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A validated LC-MS/MS method for the pharmacokinetic study of alogliptin in healthy rabbits

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ABSTRACT

A liquid chromatography-tandem mass spectrophotometric (LC-MS/MS) method was developed for quantification of Alogliptin in rabbit plasma employing Liquid-Liquid extraction technique. The developed method was validated for specificity, precision, accuracy, recovery, and stability characteristics. Chromatographic separation was achieved on Inertsil ODS 5 µm C18, 50 × 4.60 mm with 30:70 v/v of 0.1% formic acids: Organic Mixture (acetonitrile: methanol, 80:20% v/v) as an isocratic mobile phase with a flow rate of 1.0 ml/min. the developed LC-MS method was applied to assess pharmacokinetics parameters of alogliptin tablet in healthy rabbits. Alogliptin showed T_{max} of 2.6 ± 0.37 h and mean C_{max}, AUC 0-t and AUC0 for test formulation is 90.6 ± 4.41, 1675.41 ± 164 and 3726.47 ± 796 respectively.

INTRODUCTION

The International Diabetes Federation (IDF) reported about 382 million patients affected by diabetes around the world, and this number will rise to 592 million by 2035 (Guariguata *et al.*, 2014). Type 2 diabetes is a chronic and progressive disease, characterized by resistance to insulin and malfunction of b-cell (Meier, 2008; Kaneto *et al.*, 2010; Robertson *et al.*, 2004). The enzyme dipeptidyl peptidase-4 (DPP-4) widely distributed in living cell and blood circulation and could inactivate an insulin tropic endogenous hormone glucagon-like peptide-1 (GLP-1), playing an important role in glucose metabolism (Drucker, 1998; Nauck, 1999; Wang *et al.*, 1995; Kieffer *et al.*, 1995; Deacon *et al.*, 1995). The review of the literature revealed a few methods reported for determination of alogliptin benzoate. These include UV spectrophotometric (Moritoh *et al.*, 2009), HPTLC (Yadav *et al.*, 2014), HPLC methods (Sharma and Parle, 2015; El-Bagary *et al.*, 2012; Zhang *et al.*, 2015; Deng *et al.*, 2015) and chiral HPLC method (Yadav *et al.*, 2014). Hence it is essential to develop more sensitive analytical methods to determine the concentration of alogliptin from plasma samples. To best of our knowledge, no published LC-MS/MS-based methods for the pharmacokinetic study of alogliptin in healthy rabbits. Therefore an LC-MS/MS method was developed, validated and applied for quantification of Alogliptin in rabbit plasma employing liquid-liquid extraction (LLE) technique. The LLOQ of the method was found to be low for conducting a pharmacokinetic study with any marketing formulation of alogliptin in human volunteers.

EXPERIMENT

Apparatus and software

The LC-MS/MS used for the study consist of Shimadzu LC-20ADvp (Shimadzu, Japan) coupled with Applied Biosystem sampler Sciex (MDS Sciex, Canada) API 4000 Tandem mass spectrometer and the autosampler was SIL-HTC from Shimadzu, Japan. The system was operated by Analyst software (version: 1.4.2; Applied Biosystems).

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Chemicals and reagents

Alogliptin and Alogliptin D3 (IS) were procured by Hetero Drugs Ltd., Hyderabad, India, formic acid was purchased from Merck Specialities Pvt. Ltd, Mumbai, India. The water for chromatography was produced from water purification systems (Milli Q, Milli Pore, USA) installed in the laboratory. The HPLC grade methanol and acetonitrile were supplied by Baker, USA. Hyderabad. The rabbit plasma was prepared by freeze centrifugation. The study was approved by Institutional Ethical committee no: VCP/IAEC/2016-44.

Calibration standard solutions

Stock solutions (1 mg/ml) of Alogliptin and Alogliptin D3 internal standard **(IS)** were prepared in methanol further the eight calibration standards were prepared from stock by diluting with 70% methanol subsequently they are spiked with drug free plasma to give the concentrations of 4.00, 8.00, 25.00, 50.0, 100.0, 150, 300, and 600 ng/ml.

Quality control standards

Lowest quality control (LQC), median quality control (MQC) and highest quality control (HQC) standards were prepared from stock by spiking drug-free plasma with Alogliptin to give a solution containing 8, 250, and 500 ng/ml respectively. They were stored at −20°C till the time analyzed.

Chromatographic conditions

Chromatographic separation was achieved on Inertsil ODS-C18 (50 × 4,6 mm, 5 µm) with 0.1% formic acid-acetonitrile-methanol (30: 56: 14 v/v). as an isocratic mobile phase with a flow rate of 1.0 ml/min. Injection volume was 5 µl. The total chromatographic run time was fixed to 2.00 minutes and column oven and autosampler temperature was set to 40°C and 5°C, respectively.

