INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

UPLC-MS/MS ASSAY FOR ALOGLIPTIN IN HUMAN PLASMA AND ITS PHARMACOKINETIC APPLICATION

R. Muralikrishna[1] and A. Lakshmana Rao[2*]

[1]Faculty of Pharmacy, Krishna University,
Machilipatnam–521 001, Andhra Pradesh, India.
[2]V. V. Institute of Pharmaceutical Sciences,
Gudlavalleru–521 356, Andhra Pradesh, India.

ABSTRACT

This paper describes an ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) assay method for the determination of alogliptin in human plasma. A deuterated analogue alogliptin 13C D3 was employed as internal standard (IS) to quantify the alogliptin concentrations. A smallest plasma volume of 50 µL was utilized for sample processing by solid phase extraction (SPE). The processed samples were chromatographed on C_{18} column by using an isocratic mobile phase composed of 5mM ammonium formate, acetonitrile and methanol (20:40:40, v/v/v). The method was found to be linear in the range of 1.01–301 ng/mL and r^2 was ≥ 0.99. A total of five precision and accuracy batches were run in three consecutive days during the validation. Analyte stability in neat samples as well as in plasma samples were well within the specified limits. Also, this method was successfully applied to pharmacokinetic study in humans.

Keywords: Alogliptin, Human plasma, Solid phase extraction (SPE), UPLC-MS/MS.

INTRODUCTION

The enzyme dipeptidyl peptidase-4 (DPP-4) is majorly involved in the rapid degradation of incretin hormones, which plays a key role in the type 2 diabetes mellitus (T2DM) pathogenesis[1,2]. Alogliptin is an orally administered anti-diabetic drug in the DPP-4 inhibitor class, indicated as an adjunct to exercise and diet to improve glycemic control in adults with T2DM[3,4]. Like other medications for the treatment of Type 2 diabetes, alogliptin does not decrease the risk of heart attack and stroke. Alogliptin and other gliptins are commonly used in combination with metformin in patients whose diabetes cannot adequately be controlled with metformin alone. The drug is well tolerated, with no dose-limiting toxicity[5]. As per the literature, few LC-MS/MS methods has been reported for the determination of alogliptin in variety of samples like human plasma[6-8], rat plasma[9], and in river water samples[10]. Recently, Mowaka *et al.,* 2017 and Abdel-Ghany *et al.,* 2017 reported LC-MS/MS based methods for the determination of alogliptin along with metformin and alogliptin with pioglitazone, respectively in human plasma samples. Mowaka *et al.,* 2017 used 1 mL of plasma sample for processing and LLOQ was 10 ng/mL. Abdel-Ghany *et al.,* 2017 also used similar chromatographic and extraction procedures employed by the Mowaka *et al.,* 2017. Similarly, Hemavathi *et al.,* 2017, has been published a LC-MS/MS for the simultaneous determination of alogliptin and voglibose in human plasma using similar chromatographic conditions used by Mowaka *et al.,* 2017. The reported LLOQ was 5.09 ng/mL with 200 µL human plasma and the total run time was >3 min. Another author, Chen *et al.,* 2016, reported a bioavailability study of alogliptin in rat plasma by UPLC-MS/MS. Alogliptin and its internal standard (diazepam) were resolved on UPLC BEH C18 column using a gradient mobile phase. The reported LLOQ in rat plasma is 2 ng/mL. All three methods plasma (Mowaka *et al.,* 2017; Hemavathi *et al.,* 2017, Chen *et al.,* 2016), were employ direct protein precipitation (PP) technique to extract the analytes from plasma. PP is fails to sufficiently remove endogenous compounds such as lipids, phospholipids, fatty acids, etc and cause ion suppression and

changes of possible matrix effect is more[11,12]. Of late, Kai *et al.,* 2016, developed a LC-MS/MS method for the simultaneous determination of oral antidiabetic drugs including alogliptin in river water samples.

To study the tolerability and safety of new formulations of alogliptin and for bioavailability and bioequivalence assessments of alogliptin, one should have a proper analytical method to estimate the *in vivo* plasma concentrations. The reported LC-MS/MS methods in human plasma[6, 8] are having LLOQ >5 ng/mL and not sensitive enough for application of pharmacokinetic/bioequivalence studies. Moreover, the method developed by Mowaka *et al.,* 2017 utilized large plasma volumes (>1000 µL) which may not be favorable for routine drug analysis/ bioequivalence studies. Additionally, these methods are not specific for alogliptin and may create conflicts in the results due to improper characterization of selectivity. Bioanalytical method should be effective and should be satisfy the researcher in terms of sensitivity, selectivity and rapid[13-15].

To address the pharmacokinetics of alogliptin in newer formulations, a sensitive and specific method that allows exact determination *in vivo* plasma concentrations of alogliptin is desirable. With the reported lowest LLOQ (5 ng/mL) alogliptin was not quantifiable beyond 24 h post-dosing for a pharmacokinetic study in humans. Quantitation of any drug during the terminal phase for extended time points is critical to derive key pharmacokinetic parameters. Hence, in the present work we have developed and fully validated an LC-MS/MS for the determination of alogliptin in 50 µL of human plasma with an LLOQ of 1.01 ng/mL. Alogliptin was quantifiable up to 72 h of post dose using the proposed method. We have also employed a once step solid phase extraction (SPE) method with direct injection for sample preparation to get cleaner extracts for analysis with minimal or no matrix effect. Alogliptin 13C D3 was used as an IS to avoid the potential matrix effect related issues and variability in recovery between analyte and IS. This method was successfully applied to a clinical pharmacokinetic study of alogliptin in healthy male subjects under fasting condition.

