International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Issue 6, 2022

Original Article

DETERMINATION OF METFORMIN AND SITAGLIPTIN IN HEALTHY HUMAN VOLUNTEERS' BLOOD PLASMA AND ITS BIOEQUIVALENCE STUDY UNDER FASTING CONDITION

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Received: 08 May 2022, Revised and Accepted: 19 Sep 2022

ABSTRACT

Objective: Metformin hydrochloride and sitagliptin are the oral anti-hyperglycemic medications used to treat type 2 diabetes and are used in combination to treat patients. In this work, we have developed a bioanalytical method for simultaneous estimation of both the drugs form some formulation and subsequently the validation of the developed method metformin and sitagliptin in human plasma.

Methods: The stability studies were done as per USFDA and EMA guidelines. The sample extraction approach presented here was a straightforward liquid extraction. The linearity range of metformin was 11.72 ng/ml to 3000 ng/ml, and sitagliptin was 4.68 ng/ml. to 1200 ng/ml. For metformin, the LOD was 1.0 ng/ml, and LLOQ was 11.72 ng/ml. and for sitagliptin, the LOD was 0.75 ng/ml, and LLOQ was 4.68 ng/ml. LC-ESI-MS/MS was used to develop and validate this method using the Phenomenex Kinetex C18 column. Milli-Q water containing 10 mmol Ammonium Acetate (pH =3.6) and Acetonitrile containing 0.1% Formic Acid (pH =2.4) as solvent systems for the estimation of Sitagliptin in a single dose. Metoprolol is used as an Internal Standard.

Results: The total chromatographic run time was only 7.0 min, and the elute time of metformin and sitagliptin was 3.94 min and 3.97 min, respectively. Relative Bioavailability was found at 101.14% for Metformin and 96.96% for Sitagliptin. The overall results show that the Cmax, AUC0-t, and AUC0-∞ for metformin and sitagliptin were within the acceptable limit of 80%-125%.

Conclusion: This bioanalytical method was successfully applied in the bioequivalence study. The study design was a randomized, open-label, two treatment, two-period, two sequences, single-dose, crossover bioequivalence study under fasting conditions.

Keywords: Metformin, Sitagliptin, Type-2 diabetes, Method development and validation, USFDA, EMA, Liquid-liquid extraction, and Bioequivalence studies

DOI: https://dx.doi.org/10.22159/ijap.2022v14i6.45140. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Metformin hydrochloride (N, N-dimethylimidocarbonimidic diamide) is an oral anti-diabetic medicine used as a first-line treatment for type 2 diabetes, especially in persons who are overweight or obese and have normal kidney function [1]. It helps to lower blood sugar levels by suppressing hepatic gluconeogenesis and boosting glucose transfer across the cell membrane in skeletal muscle [2, 3]. Sitagliptin phosphate [f1,2,4-triazolo[4,3-a] pyrazine,7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluoro phenyl) butyl]-5, 6, 7, 8–tetrahydro–3-(trifluoromethyl)], another another anti-diabetic drug, belongs to gliptin family [4]. It works by inhibiting dipeptidyl peptidase-4 (DPP-4), an enzyme responsible for degrading and inactivating glucagon-like peptide-1 (GLP-1) [5, 6]. Because of the constant rise in the number of diabetes cases worldwide, it is critical to have good diabetes management. To improve glycemic control, a combination of two medications, metformin and sitagliptin, has recently been recommended to treat diabetes mellitus [7]. This combination was found to be successful in reversing insulin resistance, islet and adipocyte hypertrophy, and hepatic steatosis, as well as causing significant weight loss.

Metformin (fig. 1) is a highly polar molecule that is notoriously difficult to measure. For the detection of metformin or Sitagliptin (fig. 2), several analytical techniques based on UV [8-10], spectro-fluorimetry [11], RP-HPLC [12-14], HPTLC [15, 16], and LC-MS-MS [17-19] have been reported. However, for simultaneous estimation of these two drugs from a combined formulationin human plasma samples, there is hardly any sensitive, confirmed approach available [20]. Hence the objective of this work was to develop a new validated method which is sensitive and accurate method for simultaneously measuring these two medications in human plasma. We also have utilized the developed method for the quantification of these two drugs for their in pharmacokinetics and bioequivalence investigations as per the USFDA and EMA validation procedures [21, 22].

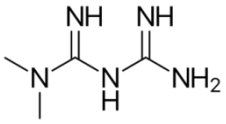

Fig. 1: Structure of metformin

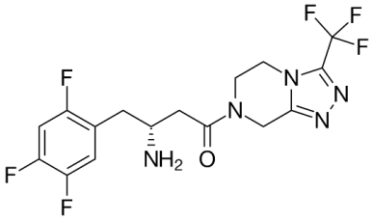

Fig. 2: Structure of sitagliptin

MATERIALS AND METHODS

Reagents

The API of metformin, sitagliptin, and metoprolol (Internal standard), Acetonitrile (ACN), Ammonium acetate and Formic acid were kindly gifted from TAAB Biostudy Services, Kolkata. Additional chemicals were purchased locally and all were of the highest purity grade. Throughout the analysis, Millipore Milli-Q water was used.