Mass spectrometric conditions

The LC eluent was split (75%), and approximately 0.25 ml/min was introduced into LC and the Quantitation was achieved through negative ion mode by employing turbo ion spray interface at 400ºC. The 5500 V voltage for ion spray and the pressure 8, 10 and 6 psi for a nebulizer, the curtain was set at 5500 V. The source parameters viz., the nebulizer, curtain and CAD gas were set at 8, 10, and 6 psi, respectively. The compound parameters viz. the declustering potential (DP), collision energy (CE), entrance potential (EP) and collision cell exit potential (CXP) for alogliptin and alogliptin-D3 were similar and are set at 18, 10, 10, and 10 V. The multiple-reaction monitoring mode (MRM) was employed for detecting and monitoring the transition pairs of m/z 340.2 → 116.0 for alogliptin, and m/z 343.3 → 196.2 for IS.

Study design

Six male albino Rabbits (weighing about 2.5 kg) were selected as the animal model. The age of the rabbits was 8–12 weeks. The rabbits selected for the study had no medication for two weeks prior to the study. Twelve hours before drug administration, food was withdrawn from the rabbits until 24 hr post-dosing, while, water was available for rabbits throughout the study. The tablet with the dose of 0.59 mg based on the animal surface area was administered to rabbits using a balling gun. The study was approved by Institutional Ethical committee no: P50/VCP/IAEC/2016/09/DBP/AE12/Rabbits. Blood samples (0.6 ml) were withdrawn from the marginal ear vein before dosing (zero time) and at time intervals of 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 24, 36, and 48 hours after administration. For each animal, the total number of blood samples drawn during the study was 16. EDTA disodium salt was used as an anticoagulant. Plasma was separated by centrifugation at 5000 rpm for 10 min and the resulting plasma sample from each blood sample was divided into two aliquots and stored in suitably labeled polypropylene tubes at –20°C until used. All the plasma samples were analyzed under the construction of standard calibration curve of alogliptin in rabbit's plasma. The alogliptin concentrations in the rabbit plasma samples was calculated using the calibration curve, obtained after linear regression of the peak area ratio (alogliptin/alogliptin-D3) versus the concentration of alogliptin.

Sample preparation method

The samples were prepared by mixing 50 µl of alogliptin D3 (50 ng/ml) and 100 µl buffer (0.05 M NaOH, pH: 4) to 250 µl of plasma. The drug was extracted by employing LLE method using 2.5 ml of ethyl acetate followed by centrifugation at 2000 rpm/min for 15 min at 4ºC. The supernatant was withdrawn and dried using lyophilizer. The residue was reconstituted with 300 µl of the mobile phase.

Pharmacokinetic analysis

The pharmacokinetic parameters for single oral dose non-compartmental study were calculated through PK Solver tool. The C_{max} (maximum plasma concentration) and T_{max} (time to maximum plasma concentration) were obtained directly from the plasma time profile curve. The linear trapezoidal rule was used to estimate all other pharmacokinetic parameters. i.e. AUC 0-t, AUC 0-∞, $t_{1/2}$ and ke.

VALIDATION

Specificity

The specificity of the method was determined by injecting 4.0 ng/ml concentration under optimized chromatographic conditions to the LC system to show the separation of alogliptin from impurities and plasma. The specificity of the method was checked for the interference from plasma.

Linearity

The linearity of the method was determined by plotting eight-point calibration curves (4 ng/ml to 600 ng/ml) between actual and calculated concentrations of samples spiked with plasma.

Recovery studies

Two sets of six HQC, MQC, and LQC samples were prepared, one is with plasma and another one is with 70% methanol. The % mean recoveries were determined by measuring the responses of the plasma quality control samples against quality control samples prepared with 70% methanol.

Precision and accuracy

Intraday precision and accuracy was obtained by analyzing the quality control standards containing concentrations 8, 250, and 500 ng/ml and LLOQ Quality control standard containing concentration 4.00 ng/ml five times a day randomly, interday precision and accuracy was achieved by determining each quality control standards (8, 250, and 500 ng/ml) and LLOQ Quality Control standards (4.00 ng/mL) once on each of five different days.

Matrix effect

The matrix effect for the established method was determined by using six lots of rabbit plasma and is used to prepare the concentrations equivalent to LQC and HQC of alogliptin the samples prepared were injected on to the column.

Stability studies

The stability of alogliptin was determined by measuring concentration change in control samples overtime under set conditions. Freeze-thaw stability study (−80ºC) of alogliptin was carried out by subjecting samples to three freeze and thaw cycles. Samples before the study and after study were analyzed by developed method. Similarly stock solution stability study of alogliptin (Stability after 6 Hours), Long-term stability (−80ºC, 30 days), benchtop stability study of alogliptin (at ambient temperature, 6 h), Dry residue stability (4ºC, 48 h) and Auto-sample stability (4ºC, 24 h) of alogliptin were carried out by subjecting samples to study conditions.

RESULTS & DISCUSSIONS

Results of method validation

The chromatograms obtained during method validation was acceptable and the representative chromatograms of standard blank, HQC, MQC, and LQC samples are shown in (Figures 1 to 4).

