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Development of an LC-MS/MS technique and its validation for the determination of infigratinib in human K2EDTA plasma; Pharmacokinetics in healthy rabbits

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ABSTRACT

A precise and linear liquid chromatography tandem mass spectrometry technique was developed for the estimation of infigratinib in human K2EDTA plasma. Chromatographic isolation of infigratinib and dasatinib was attained on orosil, 3 μm, C18, 150 × 4.6 mm stationary phase with a 0.8 ml/minute movable phase flow rate. The method was rectilinear in a concentration range of 1–1,640 ng/ml. Validation showed an $r^2 = 0.9997$ and an equation of $y = 0.0015x − 0.0063$. The average accuracies of back-assessed concentrations for all quality controls (QCs) were between 96.34 and 100.76. At medium QC, high-QC, and low-QC concentrations, infigratinib had 98.14%, 96.36%, and 97.21% mean recoveries, respectively. Retention time %CV findings were ≤0.62 for the analyte and dasatinib, respectively. The developed method was successfully applied to the pharmacokinetic studies of infigratinib in healthy rabbits. The C_{max}, T_{max} and $T_{1/2}$ of the infigratinib tablets were 87.25 ± 1.43 ng/ml, 6.0 ± 0.03 hours, and 15.24 ± 0.53 hours, respectively. $AUC_{0-\infty}$ infinity for infigratinib tablets was 291.74 ± 3.67 ng h/ml. The developed method was successfully validated and can be utilized for the assessment of infigratinib in biological matrices at industries, forensic laboratories, QC laboratories, and bioavailability studies.

INTRODUCTION

Fibroblast growth factor receptors (FGFRs) are tyrosine kinase receptors that are involved in differentiation, cell proliferation, survival, angiogenesis, and migration. Infigratinib is a kinase blocker that targets these receptors (Fig. 1). When external signals, predominantly FGFRs, bind to FGFRs, FGFR dimerizes to boost the phosphorylation of downstream molecules and the activation of the Ras-mitogen-activated protein kinase (MAPK) cascade [1,2]. This is an important step in the signaling cascade. A broad variety of neoplasms, such as prostate, urothelial, breast, liver, and ovarian cancer, have been linked to alterations in the FGFR receptors. These alterations include amplifications, fusions, and mutations in the FGFR receptors. Recent investigations have shown that up to 45% of patients with intrahepatic cholangiocarcinoma had gene reorganization that resulted in the FGFR II fusion protein. In particular, FGFR II fusion is strongly connected to intrahepatic cholangiocarcinoma [3]. The drug has a water solubility of 0.0299 mg/ml, a molecular weight of 560.48 gmol^1, and the formula $C_{26}H_{31}Cl_2N_7O_3$. It was chemically designated as 3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-[6-[4-(4-ethylpiperazin-1-yl)anilino]pyrimidin-4-yl]-1-methylurea.

Changes in FGFR that occur in tumors may lead to FGFR signals, which help malignant cells proliferate and survive. It is a reversible, noncompetitive inhibitor of all four FGFR subgroups—FGFR I, FGFR II, FGFR III, and FGFR IV—that inhibits the FGFR signal and decreases cell proliferation in cancer cell lines with activating FGFR amplification, mutations, or fusions. FGFR stands for fibroblast growth factor receptor, and there are four subtypes of FGFR. The drug has the most binding affinity for FGFR I, FGFR II, and FGFR III out of the four different FGFR subtypes. It attaches to the allosteric location between the two kinase lobes of the FGFR, or more precisely, to the ATP-binding cleft of the FGFR. This prevents the FGFR from functioning properly. By binding to this cleft, autophosphorylation of the receptor may be avoided, and

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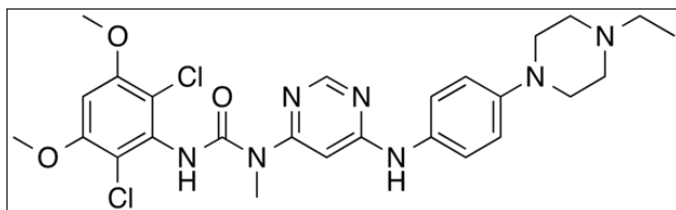E-mail: statinen @ gitam.edu, sowjanya.tatine @ gmail.com*

Figure 1. Chemical structure of infigratinib.

downstream signaling cascades, which would normally activate MAPK, are prevented from being activated [4].

