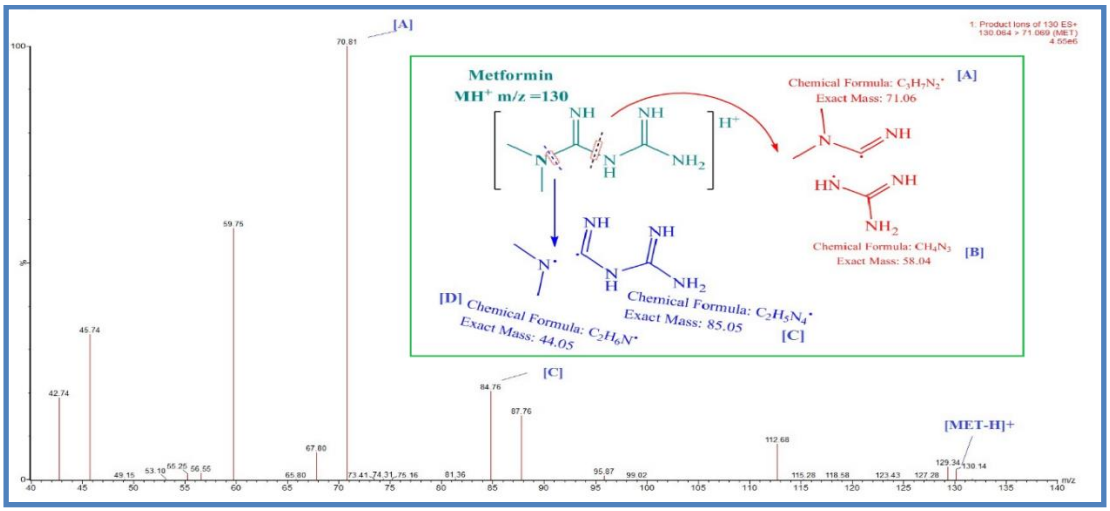
Carryover was evaluated to ensure that it would not affect the accuracy and the precision of the method. The existing carryover was deemed acceptable because it was less than 20% of the LLOQ for an analyte (0.9% and 2.5%), and less than 5% of the IS response (0.005%).

The calibration curve was validated for all analytes over the following ranges: 5–3200 ng/mL for MH and 1–799 ng/mL for SG. The calibration lines were drawn to give the linear regression equations: $y = 0.01175x - 0.05701$ for MH and $y = 0.23278x - 0.00077$ for SG, where y is the peak area ratio of the analyte and the IS, and x is the concentration of the analyte. The correlation coefficient square (R^2) was more than 0.99, while the accuracy for the calibration curve standards ranged from 96.62–107.33% for MH and 90.40–105.52% for SG; these values met the validation requirements. The LLOQ for the standard curve measured with acceptable accuracy and precision was 5 ng/mL for MH and 1 ng/mL for SG at a signal-to-noise ratio (S/N) of more than five.

The intra- and inter-day accuracy and precision of the QCs for all analytes were within the acceptable range. The intra-day precision (%CV) varied from 3.78–5.71% and the accuracy was within 92.2% and 104.52% for all analytes. Similarly, the inter-day precision (%CV) varied from 4.37–6.40% and the accuracy was within 90.90% and 102.80%.

The stability of the analytes and the IS in human plasma and stock solutions was investigated under various conditions. Analytes were stable for up to 6 hours at room temperature and for three freeze–thaw cycles below -20°C. Spiked plasma samples were stable for a period of up to 30 days during the long-term stability experiment. Autosampler stability tests confirmed that the spiked samples were stable up to 24 hours at 20°C without significant loss of the analytes.

Analyte responses were stable in plasma on the benchtop (at room temperature for 6 hours), after three freeze-thaw cycles, after 30 days of storage (at -20°C), and in the autosampler (24 hours, set at 20°C). The analyte responses for the stored stocks were within 15% of those recorded for freshly prepared solutions and were deemed acceptable.