The linearity, accuracy & precision, and stability of the method were established as per ICH guidance (ICH guidelines Q1A(R2), 2005; ICH guidelines Q2(R1), 2005).

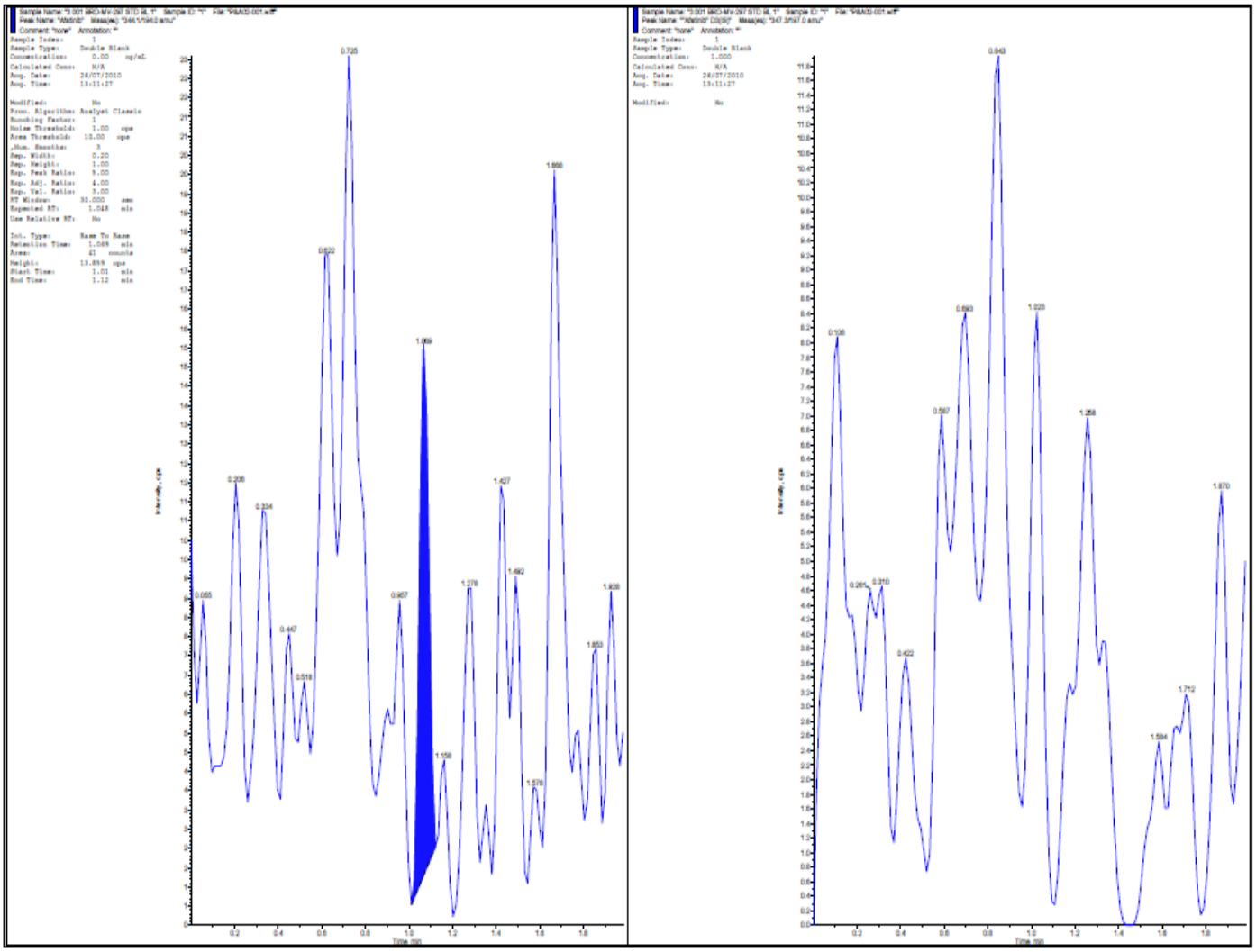

Fig. 1: blank chromatograms of alogliptin and IS in blank Plasma.