Healthy volunteers' blood was obtained in-house in vacutainers with K_2EDTA as an anti-coagulant. Plasma was separated by centrifuging for 10 min at 4 °C at 3,000 rpm.

Bioequivalence study

According to the Independent Ethics Committee's (HURIP Independent Bioethics Committee, Kolkata, West Bengal, India [Registration No.: ECR/103/Indt/WB/2013/RR-19]) preapproved protocol and other papers, the study was conducted to show the bioequivalence of the test product (a combination of metformin and sitagliptin) with the reference marketed product. 24 healthy human volunteers who freely provided informed consent for this study were recruited based on the protocol's inclusion and exclusion criteria. The research was carried out in accordance with the protocol and based on Good Clinical Practice (GCP).

Patients and methods

Study population

Healthy, non-smoking (within the preceding six months) male subjects aged 18 y to 45 y with a BMI of 32 kg/m^2 were eligible to participate. For the trial duration, participants were not allowed to consume any prescription or non-prescription medications. To avoid possible confounding effects of these compounds on the pharmacokinetics of the research, subjects agreed to restrict their use of fruit juices, alcohol, and caffeine. Key exclusion criteria were: regular use of prescription or non-prescription medications (including herbal treatments) that could not be stopped for the course of the trial, starting two weeks before the first dosage of the study drug and continuing throughout the study; prior history of stroke, neurological condition, or neoplastic disease; prior history of numerous allergies and/or hypersensitivity to medicines (including metformin) or food; and prior involvement in other investigational studies within four weeks of the study's start date.

Study design

A randomized, open-label, two treatment, two-period, two sequences, single-dose, crossover bioequivalence study under fasting conditions.

Blood sampling

In each study period, 19 blood samples will be collected in 5 ml K_2EDTA vacutainers via an indwelling catheter placed in one of the forearm veins. Blood samples will also be collected by direct venipuncture during ambulatory blood sampling visits and wherever necessary for any practical reason. A pre-dose blood sample will be taken 1 h prior to the start of the drug administration. The post-dose blood samples will be collected at 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h, 5 h, 6 h, 8 h, 10 h, 12 h, 16 h, 24 h, 32 h, 48 h, and 72 h.

For a particular sample time point, all the blood samples will be centrifuged under refrigeration at 3500 rpm and 4 °C for 10 min. For the pending assay, the resulting plasma will be separated and stored in suitably labeled polypropylene tubes at–20 ± 5 °C.

For the whole study, the total volume of blood obtained will be around 213 ml per volunteer, including the volume required for laboratory testing [screening and safety sample], PK analysis, and the volume of blood discarded before each blood draw.

Methods

Instrumentation

The mass spectrometric detection was done on an API 2000 triple quadrupole instrument (ABI-SCIEX) using multiple reaction monitoring (MRM) on the HPLC system. The software package Analyst 1.5 was used to process the data. The chromatographic and MRM conditions are described in table 1 and table 2.

Preparation of standard samples solutions

Stock solutions of 10 mg of metformin and sitagliptin were prepared in 10 ml DMSO. The potency and actual amount weighed were then used to adjust the concentration. Metformin and Sitagliptin working solutions were made by diluting the stock solution with Milli-Q water: ACN (1:1). For Internal Standard (IS), a stock solution of 10 mg of metoprolol was prepared in 10 ml DMSO. All diluted solutions were kept in the refrigerator until analysis at 2–8 °C [23, 24].

Table 1: Chromatographic condition

Column	Phenomenex Kinetex C18; 50x3 mm, Particle Size-5 µm
Mobile Phase	A: Milli-Q water containing 10 mmol Ammonium Acetate (pH =3.6) B: Acetonitrile containing 0.1% Formic Acid (pH =2.4)
Flow rate	0.3 ml/min
Injection volume	10 µl
Total run time(min)	7.0 min
Autosampler Temperature	15 °C
Retention Time (min)	Metformin: 3.94 min Sitagliptin: 3.97 min Internal Standard (Metoprolol): 3.50 min

Table 2: Multiple reaction monitoring (MRM) conditions

Compound	Q1	Q3	Dwell time (msec)	DP	CE	CXP
Analyte (Metformin)	130.20	71.00	100	30.00	27.00	4.00
Analyte (Sitagliptin)	408.10	235.20	100	40.00	30.00	4.00
Internal Standard (Metoprolol)	268.20	116.10	100	30.00	30.00	4.00
Mode	Positive (+)					

DP = Declustering Potential; CE = Collision Energy; CXP = Collision Cell Exit Potential

Preparation of mobile phase A: Water containing 10 mmol Ammonium acetate (pH =3.6)

0.7708 mg of Ammonium Acetate was dissolved in 1 L of Milli-Q water in a 1 L reagent bottle and adjusted the pH by adding formic acid. It was filtered and sonicated for 5 min. This solution was used within three days from the date of preparation and assigned a batch number.