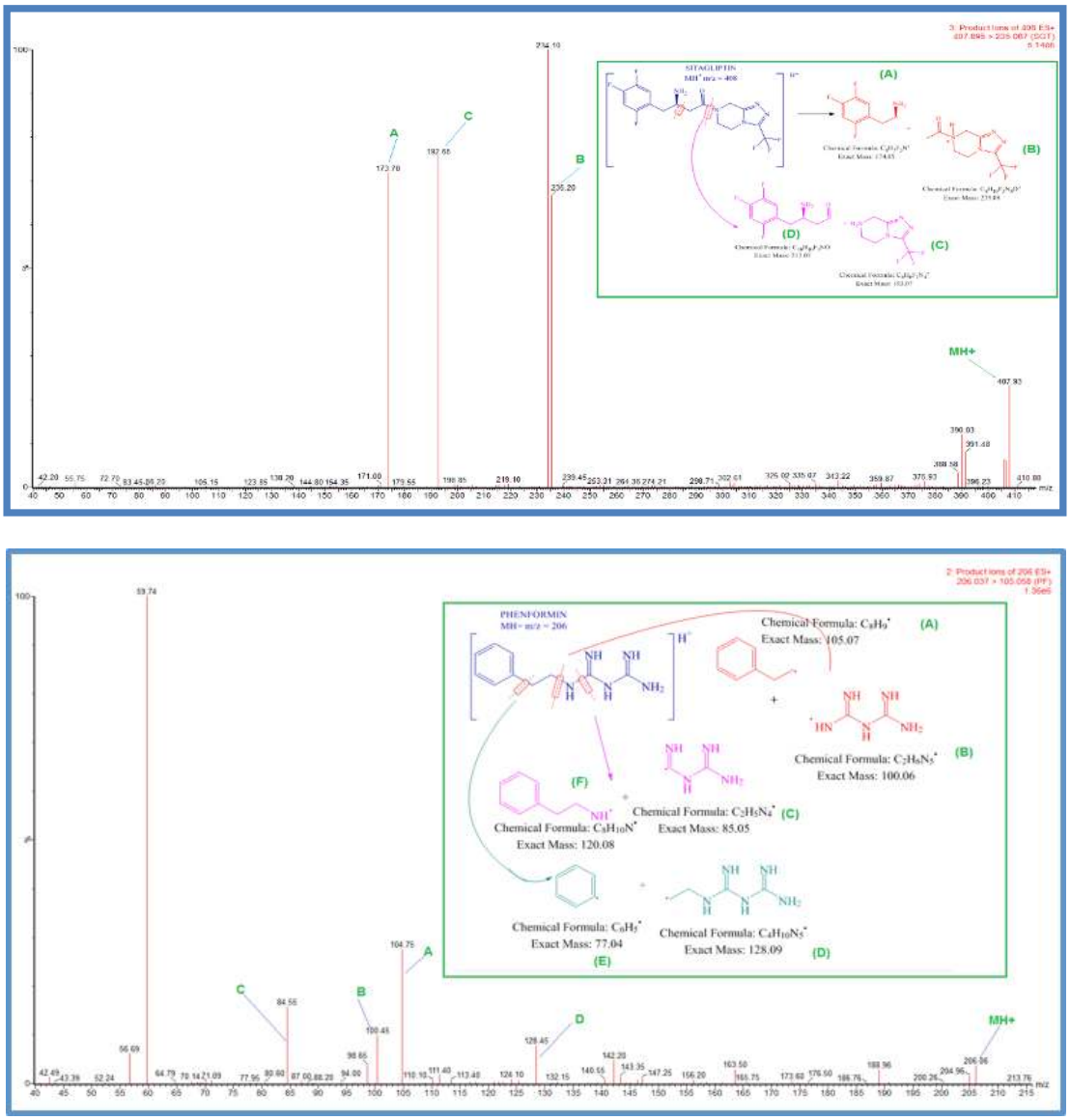


Figure 2. Precursor ion spectrum (m/z) for metformin (A), sitagliptin (B), and phenformin (C) in the positive ionization mode