MATERIALS AND METHODS

Standards and Reagents

Alogliptin reference standard (99.7% pure) was obtained from Hetero Drugs Limited (Hyderabad, India). Alogliptin 13C D3 (98.5% pure) was employed as an internal standard and was obtained from Vivan Life Sciences Pvt. Limited (Mumbai, India). Chemical structures are presented in Fig. 1. Ultra-pure methanol and acetonitrile were purchased from J.T. Baker (Phillipsburg, NJ, USA). Ammonium formate was purchased from Merck Ltd (Mumbai, India) and HPLC grade water was purchased from Rankem Ltd (Mumbai, India). The blank human plasma was obtained from Deccan's Pathological Lab's (Hyderabad, India).

UPLC–MS/MS instrument and conditions

An UPLC system (Waters Corporation, Milford, USA) consisting of a Kromasil 100-5 C18 (100 mm × 4.6 mm, 5 µm; Akzonobel, Hyderabad, Inida) column equipped with a binary pump and a 96–vial autosampler (Waters, Milford, USA) was used for the study. Aliquot of 5 µL of the processed samples were injected into the column, which was kept at 40±2ºC. An isocratic mobile phase consisting of a mixture of 5mM ammonium formate, methanol and acetonitrile (20:40:40, v/v/v) was used to separate the analyte from endogenous components and pumped at a flow rate of 0.4 mL/min.

Quantification was achieved with MS–MS detection in positive ion mode (ES+) for the analyte and the internal standard using a Waters XEVO TQ–S mass spectrometer (Manchester, United Kingdom). The cone voltage, capillary voltage and collision energy were 30 V, 3.5 V, 30 V for alogliptin and 25 V, 3.5 V, 34 V for the IS. The source temperature, desolvation temperature and desolvation gas flow were set at 150ºC, 500ºC and 1000 L/hr, respectively. The dwell time for each transition was 163 ms and argon gas operated at 3.5×10^{-3} bar. Detection of the ions was carried out in the multiple–reaction monitoring mode (MRM), by monitoring the transition pairs of *m/z* 340.2 precursor ion to the *m/z* 115.8 for alogliptin and *m/z* 344.2 precursor ion to the *m/z* 115.8 product ion for the IS. The analysis data obtained were processed by Masslynx SCN 843 (version 4.1).

Sample preparation

All the stock solutions were prepared in methanol and further working solutions were prepared in water and methanol (30:70, v/v; diluent). Two separate stock solutions were prepared for alogliptin at a concentration of 1 mg/mL and used for preparation calibration standards and quality control samples. Calibration standards were prepared at concentrations of 1.01, 2.02, 7.54, 15.1, 30.1, 60.3, 121, 181, 241 and 301 ng/mL in plasma. Similarly, quality control samples were prepared at 1.02 ng/mL (lower limit of quantitation, LLOQ), 2.55 ng/mL (low quality control, LQC), 33.1 ng/mL (medium quality control, MQC1), 150 ng/mL (MQC2) and

225 ng/mL (high quality control, HQC) concentration levels in plasma. All the prepared plasma samples were stored at –70 ± 10ºC until use.

All frozen samples were thawed in water bath and allowed to equilibrate at room temperature before processing. Each sample (50 µL) was spiked with 20 µL of IS dilution (500 ng/mL) and diluted with 100µL of HPLC grade water to dilute the sample. The sample mixture was loaded onto an Strata–X 33µm polymeric sorbent cartridge (30 mg/1 mL) that was pre–conditioned with 1.0 mL of methanol followed by 1.0 mL of water. The extraction cartridge was washed with 1.0 mL of water solution followed by 1.0 mL of 5% methanol. Then the sample was eluted with 1.0 mL of the mobile phase and injected.

Method validation procedures

We followed US FDA bioanalytical method validation guidelines to validate the present method[16]. The validation parameters include system suitability, carryover test, selectivity, sensitivity, linearity, precision and accuracy, recovery, dilution integrity and stability.

Selectivity, Matrix effect, and Sensitivity

Selectivity was assessed in eight different sources of plasma. Sensitivity was established by injecting six sets of plasma samples at LLOQ level. Matrix effect was evaluated by calculating IS normalized matrix factor (MF) at LQC and HQC levels. The mean area response of post–extraction spiked samples was compared with mean area of aqueous samples.

IS–normalized MF was calculated using the below formula:

IS normalized matrix Factor

$$= \frac{\text{Peak response area ratio in presence of matrix ions}}{\text{Mean peak response area ratio in absence of matrix ions}}$$

Sensitivity can be defined as lowest level of concentration that can be quantified with acceptable precision and accuracy. This concentration is known as LOQ and set at 1.01 ng/ mL or the present method.

Linearity, precision and accuracy

A total of five calibration curves were generated during the validation. Each calibration curve contains blank plasma, zero standard and ten non–zero concentrations. These calibration curves were analyzed individually by least square weighted ($1/x^2$) linear regression. Two batches were run in a same day to calculate intra-day precision and accuracy and remaining were run on three consecutive days. To check the ruggedness of the method, one batch was processed by the different analyst and analyzed on different column with similar dimensions of the same make. The % RSD at each QC level should not be greater than 15%, except for LLOQ QC where it should be 20%. The accuracy (%) must be within ±15% of their nominal value at each QC level except LLOQ QC where it must be within ±20%.

Recovery, dilution integrity and run size evaluation

Extraction recovery of the analyte was studied at LQC, MQC-2 and HQC (six replicates at each level). The extracted samples (mean area response) were compared with un-extracted sample (neat samples). Likewise, IS recovery was evaluated at working concentration (500 ng/mL). Dilution integrity was checked with 2.5 times concentration of ULOQ sample by diluted to 5- and 10-fold with screened blank plasma, respectively. Analytical batch size was assessed with a batch size containing 196 samples. This includes calibration curve standards with blank and zero sample (12 samples in total), bulk spiked QCs (160 samples) and 24 freshly spiked QCs.