Literature on infigratinib revealed that only one analytical procedure was reported on liquid chromatography tandem mass spectrometry (LC-MS-MS) [5]. One ultra-performance liquid chromatography tandem mass spectrometry method for the quantification of infigratinib was developed in the rat plasma [6]. No methods were reported for the pharmacokinetic estimation of infigratinib in healthy rabbits. For this reason, the LC-MS-MS technique was absolutely necessary for the investigation of biological materials, as it will be useful in pharmacokinetic, pharmacodynamic, and forensic research, respectively. In the present investigation, we created a method that is predicated on human K2EDTA plasma.

MATERIALS AND METHOD

Chemical reagents

In the research investigation, purified water for the chromatographic system was generated using the MilliQ system (Millipores, USA), which was used to manufacture the water. Chromatography-grade methyl alcohol, acetonitrile, AR-grade formic acid, and ammonia, were purchased from Merck Ltd, Mumbai, India. Both infigratinib and dasatinib were purchased from Beijing Sunflower Technology Development Co., Ltd., Beijing, China. The ethical number was obtained from the institutional ethical committee (1447/PO/Re/S/11/CPCSEA-69/A) to conduct the pharmacokinetic experiments on healthy rabbits.

Instrument

An LC-MS-MS equipment of Premier QuattrosX.E joined with the HPLC2695 isolating module was employed for the present study. The software version of Mass Lynx V 4.1 was utilized for the processing of chromatograms and data generation during the research work.

Internal standard (ISTD) preparation

The dasatinib reference component of 10 mg was added, dissolved, and filled with acetonitrile in a 10.0 ml volumetric flask. A calibrated pipette was used to transfer 0.5 ml of ISTD stock solution (1,000 µg/ml) into a 100.0 ml volumetric flask. The diluent was then added at the same volume (5.0 µg/ml). Mixed well, labeled, and kept at 5°C–10°C.

Preparation of calibration standards

Weigh and transfer 50.0 mg of infigratinib standard to a 50.0 ml flask. Dissolve in acetonitrile and make up the volume. Label and keep at 10°C. Use the process of serial

Table 1. Settings of mass instrument.

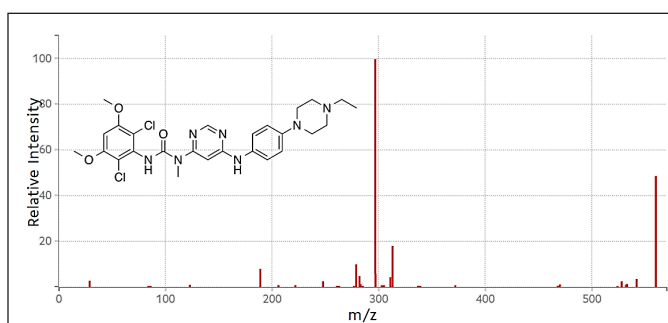| Parameter	Values
Cone voltage (V)	27
Collision energy	24
De solvation flow	650 ± 10 l/hour
Pressure in collision cell	$3.00e^{-3} - 3.75e^{-3}$ mbar
Dwell	0.200
Source temp (°C)	150
De solvation temp (°C)	250
Cone flows	110 ± 5 l/hour
Capillary	3.0 kV
Extractor	1.00 V

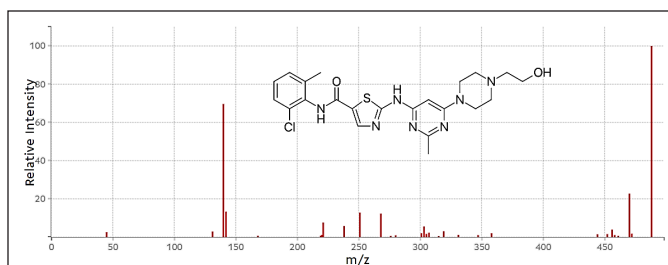**Figure 2.** Mass spectrum of infigratinib.