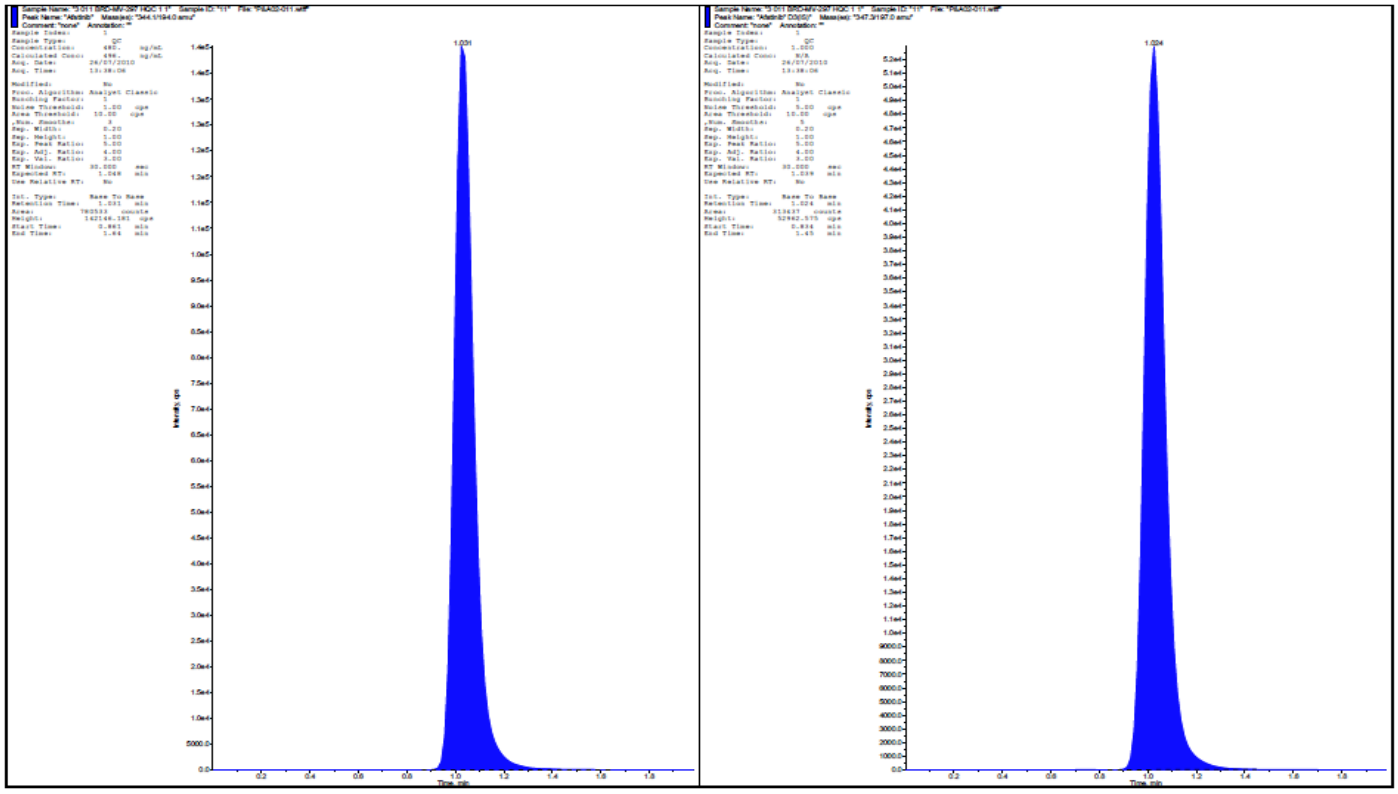

Fig. 2: HQC-chromatograms of alogliptin in plasma with internal standard.