Preparation of mobile phase B: Acetonitrile containing 0.1% Formic acid (pH =2.4)

1 ml of Formic Acid was taken in 1000 ml of Acetonitrile in a 1000 ml reagent bottle and adjusted the pH by adding formic acid. It was filtered and sonicated for 5 min. This solution was used within three days from the date of preparation and assigned a batch number.

Preparation of calibration standards and preparation of quality control samples

By spiking appropriate analytes and IS in blank human plasma, nine-point standard calibration solutions of metformin (3000 ng/ml, 1500 ng/ml, 750 ng/ml, 375 ng/ml, 187.50 ng/ml,93.75 ng/ml, 46.87 ng/ml, 23.44 ng/ml, 11.72 ng/ml) and sitagliptin (1200 ng/ml, 600 ng/ml, 300 ng/ml, 150 ng/ml, 75 ng/ml, 37.50 ng/ml, 18.75 ng/ml, 9.37 ng/ml and 4.68 ng/ml) were prepared to yield final concentrations. The QC samples of LLOQ, LQC, MQC, and HQC were

prepared (for metformin LLOQ, LQC, MQC, and HQC were 2250 ng/ml, 1125 ng/ml, 35.15 ng/ml, and 11.72 ng/ml respectively and for sitagliptin LLOQ, LQC, MQC, and HQC were 900 ng/ml, 450 ng/ml, 14.06 ng/ml and 4.68 ng/ml respectively) at three concentration levels of the analytes. The calibration curve was plotted using the concentration on the X-axis and a peak area ratio of the drug and IS on the Y-axis.

Sample extraction

Plasma extraction was performed by liquid-liquid extraction technique

A 600 µl plasma sample was transferred to a 15 ml plastic tarson tube and spiked with 100 µl of IS working solution (5 µg/ml). 1 ml ACN was added after 1 min of vortexing. After that, the material was vortex-mixed for 5.0 min before centrifuging for 10 min at 12,000 rpm. The organic layer was then transferred to a 5 ml plastic tube and evaporated at 40 °C under nitrogen steam for dryness. The dried extract was then reconstituted in 500 µl of diluents acetonitrile (ACN): Milli-Q Water (50:50), transported to an autosampler vial for LC-MS/MS analysis, and 10 µl of volume was injected. [25-27]

Selectivity and sensitivity

Researchers analyzed human blank plasma samples from various sources to test for interference at the retention periods of analytes (Volunteers). The sensitivity of the analyte was compared to its LLOQ in a blank plasma sample. The peak area of blank samples should not exceed 20% of the mean peak area of metformin and sitagliptin LLOQ samples.

Precision and accuracy

It was determined by performing replicate analyses on quality control samples (n = 5) at the LLOQ, LQC, MQC, and HQC levels. The %CV should be less than 15%, and accuracy should be within 15%, with the exception of LLOQ, which should be within 20%.

The lower limit of quantification (LLOQ) and limit of detection (LOD)

The lower limit of quantification and the limit of detection were determined of metformin and sitagliptin.

Matrix effect

The matrix effect attributable to the plasma matrix was used to estimate the ion suppression/enhancement in a signal when comparing the absolute response of QC samples after pretreatment (LLE) with the reconstitution samples extracted blank plasma sample spiking with the analyte. A 15 percent accuracy (% CV) is regarded as satisfactory.

Recovery

The six replicates of the retrieved HQC, MQC, and LQC. The extracted QC samples are inserted alongside freshly generated aqueous QC samples based on recovery. The extracted samples' mean peak area response is compared to the aqueous samples' mean peak area response. The mean % recovery, SD, and %CV are computed for each level, and the global mean % recovery of analyte and internal standard. The % recovery for analytes and IS should not exceed 115 %, and the % CV of Area at various concentrations (i.e., HQC, MQC, and LQC) should not exceed 15%. The % CV of Global Recovery should be around 20%.

Stability

To determine the drug's Stability in human plasma, various stability studies such as freeze-thaw stability, autosampler stability, and short-term and long-term stability were done. Analytes spiked blank human plasma at three quality control concentrations HQC, MQC, and LQC. Five replicates of each were stored appropriately at stability test storage settings, from which it was retrieved and examined. To assess the stability of the analytes in the stock, QC samples from the stock were freshly produced soon before the investigation. The spiking plasma sample was stored for 24 h before extraction and analysis for the short-term stability investigation. In the case of freeze and thaw stability, the sample was stored at-20°C and exposed to freeze and thaw cycles by freezing at-20°C and thawing in the laboratory's normal conditions. To determine autosampler stability, the samples were stored in an autosampler at 15 °C for 24 h before injection. The spiked plasma sample was stored at-20 C for 30 d before extraction and analysis as part of a long-term stability investigation. The stability testing accuracy against the stability samples was determined using freshly spiked QC samples at HQC, MQC, and MQC.