Figure 3. Mass spectrum of dasatinib.

dilution to get the concentrations of the solutions ready, which should range from 1 to 1,640 ng/ml, and be prepared with a movable phase. Using human K2EDTA plasma may prepare the spiked calibration standards to be used within a similar concentration range.

Processing of quality controls (QCs)

Using a calibrated balance, 50.0 mg of infigratinib was weighed and relocated into a 50-ml flask. Dissolve the contents and make up the volume with acetonitrile. A QC stock solution was used to produce low-QC (LQC) (3.0 ng/ml), medium QC (MQC) (820 ng/ml), and high-QC (HQC) (1,230 ng/ml) spiked samples.

Sample extraction

The necessary plasma solutions from the deep freezer were thawed to room temperature. Except for the STD Blank,
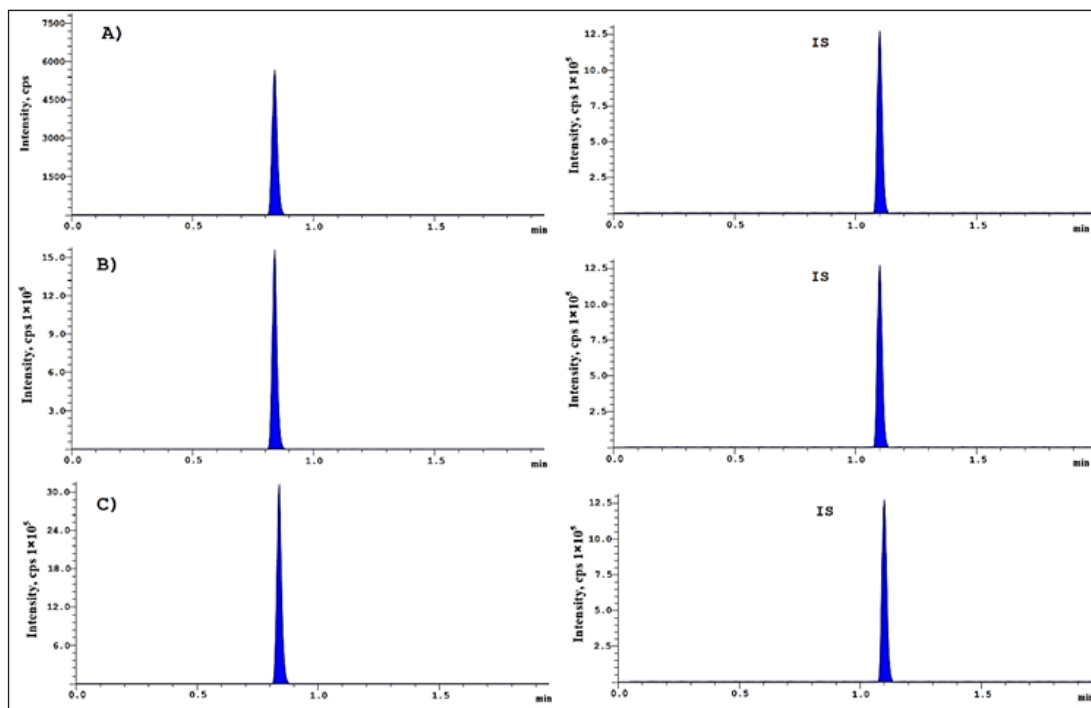
Figure 4. Representative chromatograms of aqueous A) LQC, B) MQC, and C) HQC solutions.

5.0 μg/ml IS working samples were added to pre-labeled empty tubes in a batch sequence to get the final concentration of 450 ng/ml. 200 μl of plasma was mixed for 10 seconds in an ISTD tube. Vortex all tubes by the addition of 2.50 ml of ethyl acetate as an extraction solvent and spin at 500 rpm for 10 minutes. Centrifuge all tubes at 5,000 rpm at 5°C for 5 minutes. The top clear portion was relocated to the evaporating tube, where it was evaporated under nitrogen at 45°C ± 5°C until dry. For 1 minute, vortex all tubes with a 250 μl mobile phase. Use designated autosampler vials to introduce 5.0 μl of reconstituted solution into LC-MS-MS.