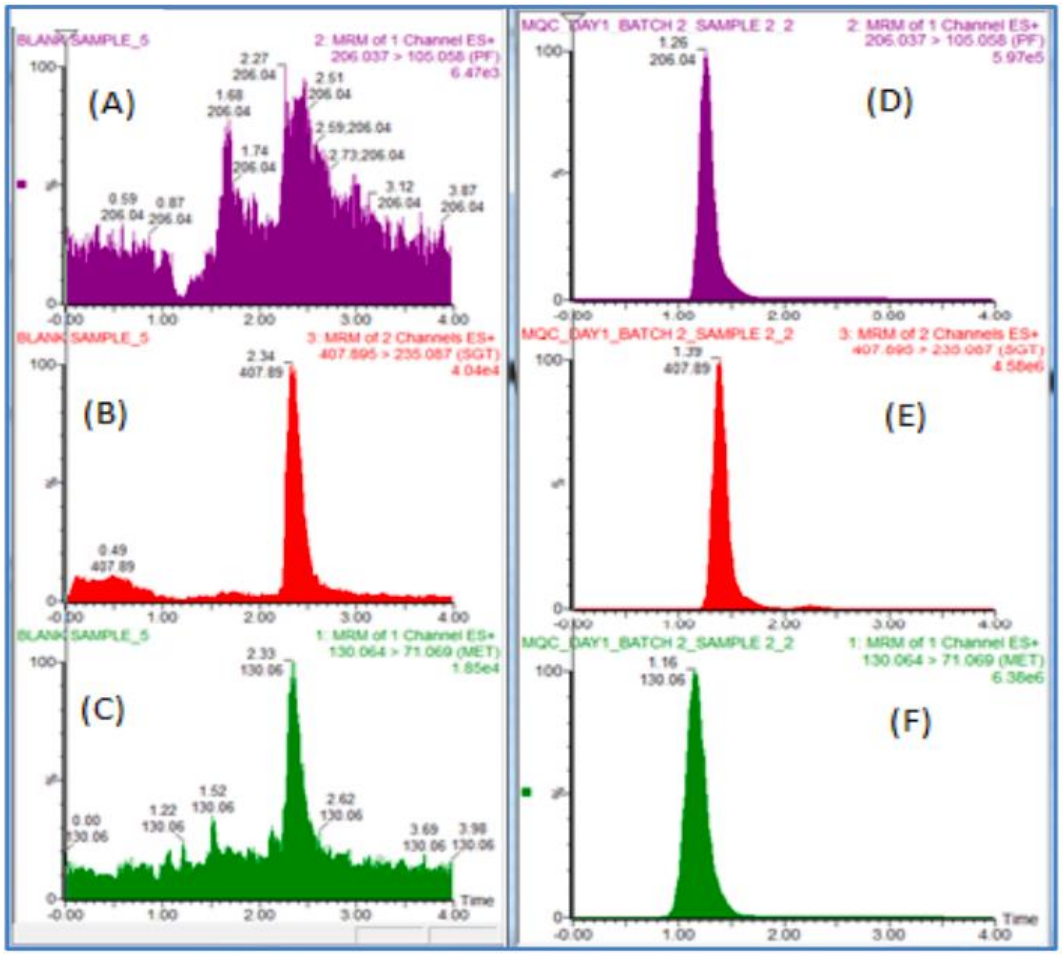


Figure 3. Chromatograms of blank plasma (A, B, C) and analytes at the lower limit of quantitation (LLOQ) level with the IS (D, metformin; E, sitagliptin, F, phenformin [IS])