Stability studies

Analyte stability in plasma as well as in neat samples was extensively evaluated using six replicate of samples at LQC and HQC levels. Plasma samples were stable up to 4 freeze–thaw cycles and 15 h at room temperature. Long term stability was also evaluated for 75 days. Processed samples were stable for 69 h in autosampler at 10°C and 65 h in refrigerator at 2-8°C. Whole blood samples were found to be stable for 2 h at similar concentration levels. The results were acceptable if the accuracy was within ±15% SD and the precision was ≤15% RSD.

Pharmacokinetic study protocol

A pharmacokinetic study of alogliptin 12.5 mg was conducted in healthy Indian male subjects (n = 12) under fasting condition. Informed consent was obtained from all the participants in the study. Each subject was screened for their healthiness before taken in to study. The subject with a body–mass index (BMI) of ≥18.0 kg/m^2 and ≤24.5 kg/m^2, with body weight not less than 50 kg with an age group of 20-40 years were selected for the study. A 4 mL aliquot of the blood was collected prior to dose to check the possible interference from *in-vivo* samples. Alogliptin 12.5 mg tablet was administered orally with 200 mL of water and

blood samples were collected at 0.33, 0.67, 1, 1.33, 1.67, 2, 2.33, 2.67, 3, 3.33, 3.67, 4, 4.33, 4.67, 5, 5.33, 5.67, 6, 8, 10, 12, 16, 24, 36, 48 and 72 h of post dose in K_2 EDTA vacutainer (4 mL) collection tubes (BD, Franklin, NJ, USA). The blood samples were centrifuged for 10 min at 4000 rpm and the supernatant plasma was collected and stored at –70 ± 10 °C till their use. WinNonlin software (Version 5.2) was used to calculate the pharmacokinetic parameters of alogliptin by employing non–compartmental model was employed for the preset study. Now a days, incurred sample reanalysis (ISR) is a necessary component of bioanalytical method validation and is intended to verify the reliability of the reported study sample analyte concentrations. Two samples were selected from each subject near to C_{max} and the elimination phase in the pharmacokinetic profile of the drug. The results were compared with the initial values. The percent change deviation allowed is ±20%[17,18].

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

The main goal of the study is to develop a sensitive LC-MS/MS method suitable for quantification of alogliptin effectively in terminal phase of its pharmacokinetic profile. Our target is to obtain high sensitivity with minimum use sample volume. For this, mass spectrometry conditions were suitably altered to get highest response from MRM channels without cross link. LC-MRM provides highest selectivity and sensitivity for the analyte and the IS[19, 20].

Tuning solution was prepared in water and methanol (40:60, v/v) at a concentration of 50 ng/mL and tuned in positive and negative ionization mode. High intense signals are obtained in positive mode than the negative mode. Various voltages like cone voltage, capillary voltage, and collision energy selectively altered to obtain highest peak response. Alternatively, desolvation temperature, source temperature, and desolvation gas flow were ideally set to obtain reproducible response. Protonated form of alogliptin and IS $[M + H]^+$ ion was the parent ion in the Q_1 spectrum and was used as the precursor ion to obtain Q_3 product ion spectra. The most sensitive mass transition was observed from m/z 340.2 to 115.82 for MMF (Fig. 2A) and from m/z 344.23 to 115.83 for the IS (Fig. 2B). The dwell time for each transition was set at 200 ms.

It's very much important to select a suitable buffer in mobile phase composition to better ionization. Unlike conventional HPLC, there are some limitations in use of buffers. Only, volatile buffers like ammonium acetate and ammonium formate can be used for LC-MS analysis. Additionally, acids like formic acid and acetic acid can also used in combination with organic solvents. Acetonitrile and methanol are most widely used organic solvents where HPLC coupled with MS detection. Ammonium acetate and ammonium formate at different strengths ranging from 2-10 mM were tested in combination with acetonitrile or methanol. But the response was insufficient to quantify or not reproducible or bad peak shape at LOQ level, with a combination of methanol or acetonitrile/ammonium formate and methanol or acetonitrile/ammonium acetate. Similarly, analytical columns of C_8 and C_{18} of different makes were tested for better selectivity and sensitivity of an assay. Although the better baseline separation was achieved on all columns, peak shape was not acceptable. Ultimately, an isocratic mobile phase composed of 5 mM ammonium formate, acetonitrile and methanol (20:40:40, v/v/v) and Kromasil 100-5 C_{18} (100 mm × 4.6 mm, 5 µm) column gave good peak shape and response even at lowest concentration level for the analyte and the IS. A combination of methanol and acetonitrile gives better sensitivity and sharp peaks for the alogliptin and the IS than alone. We observed that high proportion of (> 80%) of organic solvents helped for better resolution. Similarly, analyte response with 5 mM ammonium formate was much higher when compared with other buffers. Flow rate was altered from 0.5 to 1.5 mL/min to get optimum retention time. The total run time was set at 3.5 min, with retention time (RT) of 2.5 min alogliptin and the IS. In mass spectrometry analysis use of stable labeled isotopes as internal standard is recommended to avoid matrix effect. Also, these standards will increase the bioanalytical assay precision and accuracy. Hence, alogliptin 13C D3 was used as an internal standard.

The reported methods recommended protein precipitation (PP) method to extract the alogliptin from various biological samples. During the method development, numerous trials were carried out with LLE and SPE techniques to extract the analyte from plasma. Ethyl acetate, hexane, TBME, dichloromethane, diethyl ether, chloroform, alone and in combination under neutral and acidic conditions were used for LLE. But the recovery was poor about 60% for the analyte. Also, the recovery variation across the QCs was > 20 %. Therefore, SPE was tried on Strata X polymeric sorbent, Oasis HLB, Bond Elut Plexa, and Orpheus C_{18} cartridges to obtain consistent and reproducible recovery.

But, promising results were obtained with Starata X polymeric sorbent cartridge, which can produce a clean chromatogram for a blank sample and yields the highest recovery for the analyte and the IS from the plasma. Also, use of 5% methanol in water during washing step was essential to obtain cleaner extract with minimal or no matrix effect. Elution with mobile phase solution helped in obtaining quantitative and consistent recovery for analyte and the IS from plasma sample.