Chromatographic optimized conditions

An Orosil, 3 μm, C18, 150 × 4.6 mm column, acetonitrile, methanol, and 0.1% formic acid (60:30:10) movable system at 0.8 ml/minute was employed for the isolation of components. 5 μl volumes were employed to isolate the drug and ISTD in 2.00 minutes at 30°C ± 5°C of oven temperature. Infigratinib retention was 0.82 minutes, and ISTD was 1.11 minutes.

Mass system parameters

The settings for conducting mass spectrometry employing an electro-spray ionization source and multiple reaction monitoring (MRM) are shown in Table 1. Infigratinib's MRM transitions were m/z 561.21/297.18 and the ISTD at 488.16/140.02.

Infigratinib pharmacokinetics in rabbits

Before beginning the studies, the rabbits were allowed to go without food for a whole day. After a dosing period of 4 hours, the meals were made available again. Animals were

Table 2. Infigratinib system suitability.

S.no	Area of analyte	RT of drug (minute)	Response of ISTD	RT of IS	Ratio response
MQC1	1,446,709	0.821	1,256,935	1.11	1.150982
MQC 2	1,458,845	0.822	1,256,465	1.12	1.161071
MQC 3	1,445,285	0.821	1,255,385	1.11	1.151268
MQC 4	1,416,184	0.822	1,255,984	1.1	1.127549
MQC 5	1,550,044	0.821	1,256,308	1.12	1.233809
MQC 6	1,563,430	0.821	1,256,217	1.11	1.244554
n	6	6	1,255,412	6	6
Mean	1,480,083	0.82	1,256,032	1.11	1.17
SD	55,827.3	0.01	471.13	0.01	0.04
% CV	3.77	0.05	0.03	0.61	3.76

given an infigratinib tablet that was given orally as a single dose, and 0.5 ml of blood samples were collected at regular time intervals from the retro-orbital puncher at times 0, 0.50, 1, 1.50, 2, 2.50, 3, 4, 6, 8, 12, 16, 20, and 24 hours after the dose. These blood samples were then placed in Eppendorf tubes that contained heparin to prevent blood from clotting. Blood was centrifuged at 4,000 rpm in a cooled centrifuge for 5–10 minutes to separate the plasma, which was then frozen at 20°C until the results of the study could be analyzed [7].

Method validation

Validation of the devised technique was performed in line with the European Medicines Agency, 2011, and Food and Drug Administration, 2001 standards [8–10,19–20].
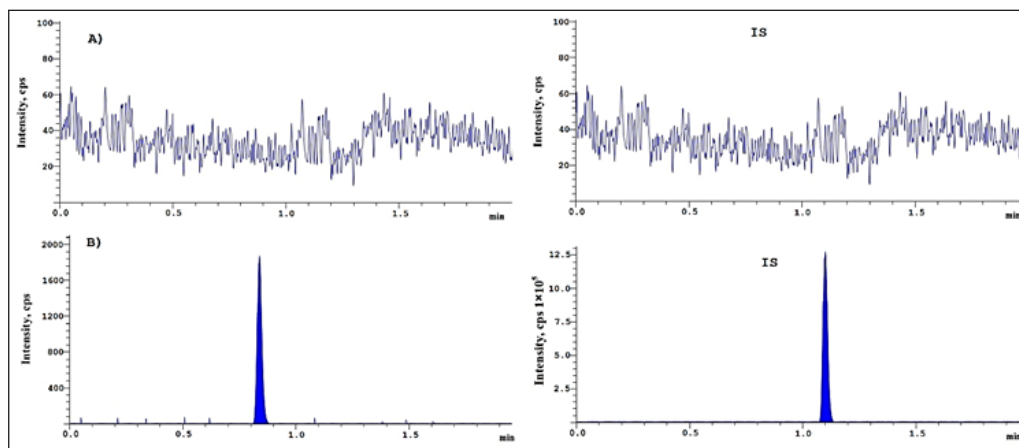
Figure 5. Chromatogram of A) blank and B) LLOQ + IS solution.

RESULT AND DISCUSSIONS

Method validation

System suitability

The sample was subjected to processing that included six consecutive infusions of an aqueous standard combination at the MQC (Fig. 2–4). concentration. On a daily basis during the technique validation process, system suitability was examined [11–14]. Retention timings % coefficient of variation (CV) values were ≤0.62 for infigratinib and IS. %CV of peak response ratios (analyte response/IS response) exhibited ≤3.8%. Table 2 summarizes the results of system suitability.