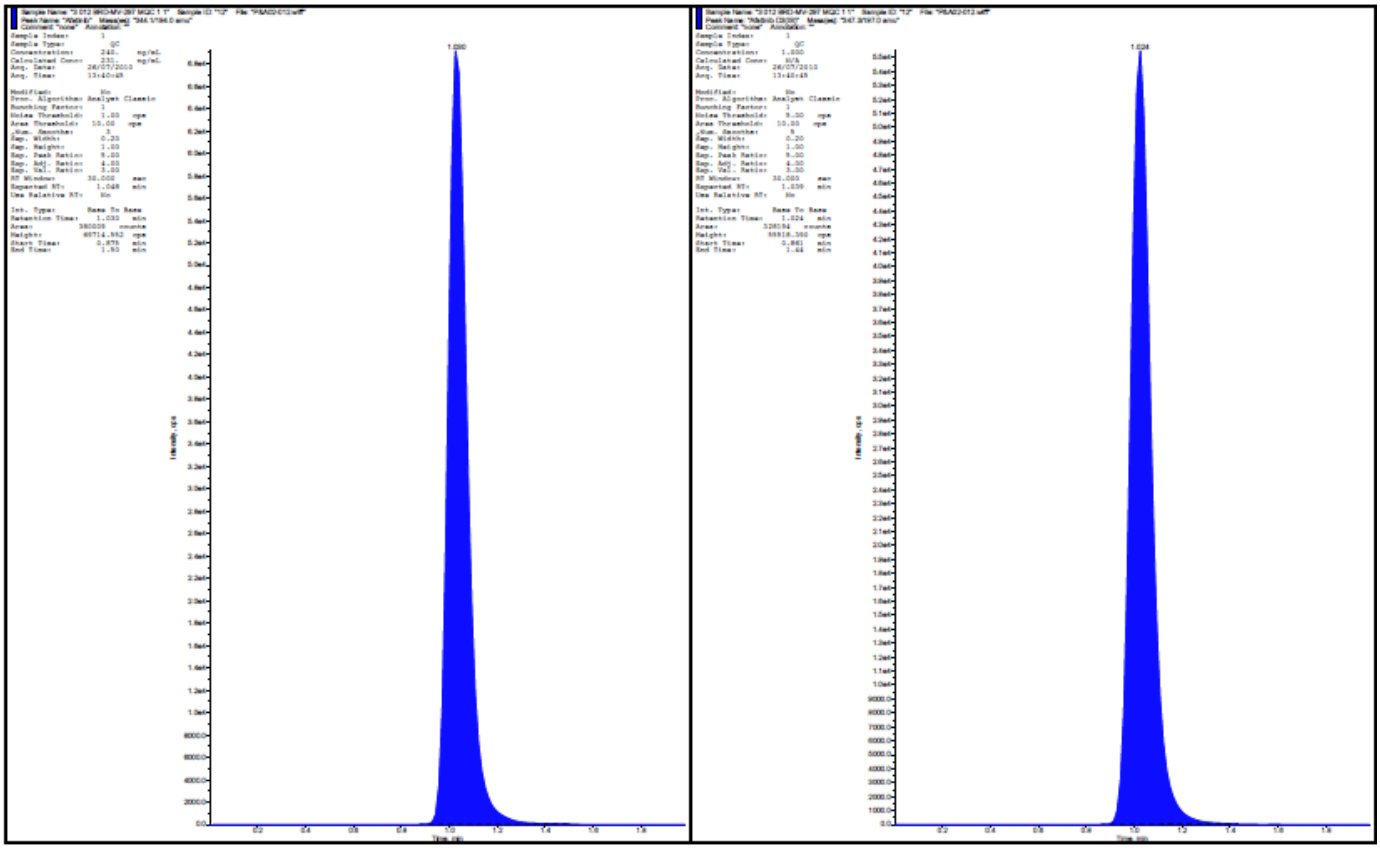

Fig. 3: MQC-chromatograms of alogliptin & its internal standard.

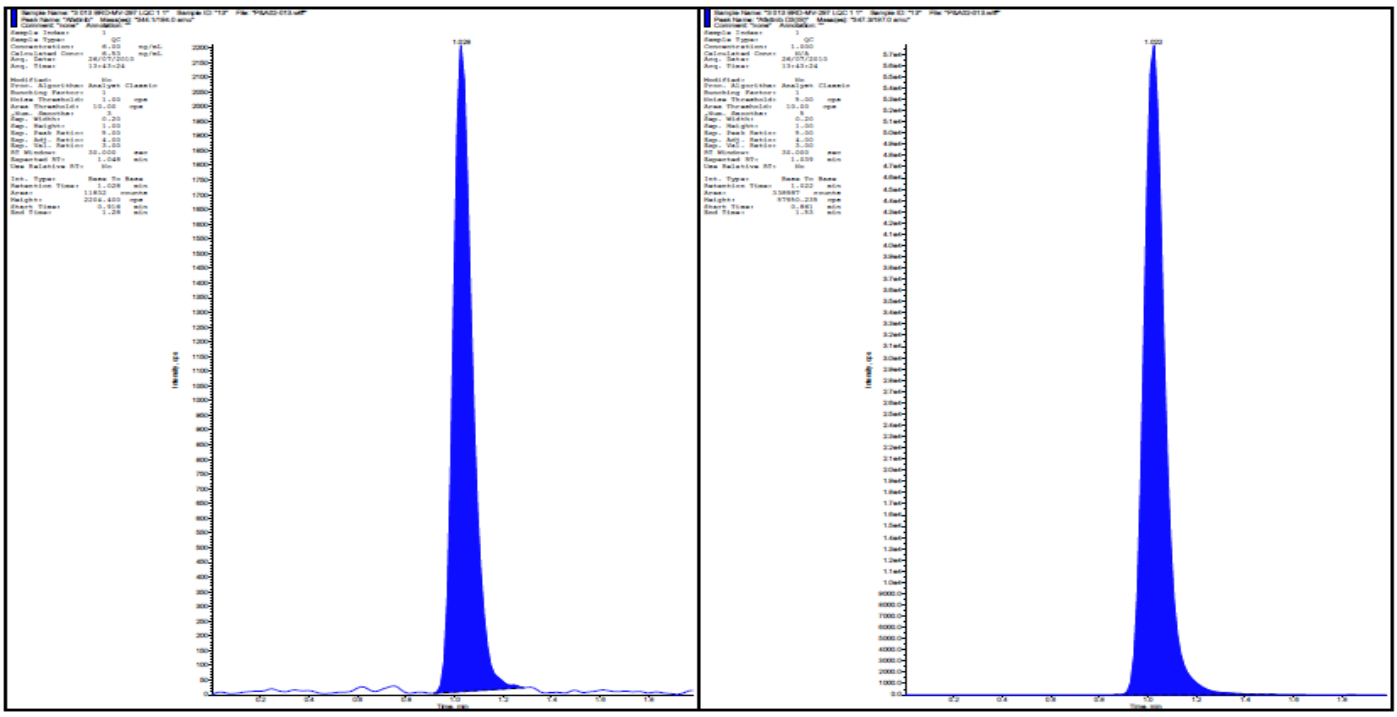

Fig. 4: Chromatograms of alogliptin & its internal standard at LQC Level.