Table 2. Summary of the validation results

Property		QC	MH	SG	IS
Selectivity	Analyte (% LLOQ)	-	1.1	2.8	
	Internal standard (% IS)	-	0.0	0.0	
Carryover	Analyte (% LLOQ)	-	1.3	2.4	
	Internal standard (% IS)	-	0.0	0.0	0.0
Calibration standard curve	R square (R^2)	-	0.9987	0.9968	
Intra-day accuracy and precision	Accuracy (%)	LQC	99.76	100.76	
		MQC	93.28	101.08	
		HQC	90.90	102.81	
	Precision, CV (%)	LQC	5.23	4.29	
		MQC	3.30	6.40	
		HQC	2.72	4.53	
Inter-day accuracy and precision	Accuracy (%)	LQC	99.56	101.83	
		MQC	95.00	103.86	
		HQC	92.24	104.54	
	Precision, CV (%)	LQC	4.37	5.71	
		MQC	3.52	5.19	
		HQC	2.52	4.24	
Matrix effect	Precision, CV (%)	LQC	5.30	4.85	
		HQC	8.02	6.08	
Recovery	Mean	LQC	79.59	89.49	77.41
		MQC	70.66	89.03	77.41
		HQC	70.92	86.96	73.45
	Precision, CV (%)	LQC	2.08	0.52	6.55
		MQC	4.85	1.39	3.52

Property		QC	MH	SG	IS
		HQC	1.68	3.07	4.74
Free-thaw stability	% of nominal concentration	LQC	98.75	99.03	
		HQC	101.19	101.23	
At room temperature for 6 hours	% of nominal concentration	LQC	95.38	105.48	
		HQC	100.38	99.13	
In autosampler for 24 hours	% of nominal concentration	LQC	100.98	97.73	
		HQC	98.54	100.31	
Long-term stability	% of nominal concentration	LQC	98.43	95.78	
		HQC	98.36	99.26	

Application

The developed and validated method was applied to determine the concentrations of MH and SG in a bioequivalence study conducted in 14 healthy Vietnamese subjects administered a single fixed dose of a test and reference tablet formulation containing 500 mg/50 mg MH/SG. This study was conducted in compliance with the Declaration of Helsinki and in accordance with Good Clinical Practice. The protocol was approved by the local institutional review board, and informed consents were signed by all subjects before study participation. The mean concentration time curve of reference and test of MH and SG in human plasma after oral administration of test and reference formulation under fasting conditions are shown in **Figure 4**.

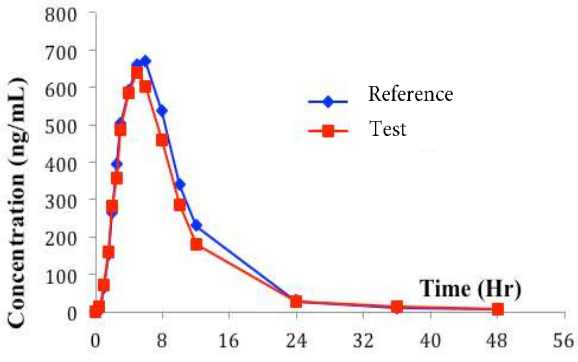
In **Table 3** the mean pharmacokinetic parameters of MH and SG are summarized.