Auto-sampler carryover effect

The carryover effect of the auto sampler was evaluated by infusing an unextracted sample solution of the movable system, the lower limit of quantification (LLOQC), and upper limit of quantification (ULOQC), as well as extracted solutions of standard blank, ULOQC, and LLOQC [15]. The results of this investigation indicated that there was no carryover impact.

Biological matrix screening and specificity

An LC-MS-MS method showed specificity in standard plasma samples. For a specificity estimate, 10 plasma batches were investigated [16]. Seven of the 10 samples included anticoagulant plasma, 1 hemolytic, 1 lipidemic, and 1 heparin. All investigated human plasma lots had no significant interferences with the drug's retention timings or IS (Fig. 5).

Sensitivity

By assessing six LLOQs, the method's sensitiveness was estimated to be 1.0 ng/ml for infigratinib. At the LLOQC level, infigratinib's accuracy and precision were discovered to be 4.04% and 98.81%, respectively [17].

Matrix effects

Six batches of chromatographically screened plasma were utilized to assess the LC-MS-MS matrix influence. Each batch of plasma was prepared with infigratinib concentrations

Table 3. Infigratinib matrix effects.

	Effect of matrix for analyte		
Analyte	Infigratinib	ISTD	Dasatinib
		HQC	LQC
S. no.	Plasma batch no.	Nominal concentrations(ng/ml)	
		1,230	3.0
		Back calculated concentration (ng/ml)	
1	P-101	1,155.83	2.75
		1,264.07	2.92
		1,274.99	2.91
2	P-102	1,179.98	3.28
		1,184.46	3.02
		1,167.83	2.82
3	P-103	1,149.66	2.81
		1,154.43	2.78
		1,275.06	2.95
4	P-104	1,179.70	3.17
		1,173.31	3.04
		1,177.57	2.81
5	(P-105)-Lipemic	1,155.95	2.75
		1,264.94	2.88
		1,273.36	3.20
6	(P-106)-Hemolyzed	1,229.27	2.83
		1,278.61	3.09
		1,175.94	2.81
	n	18	*18*
	Mean	1,206.39	2.93
	SD	49.24	0.15
	%CV	4.08	5.40
	%Mean accuracy	98.08	97.81

equal to the LQC and HQC and delivered in triplicate at each stage [18]. The overall CV of the back-calculated concentration was 4.08 and 5.40 for the higher and lower QC samples of all the lots. Back-calculated outcomes were 98.08
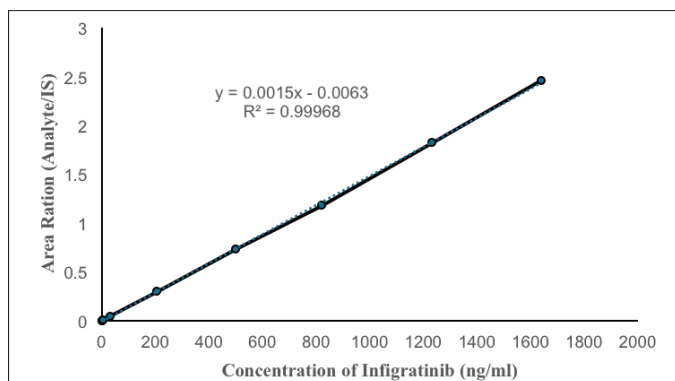
Figure 6. Infigratinib linearity.

Table 4. Linearity of infigratinib.

Concen (ng/ml)	Analyte response	IS response	Analyte/IS
1	1,839	1,256,935	0.0014631
3	5,598	1,256,465	0.0044554
30	55,945	1,255,385	0.044564
205	374,587	1,255,984	0.2982419
500	919,854	1,256,308	0.7321883
820	1,482,237	1,256,217	1.1799211
1,230	2,286,478	1,255,412	1.8212969
1,640	3,092,547	1,256,032	2.4621562

Table 5. Precision and accuracy of infigratinib.