Linearity

The calibration curve (calculated concentrations Vs actual Concentration) obtained (n = 3) were linear over working range of 4 ng/ml to 600 ng/ml (Figure 5 and Table 1). The linear regression equation for the analysis was 0.994x-2.692 with coefficient of correction (r^2) = 0.999.

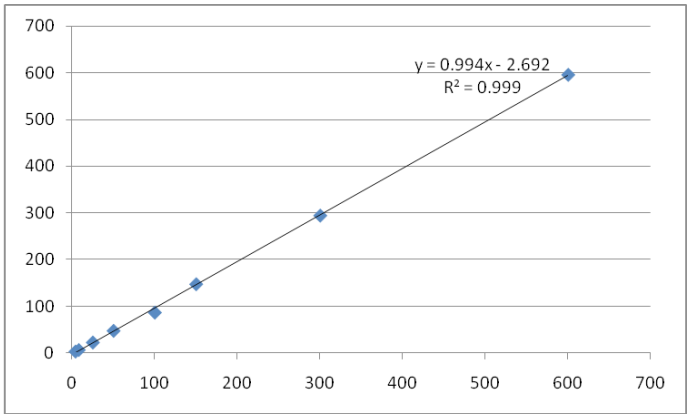

Fig. 5: Calibration curve.

Table 1: Summary of Linearity Standards for Alogliptin.

Actual conc. (ng/mL)	4	8	25	50	100	150	300	600
Set I	4.086	7.613	22.98	48.88	85.2	150.08	291.54	592.39
Set II	3.983	7.384	23.21	47.9	88.59	143.01	292.25	594.3
Set III	4.08	7.259	24.09	48.11	88.27	150.96	300.36	600.14
Mean	4.04	7.41	23.42	48.29	87.35	148.01	294.71	595.61
± SD	0.05	0.17	0.58	0.51	1.87	4.35	4.90	4.03
% CV	1.43	2.42	2.50	1.07	2.14	2.94	1.66	0.68
% Accuracy	101.2	92.7	93.7	96.5	87.3	98.6	98.23	99.2

Table 2: The % Mean Recovery of alogliptin for LQC, MQC, and HQC.

ID	LQC			MQC			HQC		
	Unextracted (area ratio)	Extracted (area ratio)	% Recovery	Unextracted (area ratio)	Extracted (area ratio)	% Recovery	Unextracted (area ratio)	Extracted (area ratio)	% Recovery
1	0.086	0.098	113.95	0.96	1.044	108.75	2.017	2.098	104.02
2	0.086	0.1	116.28	0.974	1.052	108.01	2.057	2.1	102.09
3	0.09	0.103	114.44	0.981	1.037	105.71	1.854	2.084	112.41
4	0.095	0.104	109.47	0.926	1.04	112.31	1.988	2.091	105.18
5	0.093	0.103	110.75	0.931	1.045	112.24	1.929	2.036	105.55
6	0.095	0.103	108.42	0.936	1.049	112.07	1.913	2.106	110.09
Mean	0.091	0.102	112.221	0.951	1.045	109.849	1.960	2.086	106.555
± SD	0.004	0.002	3.116	0.023	0.006	2.774	0.075	0.026	3.899
% CV	4.59	2.27	2.78	2.47	0.53	2.53	3.81	1.23	3.66

Recovery

The % mean recovery of the method for LQC(8 ng/ml), MQC (250 ng/ml) and HQC (500 ng/ml) was found to be 112.2%, 109% and 106% respectively (Table 2).

Intraday and Inter-day Precision

The mean intraday and inter-day precision of the method for alogliptin was found to be 1.26 to 4.97% (≤15%) for LQC, MQC, HQC, and LLOQC (Table 3).

Table 3: Intra-day and Inter-day quality control samples for alogliptin.

QC	Alogliptin (ng/mL)			
Intra-batch	LLOQ QC (4 ng/mL)	LQC (8 ng/mL)	MQC (250 ng/mL)	HQC (500 ng/mL)
Mean	3.91	8.25	230.00	520.00
SD	0.084	0.23	2.9	7.0
% CV	2.14	2.78	1.26	1.34
Mean	3.64	8.64	227.00	516.00
SD	0.085	0.22	4.30	6.80
% CV	2.33	2.54	1.89	1.31
Mean	3.82	7.83	222	493
SD	0.183	0.30	4.4	11.5
% CV	4.71	3.84	1.98	2.33
Inter-batch	LLOQ QC (4 ng/mL)	LQC (8 ng/mL)	MQC (250 ng/mL)	HQC (500 ng/mL)
Mean	3.79	8.24	231.00	502.00
SD	0.16	0.41	6.2	24.3
% CV	4.21	4.97	2.68	4.84

Matrix effect

After the analysis of HQC and LQC from six different plasma lots the % CV was observed 0.1% and 2.8% (≤15%) respectively (Table 4).