Chromatography

A blank sample and blank plasma sample with the IS was shown in Figure 3A and 3B, respectively. These chromatograms show no interference at RT of analyte and the IS from the plasma as well as from the IS. A total of 8 blank plasma lots including hemolytic and lipemic plasma were screened for selectivity test using proposed SPE procedure. All the lots were found to be free from signification interference. Also, selectivity was evaluated in presence of Over-the-Counter drugs (OTC). No interference peaks at the retention time of analyte and the IS were observed in the screened plasma samples spiked with co-administered drugs such as caffeine, paracetamol, dicyclomine, nicotine, ibuprofen, pantoprazole, and diphenhydramine (data not presented).

The lowest level of quantifiable concentration was set at 1.01, which is known as LOQ. At this concentration, the signal-to-noise (S/N) was ≥10. The precision and accuracy results at LLOQ level were found to be 4.19% and 95.5%, respectively. A model chromatogram of an LLOQ sample of alogliptin along with the IS is shown in the Fig. 3C. Fig. 4A and 4B shows the subject blank sample and a 2 h subject plasma sample obtained from a pharmacokinetic study after administration of 12.5 mg alogliptin tablet, respectively.

Matrix effect and Recovery

The extraction with the SPE gives cleaner samples with no significant matrix effect. For matrix effect post extraction spiked samples were compared with neat samples and was expressed as IS-normalized matrix factor. The matrix effect values at LQC and HQC level was shown in Table 1, which are within the acceptable limits. The average matrix factor valve at LQC and HQC level was 1.01 and 1.00. These values indicating that the response in the neat samples as well as plasma extract were almost the same with no significant matrix effect.

The mean overall recovery of alogliptin was 97.5±2.39% with the %RSD range of 0.96–3.14% and the recovery of the IS was 96.77%. The recoveries of analyte and the IS were good and reproducible. The results are presented in Table 2.

Linearity, precision and accuracy

A total of five calibration curves were generated during the validation in the range of 1.01–301 ng/mL. The correlation coefficient (r^2) for all the batches were ≥0.99. The mean linear equation obtained for alogliptin was y = (0.0192±0.0009)x+(0.0025±0.0008), where y is the peak area ratio of the analyte/IS and x the concentration of the analyte. The accuracy and %RSD for the calibration standards ranged from 98.3% to 101% and 0.51% to 2.81%.

The results for intra–day and inter–day precision and accuracy obtained from analysis of five validation runs in plasma quality control samples are summarized in Table 3. The intra–day precession ranged from 0.34 to 2.09% and the accuracy was within 92.4–95.8%. For inter–day test, the precision varied from 0.63 to 6.17% and the accuracy was within 92.4–96.5%.

Dilution integrity

The real-time study samples concentration above the ULOQ can be analyzed by diluting the samples with blank plasma. The unknown concentrations can be extended to 753 ng/mL by using dilution factor 5 and 10. The precision for dilution integrity of 5 and 10 dilution was found to be 0.08% and 0.77%, while the accuracy results were found to be 101% and 101%, respectively.

Long run evaluation

The main aim of the long run evaluation is to evaluate the maximum number of samples can be analyzed in a single run under a calibration curve. This will help to determine the length of a run during study sample analysis. As per the present validation, a total of 196 samples can be analyzed in a single run. A run size batch consists of 24 freshly spikes QC samples (6 sets at each level), 40 sets each of bulk spiked QCs (LQC, MQC-1, MQC-2 and HQC), and 12 calibration curve samples including blank sample and blank plasma spiked with the IS sample were analyzed in a single run. All the freshly spiked QCs were within 15% of their respective nominal (theoretical) values. Similarly, 155 out of 160 bulk spiked samples were passing the above criteria.

Stability studies

Alogliptin and the IS stock solutions were stable for 30 days when stored in refrigerated condition at 2-8 °C. The % stability (with the precision range) of alogliptin and the IS was

101 (0.30-0.76%) and 102% (0.32-0.89%), respectively. The stability results of alogliptin were presented in the Table 4. All the stability results were well within the acceptable results as specified in the recent US FDA guidelines.

Pharmacokinetic results

The suitability of the proposed method for *in-vivo* use was demonstrated by analyzing human plasma samples for a pharmacokinetic study in 12 healthy Indian male subjects. The mean concentration–time profiles of alogliptin after a single oral dosage of 12.5 mg alogliptin tablets is shown in Fig. 5 and the corresponding pharmacokinetic parameters are listed in Table 5. The alogliptin concentration were quantifiable from 15 min and up to 76 h of post dose.

Incurred sample reanalysis

The method reproducibility was further evaluated by reanalysis of subject samples. The % change in the study sample concentration was within ±15% from the initial results (Table 6). The ISR results encourage the proposed method for its use in clinical application.

CONCLUSION

An improved and sensitive UPLC-MS/MS method was proposed for the determination of alogliptin in human plasma using a deuterated analog as an internal standard. The method was fully validated as per the US FDA guidelines. The method provided good linearity in the range of 1.01-301 ng/mL. The optimized SPE method gave high and reproducible recovery for the alogliptin with no interference or matrix effect from the endogenous components. The method was successfully applied to a pharmacokinetic study with 12.5 mg of alogliptin in 12 healthy Indian subjects and the data was confirmed by ISR.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge PCR Laboratories (Hyderabad, India) for providing necessary facilities to carry out this work.