	Precision and accuracy			
Analyte	Infigratinib		ISTD	Dasatinib
	LLOQ	MQC	LQC	HQC
I				
Mean ± SD	0.99 ± 0.04	804.88 ± 30.42	2.99 ± 0.08	1,204.57 ± 46.95
%CV	4.11	3.78	2.64	3.89
%Mean accuracy	98.81	98.16	99.99	97.93
II				
Mean ± SD	1.01 ± 0.04	804.76 ± 30.39	2.99 ± 0.16	1,185.03 ± 41.91
%CV	3.763	3.77	5.29	3.54
%Mean accuracy	100.75	98.14	99.63	96.34
III				
Mean ± SD	0.99 ± 0.04	804.86 ± 30.20	3.02 ± 0.18	1,229.79 ± 48.13
%CV	4.08	3.75	6.09	3.91
%Mean accuracy	98.98	98.15	100.77	99.98
Inter batch accuracy and precision				
n	18	18	18	18
Means ± SD	0.99 ± 0.04	3.00 ± 0.14	804.61 ± 30.39	1,206.46 ± 49.29
%CV	4.04	4.79	3.78	4.09
%Mean accuracy	99.83	100.03	98.12	98.09

Table 6. Recovery of infigratinib.

	LQC (3 ng/ml)			HQC (1,230 ng/ml)			MQC (820 ng/ml)		
S. no	Un extracted response	Extracted response	Concentreation obtained	Un extracted response	Extracted response	Concentreation obtained	Un extracted response	Extracted response	Concentreation obtained
1	5,346	5,004	2.80	2,262,045	2,170,062	1,179.98	1,508,021	1,550,044	842.85
2	5,739	5,229	2.73	2,262,009	2,178,267	1,184.46	1,508,176	1,563,430	850.04
3	5,619	5,434	2.90	2,262,264	2,147,926	1,167.83	1,508,066	1,446,402	786.47
4	5,636	5,761	3.06	2,262,010	2,114,275	1,149.66	1,508,089	1,458,590	793.08
5	5,431	5,317	2.93	2,262,032	2,123,066	1,154.43	1,508,046	1,445,109	785.77
6	5,421	5,521	3.05	2,262,264	2,345,144	1,275.06	1,508,040	1,417,262	770.63
n	6	6	6	6	6	6	6	6	6
Mean		2.91			1,185.24			804.81	
SD		0.12			42.05			30.26	
%CV		4.14			3.54			3.76	
%Mean Accuracy		97.21			96.36			98.14	
% Overall mean recovery					97.23				

and 97.81 (Table 3) for higher and lower QC samples of all lots, respectively.

Calibration curve

      A $1/x^2$ weighted least squares regression study of calibration graphs from an 8-point linear curve verified the method's linearity [11]. During validation, all calibration curves were linear for standard concentrations ranging from 1 to 1,640 ng/ml. Figure 6 shows an example calibration curve from the first precision and accuracy batch. Validation showed an $r^2 = 0.9997$ (Table 4) and an equation of $y = 0.0015x - 0.0063$.

Precision

The validation approach examined the accuracy of the LC-MS-MS technique using %CV at varied LQC, LLOQC, HQC, and MQC concentrations [11,18]. All QC samples had back-calculated concentration solution CVs between 2.63 and 6.09, within the 15% range. All LLOQ samples had a CV of back-calculated concentrations of 4.04, within the permitted 20.00% range. Table 5 provides a summary of the findings.

Table 7. Dilution integrity of infigratinib.

	1/10th 492 ng/ml			1/5th 984 ng/ml		
	Nominal con.	Peak area	Con found	Nominal con.	Peak area	Con found
	492	891,621	484.84	984	1,703,254	926.18
	492	882,654	479.96	984	1,702,567	925.81
I	492	872,032	474.18	984	1,807,945	983.11
	492	875,321	475.97	984	1,712,367	931.14
	492	882,147	479.68	984	1,768,547	961.68
	492	890,329	484.13	984	1,765,482	960.02
n	6	6	6	6	6	6
Mean	492	882,350.7	479.79	984	1,743,360	947.99
SD			3.88			21.66
%CV			0.80			2.28
% Mean accuracy			97.52			96.34

Accuracy

The accuracy of the assay was evaluated based on the ratio of the estimated average readings of QCs to the nominal values associated with those readings. This ratio was expressed as a percentage. Back calculations of concentration levels showed that all control solutions had accuracies in the range of 96.34%–100.76% (Table 5) on average [16].