RESULTS AND DISCUSSION

Development of a method, various approaches for optimizing mass spectrometer detection settings, chromatography, and sample extraction were examined throughout method development.

Mass spectrometry detection parameters optimization

Electrospray ionization (ESI) was used for this approach because it provided the best response over atmospheric pressure chemical ionization (APCI). During infusion of the analyte in a continuous mobile phase flow to an electrospray ion source operating at both polarities at a flow rate of 0.3 ml/min, the instrument was tuned for sensitivity and signal stability. Metformin and Sitagliptin had a higher response than in the negative ion mode in the positive ion mode. The mass transitions for m/z (Metformin) 130.2 → 71 and m/z (Sitagliptin) 408.1 → 235.2 were used in the Turbo ion spray (API) positive mode (Electron spray ionization) with Resolution using MRM positive ion mode. The mass spectras of parent and product ions of Metformin and Sitagliptin are shown in fig. 3, fig. 4, fig. 5, and fig. 6, respectively. Mass parameters were optimized as Source temperature 400 °C, Curtain gas 10 (nitrogen) psi, CAD gas 8 (nitrogen) psi, Ion Spray (IS) voltage 5000 volts, Entrance potential 11 V, Declustering potential of Metformin, Sitagliptin, and Metoprolol (Internal Standard) were 30 V, 40 V, 30 V respectively, Collision energy of Metformin, Sitagliptin, and Metoprolol (Internal Standard) were 27 V, 30 V, 30 V respectively, Collision cell exit potential of Metformin, Sitagliptin, and Metoprolol (Internal Standard) were 11.8 V, 18.7 V, 15.2 V respectively.