Table 4: Matrix effect obtained with six different lots of plasma.

QC ID	LQC	HQC
Actual conc.	8 (ng/mL)	500 (ng/mL)
1	8.026	498.80
2	8.028	499.89
3	8.028	499.90
4	8.028	499.86
5	8.027	499.86
6	8.031	498.86
Mean	8.0280	499.86
± SD	0.00167	0.5099
% CV	2.80	0.1

Stability studies

The quality control samples (HQC and LQC) were used for stability studies. The % mean stability for HQC and LQC were presented in table 5 and are within the acceptance limits of 85 to 115%. (Table 5).

PHARMACOKINETIC STUDIES

The Pharmacokinetic parameter of alogliptin was calculated from the plasma concentration-time curves using pk solver software. Also, the area under the plasma concentration-time curve from 0 to 48 hr ($AUC_{0\text{-}48}$) was calculated using trapezoidal rule. Alogliptin showed T_{max} of 2.6 ± 0.37 and mean $C_{max,}$ AUC_{0-t} and $AUC_{0-\alpha}$ for Test formulation is 90.6 ± 4.41, 1675.41 ± 164 and 3726.47 ± 796 respectively The results were presented in Table 6, Table 7 and Figure 6.

Table 5: Results of stability studies.

Stability Condition	Nominal	Calculated concentration	
	Concentration (ng/mL)	Mean ± SD	% Bias
Freeze-thaw stability (−80°C)	8	8.77 ± 0.211	9.625
	500	594 ± 7.86	18.8
Long-term stability (−80°C, 30 days)	8	8.83 ± 0.169	10.375
	500	584 ± 20.8	16.8
Autosampler Stability (4°C, 24 h)	8	8.69 ± 0.204	8.625
	500	583 ± 12.0	16.6
Bench Top stability (6 h)	8	8.22 ± 0.411	2.75
	500	562 ± 6.56	12.4
Dry residue stability (4°C, 48 h)	8	8.83 ± 0.121	10.375
	500	571 ± 10.4	14.2
Autosampler Stability (4°C, 24 h)	8	8.44 ± 0.221	5.5
	500	569 ± 8.0	13.8

Table 6: Calculated plasma concentrations in rabbits at each time point.

Time points in hours	Calculated concentrations (ng/ml)							
	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Rabbit 5	Rabbit 6	Avg	SD
0		0	0	0	0	0	0	0
0.5	16	18	19	12	15	18	16.33	2.58
1	34	29	32	31	37	34	32.83	2.79
1.5	52	58	60	58	52	56	56	3.35
2	71	81	79	69	65	69	72.33	6.28
2.5	84	96	91	87	93	89	90	4.29
3	77	83	89	82	88	93	85.33	5.75
4	68	75	76	70	76	81	74.33	4.68
5	63	58	73	66	69	78	67.83	7.14
6	58	54	64	62	62	67	61.17	4.58
8	55	61	61	57	54	49	56.17	4.58
12	52	56	57	49	47	41	50.33	5.99
16	49	53	50	42	36	35	44.17	7.63
24	46	49	45	40	33	32	40.83	7.08
36	34	38	38	31	29	33	33.83	3.66
48	0	0	0	0	0	0	0	0

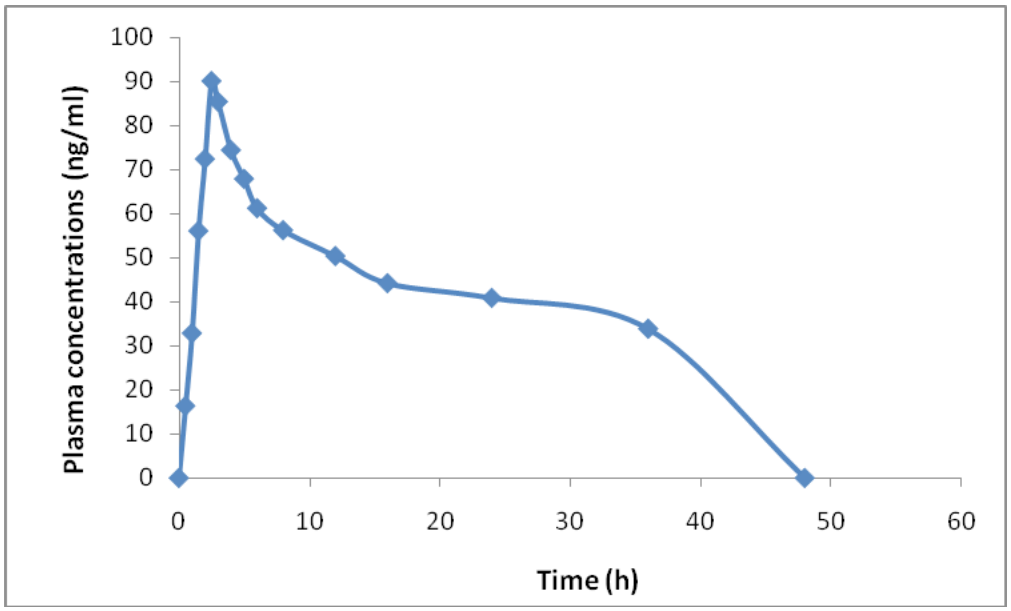

Fig. 6: Plasma concentration time profile of test animals.