Table 1: Matrix effect of Alogliptin in human plasma (n = 3)

Plasma lot	LQC (2.55 ng/mL)			HQC (225 ng/mL)		
	Concentration found (mean ± SD; ng/mL)	% Accuracy	IS normalized MF	Concentration found (mean ± SD; ng/mL)	% Accuracy	IS normalized MF
Lot 1	2.39 ± 0.21	94.0	1.010	240 ± 10.7	106	0.998
Lot 2	2.42 ± 0.28	95.1	1.001	243 ± 7.47	108	1.002
Lot 3	2.67 ± 0.05	105	1.007	234 ± 5.89	104	0.999
Lot 4	2.49 ± 0.13	97.7	1.009	234 ± 2.93	104	1.001
Lot 5	2.61 ± 0.22	102	1.009	232 ± 8.20	103	0.999
Lot 6	2.66 ± 0.18	105	1.004	236 ± 4.29	102	0.998
Lot 7	2.64 ± 0.01	105	1.002	236 ± 4.61	97.8	1.000
Lot 8	2.63 ± 0.20	103	1.006	230 ± 4.59	102	1.001

Table 2: Recovery results of Alogliptin and Alogliptin 13C D3 (IS)

Compound name	Sample concentration (ng/mL)	Response extracted (mean ± SD)	Response Un-extracted (mean ± SD)	Recovery (%)	Mean ± SD (% CV) recovery
Alogliptin	2.55	19549 ± 260	20572 ± 330	95.0	97.5 ± 2.38 (4.77%)
	150	1233036 ± 29777	1263860 ± 39659	97.6	
	225	1737974 ± 16639	1741591 ± 34489	99.8	
Alogliptin 13C D3	500	454576 ± 8296	469922 ± 15924	96.7	-

Table 3: Precision and Accuracy data for Alogliptin

Quality control	Run	Concentration found (mean ± SD; ng/mL)	Precision (%)	Accuracy (%)
Intra–day variations (Six replicates at each concentration)				
LLOQ		0.94 ± 0.02	2.09	92.4
LQC		2.40 ± 0.03	1.21	94.3
MQC1		31.5 ± 0.11	0.34	95.4
MQC2		144 ± 0.38	0.26	95.8
HQC		216 ± 1.00	0.46	95.8
Inter–day variations (Eighteen replicates at each concentration)				
LLOQ		0.94 ± 0.06	6.17	92.4
LQC		2.37 ± 0.10	4.42	93.2
MQC1		31.9 ± 0.34	1.07	96.5
MQC2		144 ± 1.90	1.31	96.4
HQC		215 ± 1.35	0.63	95.6
Spiked concentrations of LLOQ, LQC, MQC1, MQC2 and HQC are 1.02, 2.55, 33.1, 150 and 225 ng/mL, respectively.				

Table 4: Stability data for Alogliptin in plasma (n=6)

Stability test	QC (spiked concentration, ng/mL)	Mean±SD (ng/mL)	Accuracy/ Stability (%)	Precision (%)
Aautosampler stability (at 15°C for 70 h)	2.55 225	2.52 ± 0.12 217 ± 0.52	99.1 96.4	4.75 0.24
Wet extract stability (at room temperature for 66 h)	2.55 225	2.46 ± 0.02 218 ± 0.40	96.6 96.7	0.67 0.18
Bench top stability (15 h at room temperature)	2.55 225	2.52 ± 0.09 218 ± 0.56	99.0 96.9	3.56 0.25
Freeze–thaw stability (5 cycles)	2.55 225	2.57 ± 0.17 218 ± 0.80	101 96.7	6.75 0.37
Reinjection stability (26 h)	2.55 225	2.48 ± 0.04 233 ± 0.63	97.5 103	1.62 0.27
Long–term Stability (at –70°C for 60 days)	2.55 225	2.54 ± 0.08 220 ± 1.34	99.9 97.6	2.96 1.34

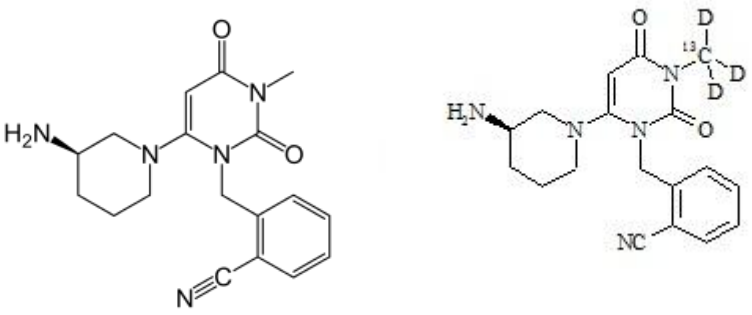
Table 5: Pharmacokinetic parameters of Alogliptin after single oral administration of 12.5 mg Alogliptin tablet to healthy south Indian Male Subjects (n=12, MEAN ± SD)

Parameter	Mean ± SD
C_{max} (ng/mL)	48.7 ± 3.48
t_{max} (h)	3.03 ± 1.18
AUC_{0-t} (ng h/mL)	790 ± 67.9
AUC_{0-inf} (ng h/mL)	831 ± 77.6
$t_{1/2}$ (h)	18.7 ± 2.09
Kel (h^{-1})	0.04 ± 0.004