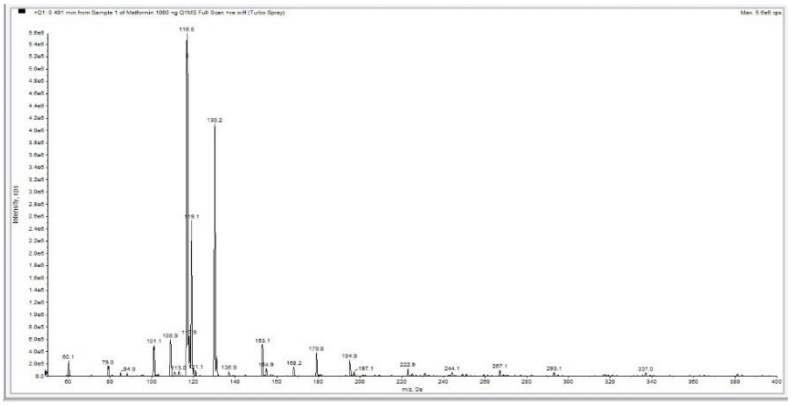

Fig. 3: Q1 MS scan of metformin

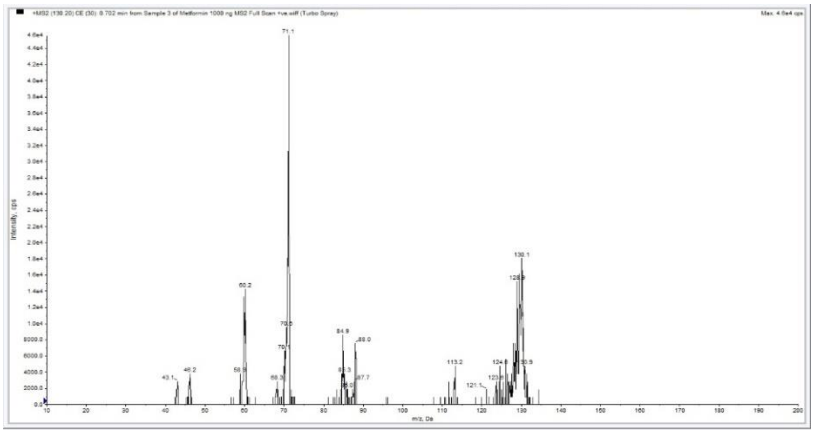

Fig. 4: MS2 scan of metformin

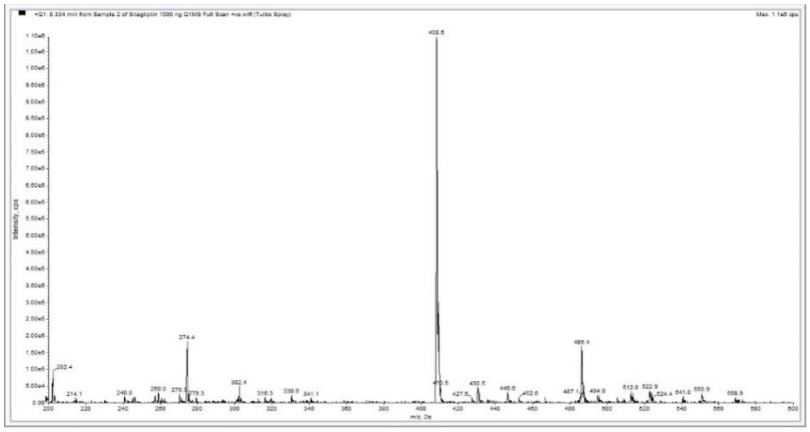

Fig. 5: Q1 MS scan of sitagliptin

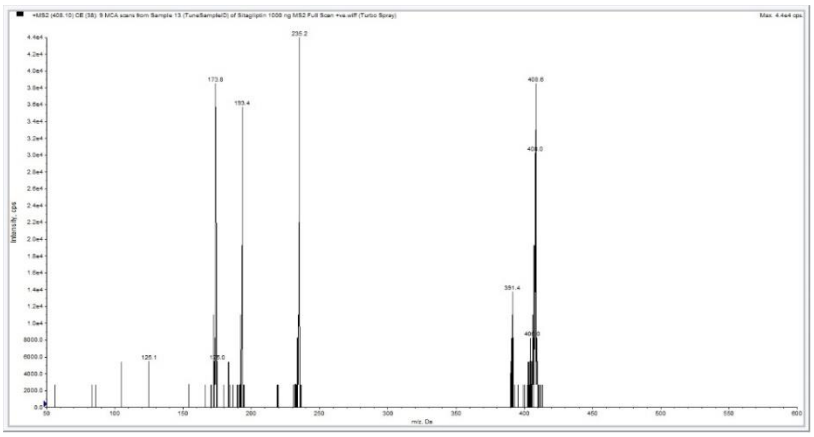

Fig. 6: MS2 scan of sitagliptin

Chromatography optimization

A mobile phase consisting of 5 mmol ammonium acetate and Milli-Q water in varying combinations was tried, but an inadequate response was observed. Then the mobile phase containing 10 mmol ammonium acetate and Milli-Q water gives a better response in pump A (pH =3.6). Then, the pump B mobile phase of 0.1% formic acid in combining Acetonitrile was tried (pH =2.4). Moreover, it gave the best signal and a marked improvement in the peak shape observed for metformin and sitagliptin. Short-length columns such as Phenomenex Kinetex C18; 50x3 mm, Particle Size-5m were used during the method development. This column produced an excellent peak shape with the best signal. It produced suitable peak shapes for metformin and sitagliptin. In binary flow rate of 0.3 ml/min without a splitter was utilized and reduced the run time. The metformin, sitagliptin, and IS were eluted for a shorter time at 3.94 min, 3.97 min, and 3.50 min. When a strong matrix effect is feasible in an LC-MS/MS analysis, using stable isotope-labeled or appropriate analog drugs as an internal standard is beneficial and cannot interfere with the drugs. Metoprolol was shown to be the best option in our situation. The temperature in the column oven was kept constant at around 40 °C. For improved ionization and chromatography, the injection volume of a 10 µl sample is injected.

Extraction optimization

Before loading the sample for LC injection, the co-extracted proteins should be removed from the prepared solution. Initially,

we experimented with different extraction processes such as Protein Precipitation Technique (PPT) and Liquid Liquid extraction (LLE). We discovered an ion suppression effect in the protein precipitation method for both the drugs and the internal standard. We also experimented with LLE. According to all of us, LLE is suitable for drugs and IS extraction. To extract analyte from plasma, we explored numerous organic solvents (Acetonitrile, ethyl acetate, chloroform, and methyl tertiary butyl ether) singly and in combination in LLE. In our case, a 50:50 mixture of Acetonitrile and Milli-Q water worked well as an extraction solvent. The Liquid-Liquid extraction method yielded high recovery and selectivity. Reduced analysis time and reliable and exact detections of metformin and sitagliptin in human plasma were achieved using these modified detection parameters, chromatographic conditions, and extraction techniques.

Method validation

Metformin and sitagliptin method validation in human plasma was carried out in accordance with USFDA and EMA criteria. Selectivity, sensitivity, matrix effect, linearity, precision and accuracy, recovery, and stability were tested [23-26].

Selectivity and specificity

Metformin and sitagliptin were highly selective in the MRM function, with no interfering substances. Different batches of human plasma were used to test specificity. Plasma was spiked with metformin (11.72 ng/ml) and Sitagliptin (4.68 ng/ml) chromatograms.

Linearity

The peak area ratio vs. concentration was plotted on calibration curves. The calibration results out to be linear. The concentration range of 11.72 ng/ml-3000 ng/ml for metformin and 4.68 ng/ml-1200 ng/ml for sitagliptin. For curves, the determination coefficients (r^2) for metformin and sitagliptin were 0.9917 and 0.9994, respectively (fig. 7 and 8).