Table 7: Calculated mean values of PK parameters for test animals.

Test animals	Tmax	Cmax	t1/2	Lambda_z	AUC 0-t	AUC 0-inf_obs
T1	3	84	41.80	0.016579	1736	3786.754388
T2	2.5	96	43.03	0.016106	1860.25	4219.609708
T3	2.5	91	50.40	0.013751	1841.25	4604.708854
T4	3	87	31.78	0.02181	1628.75	3050.114539
T5	2.5	93	64.13	0.010808	1505	4188.267063
T6	2	93	21.59	0.032096	1481.25	2509.407052
Mean	2.6	90.6	42.12	0.018525	1675.417	3726.476934
Standard deviation	0.37	4.41	14.71	0.007576	163.8626	796.2512068

CONCLUSION

The bio-analytical method developed for the determination of alogliptin is highly specific, rugged and rapid for assessing single dose or multiple dose pharmacokinetics and also for clinical trial samples with desired sensitivity, precision, accuracy and high throughput. The overall runtime is promising compared to other reported procedures for alogliptin. The established LLOQ is found to be low for conducting the pharmacokinetic studies of any marketing formulation of alogliptin in healthy rabbits.

REFERENCES

Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shawet JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract, 2014;103(2):137-149.

Meier JJ. Beta cell mass in diabetes: a realistic therapeutic target? Diabetologia, 2008;51(5):703-713.

Kaneto H, Katakami N, Matsuhisa M, Taka-aki M. Role of reactive oxygen species in the progression of type 2 diabetes and atherosclerosis. Mediat Inflamm, 2010; 2010:1-12.

Robertson RP, Harmon J, Tran POT. B-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. Diabetes, 2004; 53:119-24.

Drucker DJ. Glucagon-like peptides. Diabetes,1998; 47:159-169.

Nauck MA. Is glucagon-like peptide-1 an incretin hormone? Diabetologia, 1999; 42(3):373-379.

Wang Y, Egan JM, Raygada M. Glucagon-like peptide-1 affects gene transcription and messenger ribonucleic acid stability of components of the insulin secretory system in RIN 1046-38 cells. Endocrinology, 1995; 136:4910-4917.

Kieffer TJ, McIntosh CH, Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 *in vitro* and *in vivo* by dipeptidyl peptidase IV. Endocrinology, 1995; 136(8):3585-3596.

Deacon CF, Nauck MA, Toft-Nielsen M. Both subcutaneously and intravenously administered glucagonlike peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects. Diabetes, 1995; 44(9):1126-1131.

Moritoh Y, Takeuchi K, Asakawa T, Kataoka O, Odaka H. The dipeptidyl peptidase-4 inhibitor alogliptin in combination with pioglitazone improves glycemic control, lipid profiles, and increases pancreatic insulin content in ob/ob mice**.** Eur. J. Pharmacol, 2009; 602:448–54..

Sharma K and Parle A., Development and validation of HPTLC method for estimation of alogliptin benzoate in bulk drugs and tablet dosage forms. Int. Bull. Drug Res, 2015; 5: 81–89.

El-Bagary R, I Elkady E, F Ayoub BM., Liquid Chromatographic Determination of Alogliptin in Bulk and in its Pharmaceutical Preparation**.**

Int. J. Biomed. Sci, 2012; 8: 215-218.

Zhang K, Ma P, Jing W, Zhang X, A developed HPLC method for the determination of Alogliptin Benzoate and its potential impurities in bulk drug and tablets. Asian J. Pharm. Sci, 2015; 10:152–158.

Deng J, Guo J, Dai R, Zhang G, Xie H., Determination of a novel dipeptidyl peptidase IV inhibitor in monkey plasma by HPLC-MS/MS and its application in a pharmacokinetics study. J. Pharm. Biomed. Anal, 2015; 117:99–103.

Yadav J, Jadhav S, Mohite K., Development and validation of rp-hplc method for alogliptin benzoate in bulk drug and dosage form Int. J. Pharm. Pharm. Res, 2014; 1:1–9.

ICH guidelines for the stability of new drug substances and products. Q1A(R2) ICH, Geneva; 2005. p. 1-13.

ICH guidelines for validation of analytical procedures: text and methodology. Q2(R1) ICH, Geneva; 2005. p. 1-14.

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