Table 6: Incurred samples re–analysis data of Alogliptin

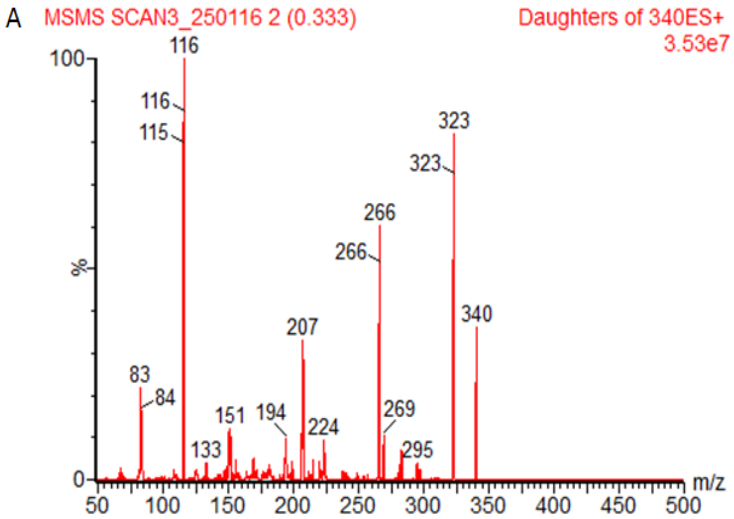
Sample	Initial conc. (ng/mL)	Re–assay conc. (ng/mL)	Difference [a] (%)
1	46.4	50.2	-7.79
2	3.01	2.99	0.57
3	54.1	55.2	-2.09
4	4.45	4.54	-2.00
5	46.5	41.2	12.0
6	4.74	4.62	2.5
7	45.2	42.2	6.86
8	5.39	5.56	-3.2
9	48.0	44.9	6.50
10	4.74	4.61	2.8
11	45.5	44.6	1.93
12	5.39	4.93	8.88
13	39.8	42.3	-6.14
14	6.90	7.91	-13.8
15	44.8	44.5	0.56
16	6.68	5.85	13.2
17	40.4	43.2	-6.72
18	4.87	4.93	-1.23
19	47.7	50.2	-5.12
20	3.79	3.97	-4.87
21	51.7	49.2	4.90
22	5.94	6.04	-1.75
23	44.9	43.3	3.65
24	5.73	5.61	2.11

[a] Expressed as [(initial conc.−re–assay conc.)/average]×100%.



(A) Alogliptin **(B) Alogliptin 13C D3**

Fig. 1: Chemical structures of (A) alogliptin and (B) alogliptin 13C D3 (IS).



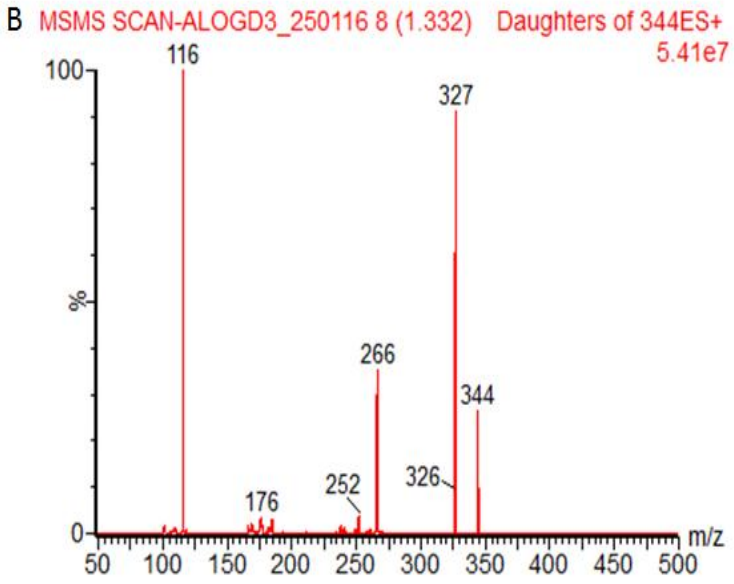


Fig. 2: Product ion mass spectra of $[M+H]^+$ of (A) alogliptin and (B) alogliptin 13C D3 (IS).