Precision and accuracy

Calculating the inter-day and intra-day batch variations at four concentrations (11.72, 35.15, 1125 and 2250 ng/ml [Metformin]), (4.68, 14.06, 450 and 900 ng/ml [Sitagliptin]), of QC samples in five replicates. As shown in (table 3, table 4, table 5, and table 6). These findings demonstrate that this procedure is reliable and repeatable within the analytical range.

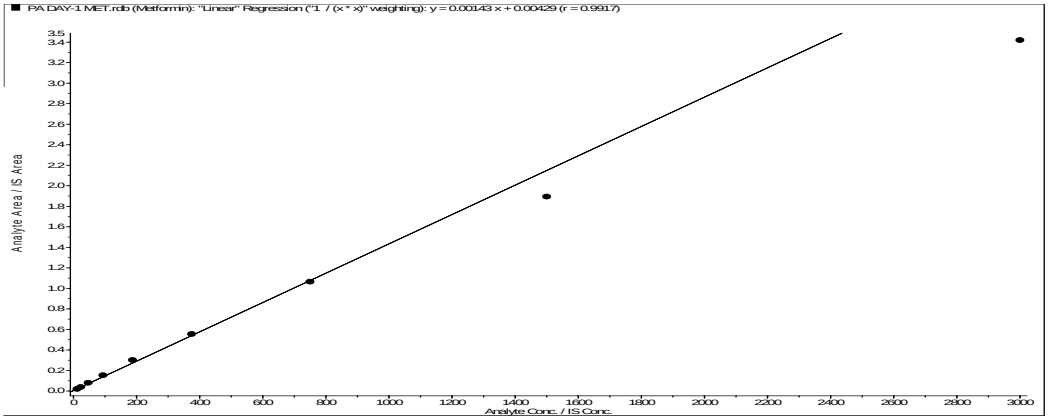

Fig. 7: Calibration curve of metformin

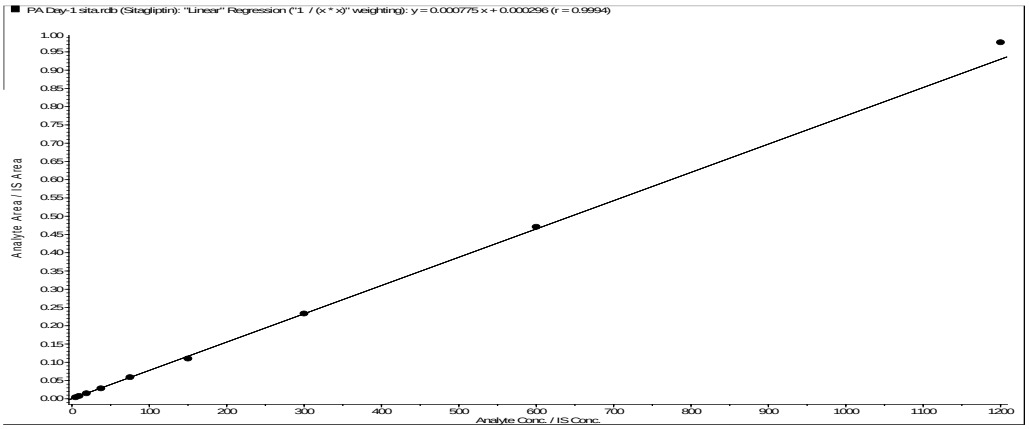

Fig. 8: Calibration curve of sitagliptin

Table 3: Inter day precision and accuracy results (Metformin)

QC samples (ng/ml)		Inter day (between run)			
		Mean concentration (ng/ml)	SD	%CV	Accuracy (%)
LLOQ	11.72	11.49	0.47	4.08	98.03
LQC	35.15	39.55	2.69	6.79	112.52
MQC	1125	1095.26	88.94	8.12	97.36
HQC	2250	2151.51	145.49	6.76	95.62

n= 5

Table 4: Intra-day precision and accuracy results (Metformin)

QC samples (ng/ml)		Intra-day (within run)			
		Mean concentration (ng/ml)	SD	%CV	Accuracy (%)
LLOQ	4.68	4.89	0.13	2.64	104.53
LQC	14.06	12.10	0.11	0.87	86.05
MQC	450	443.94	8.14	1.83	98.65
HQC	900	1012.72	20.15	1.99	112.52

n= 5

Table 5: Inter day precision and accuracy results (Sitagliptin)

QC samples (ng/ml)		Inter day (between run)			
		Mean concentration (ng/ml)	SD	%CV	Accuracy (%)
LLOQ	4.68	4.45	0.36	8.17	95.03
LQC	14.06	13.42	0.41	3.08	95.45
MQC	450	416.71	26.70	6.41	92.60
HQC	900	902.31	41.01	4.55	100.26

n= 5

Table 6: Intra-day precision and accuracy results (Sitagliptin):

QC Samples (ng/ml)		Intra-day (within run)			
		Mean concentration (ng/ml)	SD	%CV	Accuracy (%)
LLOQ	11.72	12.57	0.15	1.16	107.29
LQC	35.15	36.95	0.85	2.31	105.13
MQC	1125	1109.18	14.91	1.34	98.59
HQC	2250	2102.34	147.07	7.00	93.44

n= 5

The lower limit of quantification (LLOQ) and limit of detection (LOD)

Metformin's lower limit of quantification was 11.72 ng/ml. and the limit of detection was determined of metformin 1 ng/ml. Moreover, Sitagliptin's lower limit of quantification was 4.68 ng/ml, and the limit of detection was determined of sitagliptin 0.75 ng/ml.