DISCUSSION

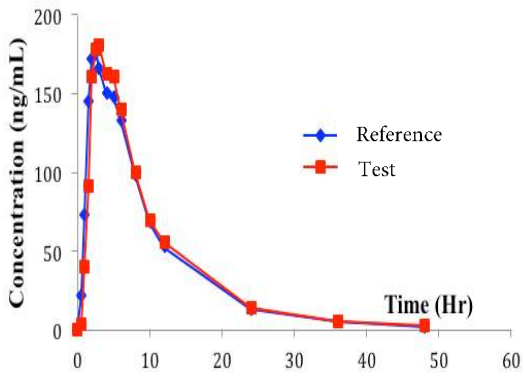
In a previous publication, Bonde et al. used a simple, inexpensive method of sample handling, namely protein precipitation with acetonitrile. However, this method has too low an extraction efficiency for MH (38.06–42.57%).[4] Swales et al. used FTA DMPK-C blood spot cards as a biological fluid sample handling kit to remove impurities; however, this method is expensive and uncommon.[8] Routine methods should be chosen carefully, considering the balance between cost and efficiency, and the manufacturer of a kit should be qualified before the kit is implemented for evaluation of bioavailability and bioequivalence.[8] Compared with solid phase extraction (SPE) techniques and other sample handling methods for mixtures of difficult-to-evaporate extraction solvents, the process for sample handling used in the present study is simple and requires no special equipment. It is easy to implement and economical when large numbers of samples require analysis in a bioavailability/ bioequivalence study. Some studies have used isotopic internal standards for both MH and SG (metformin d-6 HCl and sitagliptin d-4, respectively) and have shown an equivalent signal strength to that of the analytes.[4,5] However, these internal standards are also very costly, have a limited scope with some detectors, and are difficult to preserve, resulting in decreased economic efficiency, especially given the large number of actual samples carried out when evaluating bioavailability and bioequivalence.

In the present study, we have described a novel, simple, economical, and rapid method for simultaneous quantification of MH and SG by liquid chromatography tandem mass spectrometry. The process involved protein precipitation with methanol as the agent. The recovery was 81.39%, 99.23%, and 72.73% for MH, SG, and the IS, respectively. A single IS (phenformin) was capable of monitoring all the analytes in terms of their chromatographic separation, which was performed on a Gemini® C18 column (150 × 4.6 mm; 5 µm). The mobile phase consisted of 0.1% formic acid and methanol (45:55, v/v) and was delivered at a flow rate of 1.0 mL/min. The total chromatographic run time was 4.0 min, which was sufficiently short for use in routine analysis where large numbers of samples need quantification. Our method was fine-tuned to be linear in the range of 5–3200 ng/mL (for MH) and 1–799 ng/mL (for SG). The accuracy and precision at the LLOQ were all within the acceptable range, revealing a prodigious sensitivity of the method.

This lower LLOQ was deemed adequate for application in studies on the bioequivalence of formulations containing MH and SG at low strengths.



A. Metformin hydrochloride



B. Sitagliptin

Figure 4. Mean plasma concentration-time curves of metformin hydrochloride (A), sitagliptin (B) after oral administration of 500 mg metformin hydrochloride and 50 mg sitagliptin fixed dose tablet formulation to 14 healthy Vietnamese subjects

Table 3. Mean pharmacokinetic parameters (± SD) of 36 healthy Vietnamese subjects after oral administration of 50 mg sitagliptin and 500 mg metformin hydrochloride in a fixed-dose tablet formulation

Parameter	Product	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h.ng/mL)	AUC_{0-inf} (h.ng/mL)
Metformin hydrochloride	T	707.3±114.7	5.2	5.76	6137.4±816.1	6225.5±825.1
	R	741.1± 124.0	5.4	5.53	6951.2±1632.1	7020.2±1646.0
Sitagliptin	T	216.3±52.1	2.9	8.71	1885.3±226.8	1920.0±234.1
	R	200.7±46.6	3.5	8.63	1857.4±307.9	1888.3±311.9

Abbreviations: T = Test, R = Reference, C_{max} = Maximum plasma concentration, T_{max} = time point of maximum plasma concentration, $t_{1/2}$ = half-life of drug, AUC_{0-t} = area under the plasma concentration-time curve from zero hours to 48 hours; AUC_{0-inf} = area under the concentration-time curve from zero hours to infinity, SD = standard deviation

CONCLUSION

We have developed and validated a novel, sensitive assay for the quantification of MH and SG in human plasma. The described method offers several advantages, such as a simple extraction procedure and a short chromatographic run time, which makes the method suitable for the analysis of large sample batches resulting from studies of products containing MH and SG.

CONFLICT OF INTEREST

The authors declare "no conflicts of interest with respect to the research, authorship, and/or publication of this article."

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