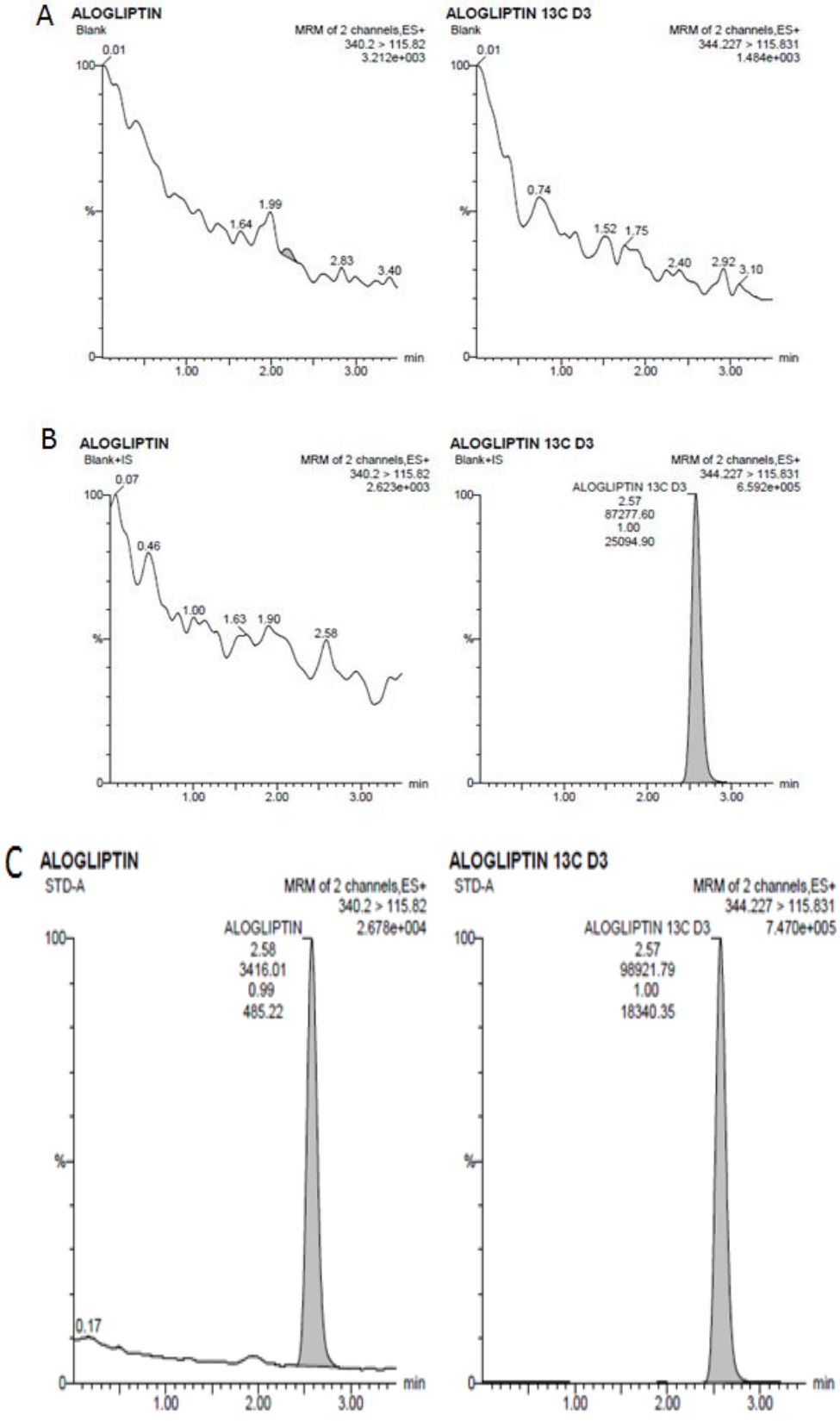


Fig. 3: Representative MRM ion–chromatograms of alogliptin (left panel) and the IS (right panel) in human blank plasma (A), human plasma spiked with IS (B) and a LLOQ sample along with IS (C).

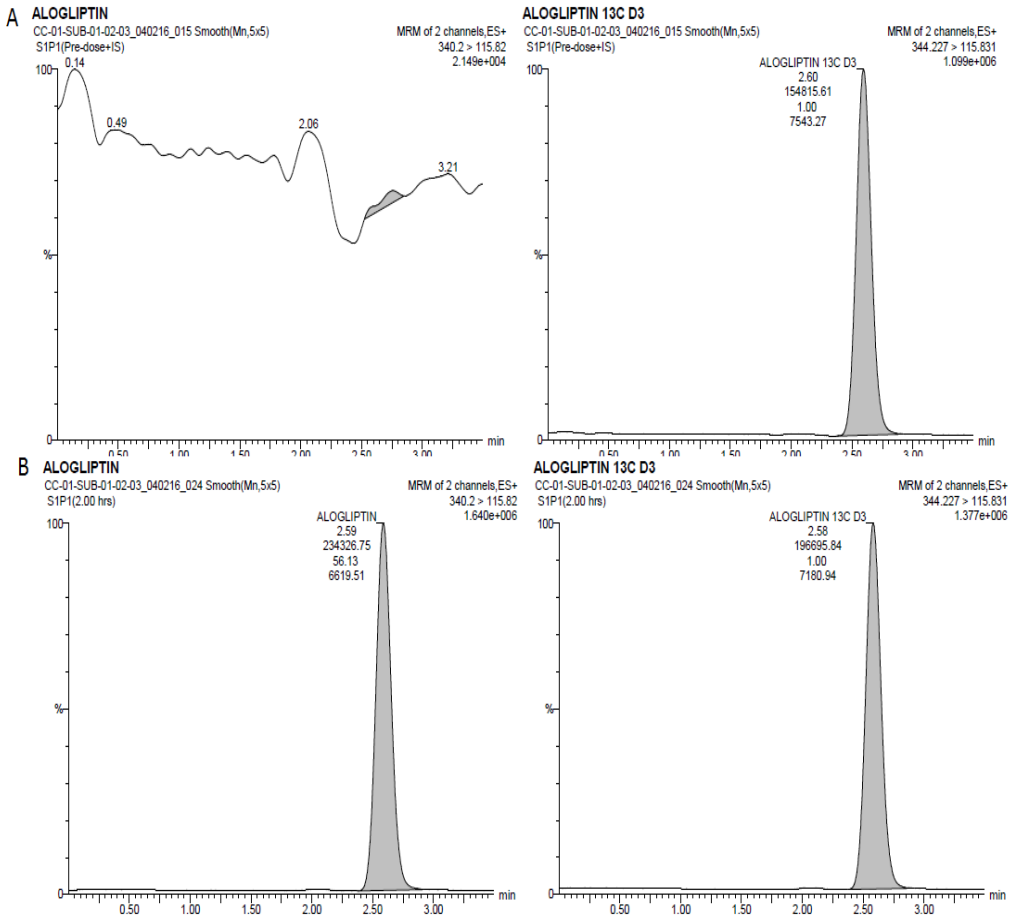


Fig. 4: MRM chromatograms resulting from the analysis of subject blank plasma sample (A) and 2 h subject plasma sample (B).

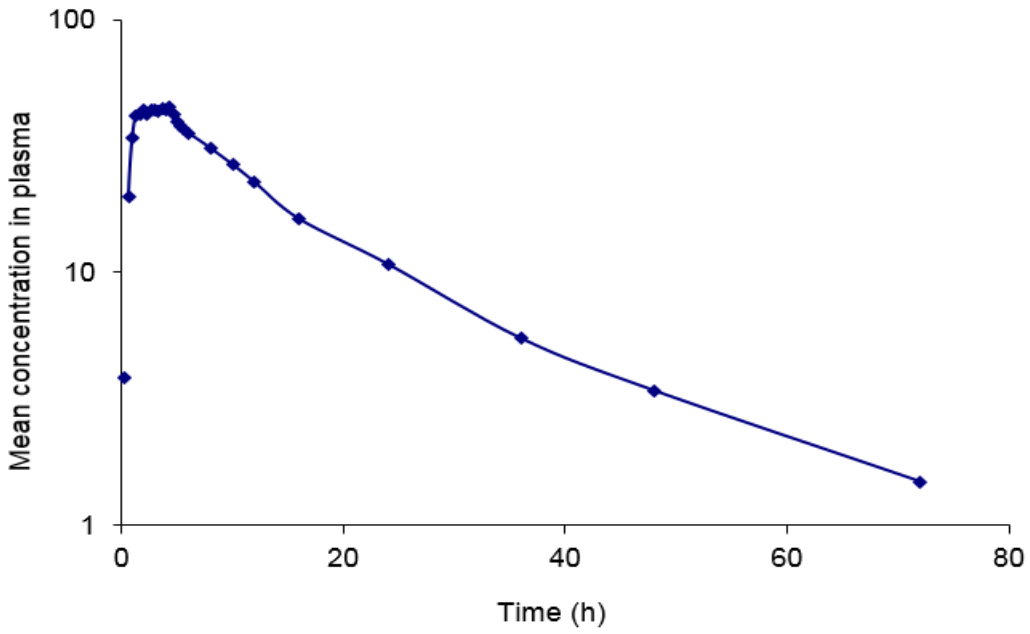


Fig. 5: Mean plasma concentration–time profile of alogliptin in human plasma following oral administration of alogliptin 12.5 mg tablet to healthy volunteers (*n*=12).