Matrix effect

When the Area under curve ratios of extracted quality controls and Internal Standard were compared to the Area under curve ratios of unextracted quality controls, and Internal Standard obtained from the injecting aqueous solution prepared at the same concentrations, there was no significant Matrix effect, ion suppression, or enhancement recorded.

Recovery

The Liquid-liquid extraction technique method shows acceptable recoveries for the metformin and sitagliptin. The recovery results securely for metformin at LQC, MQC, and HQC concentrations were 98.45%, 95.08%, and 93.47%, respectively. Sitagliptin at LQC, MQC, and HQC concentrations were 104.65%, 106.43%, and 108.74%.

Stability

The stability studies results of metformin and sitagliptin gave below in table 7 and table 8, respectively.

Table 7: Stability studies (Metformin)

Storage conditions	QC Samples (ng/ml)		Mean (ng/ml)	Accuracy (%)
Freshly thawed	LQC	35.15	37.91	
	MQC	1125	1184.36	
	HQC	2250	2064.26	
Freeze and thaw stability at-20 °C	LQC	35.15	31.06	83.35
	MQC	1125	1207.64	101.97
	HQC	2250	2371.27	114.87
Autosampler stability 24 h	LQC	35.15	39.45	104.06
	MQC	1125	1087.89	91.85
	HQC	2250	2181.34	105.67
Short term stability 24 h	LQC	35.15	32.71	86.28
	MQC	1125	1135.56	95.88
	HQC	2250	2253.78	109.18
Long term stability at-20 °C for 30 d	LQC	35.15	40.40	106.58
	MQC	1125	1003.69	84.75
	HQC	2250	2204.96	106.82

n= 5

Table 8: Stability studies (Sitagliptin)

Storage conditions	QC Samples (ng/ml)		Mean (ng/ml)	Accuracy (%)
Freshly thawed	LQC	14.06	13.35	
	MQC	450	388.40	
	HQC	900	885.41	
Freeze and thaw stability at-20 °C	LQC	14.06	12.67	94.94
	MQC	450	414.98	106.85
	HQC	900	900.91	101.75
Autosampler stability 24 h	LQC	14.06	13.27	99.39
	MQC	450	398.04	102.48
	HQC	900	881.07	99.51
Short term stability 24 h	LQC	14.06	12.31	92.19
	MQC	450	446.23	114.88
	HQC	900	844.81	95.41
Long term stability at-20 °C for 30 d	LQC	14.06	13.51	101.23
	MQC	450	401.21	103.30
	HQC	900	865.24	97.72

n= 5

Table 9: Bioequivalence study results of metformin and sitagliptin

Pharmacokinetic parameters	Reference formulation		Test formulation		Reference formulation		Test formulation	
	Metformin				Sitagliptin			
C_{max} (ng/ml)	Mean	2039.85	Mean	1961.48	Mean	137.01	Mean	125.84
	± SD	321.60	± SD	140.78	± SD	34.15	± SD	11.49
t_{max} (h)	Mean	2.52	Mean	2.48	Mean	2.48	Mean	2.50
	± SD	0.28	± SD	0.28	± SD	0.28	± SD	0.26
AUC $_{0-t}$ (ng. h/ml)	Mean	11789.74	Mean	11923.65	Mean	705.73	Mean	684.25
	± SD	1730.57	± SD	676.66	± SD	72.98	± SD	49.12
AUC $_{0-\infty}$ (ng. h/ml)	Mean	12209.14	Mean	12320.47	Mean	807.01	Mean	774.07
	± SD	1801.23	± SD	663.12	± SD	77.13	± SD	61.36
K_{el} (h^{-1})	Mean	0.057	Mean	0.058	Mean	0.041	Mean	0.042
	± SD	0.003	± SD	0.002	± SD	0.003	± SD	0.002
$t_{1/2}$ (h)	Mean	12.099	Mean	11.987	Mean	17.056	Mean	16.648
	± SD	0.591	± SD	0.422	± SD	1.412	± SD	0.817
Relative Bioavailability (%)	100 %		101.14%		100%		96.96%	