REFERENCES

1. Freeman JS. Role of the incretin pathway in the pathogenesis of type 2diabetes mellitus. Cleve Clin J Med. 2009;76:S12–9.
2. Dudkowski C, Tsai M, Liu J, Zhao Z, Schmidt E and Xie J. The pharmacokinetics and pharmacodynamics of alogliptin in children, adolescents, and adults with type 2 diabetes mellitus. Eur J Clin Pharmacol. 2017;73:279 – 88.
3. NESINA (alogliptin) tablets. Full prescribing information. Available at: http://general.takedapharm.com/content/file.aspx?FileTypeCode=NESINAPI&cacheRandomizer=0848c290-fc71-4f10-aa76-c0aa36b27802. Takeda Pharmaceuticals America, Inc. Deerfield, IL, USA. 2016.
4. Kay S, Strickson A, Puelles J, Selby R, Benson E and Tolley K. Comparative effectiveness of adding alogliptin to metformin plus sulfonylurea with other DPP-4 inhibitors in type 2 diabetes: A systematic review and network meta-analysis. Diabetes Ther. 2017;8:251–73.
5. Covington P, Christopher R, Davenport M, Fleck P, Mekki QA and Wann ER. Pharmacokinetic, pharmacodynamic, and tolerability profiles of the dipeptidyl peptidase-4 inhibitor alogliptin: a randomized, double-blind, placebo-controlled, multiple-dose study in adult patients with type 2 diabetes. Clin Ther. 2008;30:499–512.
6. Mowaka S, Elkady EF, Elmazar MM and Ayoub BM. Enhanced LC-MS/MS determination of alogliptin and metformin in plasma: Application to a pharmacokinetic study. Microchemical J 2017;130:360–65.
7. Abdel-Ghany MF, Ayad MF and Tadros MM. Enhanced LC-MS/MS analysis of Alogliptin and pioglitazone in human plasma: Applied to a preliminary pharmacokinetic study. J Chromatogr B. 2017;1058:93–101.
8. Hemavathi G and Hipparagi SM. Sensitive LC-MS/MS method for the simultaneous determination of alogliptin and voglibose in human plasma. J Anal Bioanal Tech. 2017;8:354.
9. Chen H, Xia X, Li L, Jiang W, Wang Y, Xia H, Wang Z and Wang Y. Pharmacokinetic and bioavailability study of alogliptin in rat plasma by UPLC-MS/MS. Lat Am J Pharm. 2016;35: 233–8.
10. Kai S, Ishikawa K, Ito H, Ogawa T, Yamashita H and Nagata Y. Simultaneous Analysis of Oral Antidiabetic Drug by LC-MS/MS. Chromatography. 2016;36:19–24.
11. Kole PL, Venkatesh G, Kotecha J and Sheshala R. Recent advances in sample preparation techniques for effective bioanalytical methods. Biomed Chromatogr. 2011;25:199–217.
12. Van Eeckhaut A, Lanckmans K, Sarre S, Smolders I and Michotte Y. Validation of bioanalytical LC–MS/MS assays: evaluation of matrix effects. J Chromatogr B. 2009;877:2198–2207.
13. Matta M, Burugula L, Pilli N, Inamadugu J and Rao JVLNS. A novel LC-MS/MS method for simultaneous quantification of tenofovir and lamivudine in human plasma and its application to a pharmacokinetic study. Biomed Chromatogr. 2012;26:1202–9.
14. Matta M, Pilli NR, Inamadugu J and Rao JVLNS. Simultaneous quantitation of lamivudine, zidovudine and nevirapine in human plasma by liquid chromatography–tandem mass spectrometry and application to a pharmacokinetic study. Acta Pharmaceutica Sinica B. 2012; 2:472–480.
15. Matta M, Pilli NR and Rao JVLNS. A validated liquid chromatography and tandem mass spectrometric method for simultaneous quantitation of tenofovir, emtricitabine, and efavirenz in human plasma and its pharmacokinetic application, Acta Chromatogr. 2013;27:27–39.
16. US DHHS, FDA, CDER. Guidance for Industry (draft). Bioanalytical Method Validation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), a Center for Veterinary Medicine (CV). 2013. Available at: http://www/fda.gov/cder/guidance/index.htm.
17. Fast DM, Kelley M, Viswanathan CT, O'Shaughnessy J, King SP and Chaudhary A. Workshop report and follow-up AAPS Workshop on current topics in GLP bioanalysis: Assayreproducibility for in

curred samples implications of Crystal City recommendations. AAPS J. 2009;11:238 – 41.
18. De Boer T and Wieling J. Incurred sample accuracy assessment: design of experiments based on standard addition. Bioanalysis. 2011;3:983–992.
19. Matta MK, Chockalingam A, Gandhi A, Stewart S, Xu L and Shea K. LC-MS/MS based quantitation of ciprofloxacin and its application to antimicrobial resistance study in Balb/c mouse plasma, urine, bladder and kidneys. Anal Methods. 2018;10:1237– 46.
20. Matta MK, Narayanasamy S, Thomas DC, Xu L, Stewart S and Chockalingam A. A sensitive UPLC-APCI-MS/MS method for determination of dexamethasone and its application in an ocular tissue distribution study in rabbits following topical administration. Anal Methods. 2018.