n= 24 volunteers samples

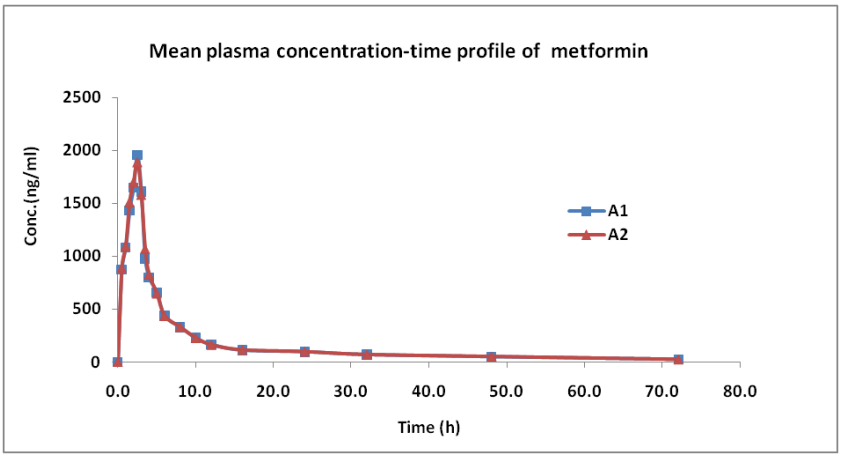

Fig. 9: Mean plasma concentration-time profile of metformin

As compared to previous literature reports; fast, sensitive, and cost-effective stability-indicating analytical method was developed for the simultaneous estimation of metformin and sitagliptin [28, 29]. The analytical method was developed and optimized to determine suitable chromatographic conditions for obtaining sharp and well-resolved peaks of metformin and sitagliptin with minimal tailing. After several trials on different RP columns such as Zodiac C18 (150 mm x 4.6 mm, 5 µm), X-bridge Phenyl (150 mm x 4.6 mm, 5 µm), and Eclipse XDP-C18 (250 mm x 4.6 mm, 5 µm), the optimum separation between the analytes was achieved on Phenomenex Kinetex C18 column. Along with columns, different mobile phase compositions at different pH and flow rates were also evaluated and optimum separation was achieved by using Milli-Q water containing 10 mmol Ammonium Acetate (pH =3.6) and Acetonitrile containing 0.1% Formic Acid (pH =2.4) as a mobile phase at a flow rate of 0.3 ml/min. In a previously reported method, it required a long run time 18 min [30], whereas in this method, the overall run time was 7 min because of elution of analyte peaks at a shorter possible time without compromising resolution and this signifies that the proposed method is rapid and cost-effective. All the system suitability parameters were within the acceptable limit of ICH, USFDA and EMA guidelines [31, 32].

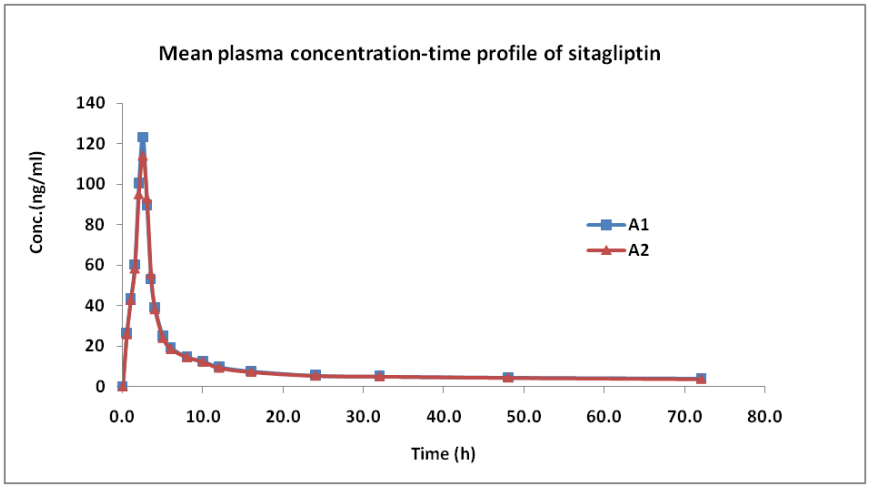

Fig. 10: Mean plasma concentration-time profile of sitagliptin

CONCLUSION

It has been reported that an accurate, simple, sensitive, precise, reproducible, and robust LC-MS/MS technique for the simultaneous measurement of metformin and sitagliptin in healthy human plasma has been developed and validated. The sample extraction approach is a straightforward liquid-liquid extraction (LLE) method that generates cleaner samples with less matrix influence and higher percentage recovery. Furthermore, combining an atmospheric pressure ionization approach with a Phenomenex Kinetex C18 column significantly lowers the matrix effect. In pharmacokinetic and bioequivalence studies, metformin and sitagliptin were successfully quantified using this method. The method was validated for usage in a pharmaceutical environment by analyzing real pharmacokinetic study samples and demonstrating its accuracy and precision. This technique could be used for therapeutic medication monitoring as well.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to M/S, TAAB Biostudy Services, Jadavpur, Kolkata-700032, India, for providing blank human plasma samples and the necessary instrumental facilities to complete the study.

FUNDING

This research work did not receive any specific grant from any funding agencies or commercial sector.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors report no financial or other conflicts of interest in this work.

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