Recovery

The QCs that were extracted from plasma were compared to the ones that were not extracted at the LQC, HQC, and MQC [17] levels to find the average recoveries, which were shown as a percentage. Infigratinib exhibited a mean recovery of 98.14%, 96.36%, and 97.21% (Table 6) when tested at MQC, HQC, and LQC concentrations, respectively. Every single QC level had a mean recovery of 97.23.

Integrity of dilution

The dilution integrity of the procedure was evaluated by first diluting it 1/5th and then 1/10th times to get to 3ULOQ. It was found that the accuracies for the integrity of dilutions at the 1/5th and 1/10th concentrations were 2.28% and 0.80%, respectively (Table 7).

Stability studies

The infigratinib and IS were kept out of the fridge for 8 hours at room temperature to ensure their short-term stability. LQC and HQCs were tested for a 10-day, 16-hour, and 20-minute period at temperatures ranging from 2.0°C to 8.0°C for long-term stability. The samples were frozen at −28°C ± 5°C and at −70°C ± 10°C and thawed at room temperature (25°C)

Table 8. Stability data of infigratinib.

| Stabilities level	Concentration level	Mean comparison sample area	Mean stability sample area	%Mean stabilities
Short-term	HQC	2,262,144	2,174,796	96.13
	LQC	5,670	5,550	97.89
Long-term	HQC	2,262,145	2,196,422	97.09
	LQC	5,670	5,515	97.26
Freeze and thaw at −28°C ± 5°C	HQC	2,262,145	2,164,793	95.69
	LQC	5,670	5,601	98.77
Freeze and thaws at −70°C ± 10°C	HQC	2,262,145	2,176,463	96.21
	LQC	5,670	5,588	98.55
Bench top	HQC	2,262,145	2,174,884	96.14
	LQC	5,670	5,598	98.73
Auto sampler	HQC	2,262,145	2,196,447	97.09
	LQC	5,670	5,484	96.72
Wet extract stability RT	HQC	2,262,145	2,174,860	96.14
	LQC	5,670	5,454	96.18
Wet extract stability (2°C–8°C)	HQC	2,262,145	2,174,562	96.12
	LQC	5,670	5,602	98.80
Dry extract	HQC	2,262,145	2,178,971	96.32
	LQC	5,670	5,486	96.75
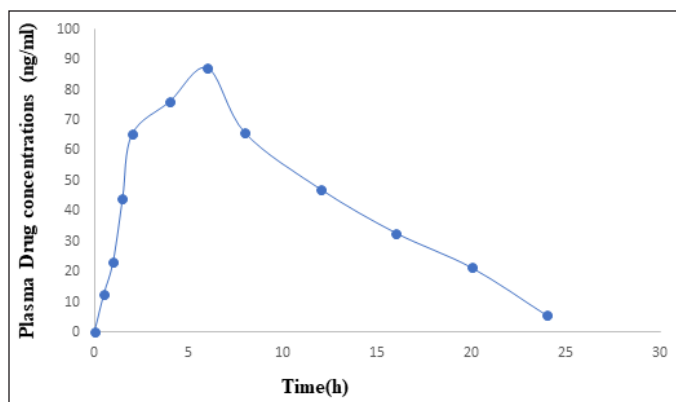
Figure 7. Average plasma concentrations-time profile for infigratinib in rabbits ($n = 6$).

Table 9. Plasma concentration profiles of Infigratinib.

Time (hour)	Infigratinib (ng/ml)
0	0
0.5	12.54 ± 1.53
1	23.43 ± 1.26
1.5	44.32 ± 1.62
2	65.42 ± 1.14
4	76.24 ± 1.75
6	87.25 ± 1.43
8	65.76 ± 1.63
12	47.25 ± 1.53
16	32.76 ± 1.24
20	21.34 ± 1.21
24	5.51 ± 0.32

$n = 6$.

three times. QC sample solutions were spiked and left to stand for 17 hours and 28 minutes on the benchtop [15]. To test their durability, the prepared controls were kept in an auto sampler for 2 days, 20 hours, and 27 minutes at 5°C ± 3°C. Wet extract stability was assessed by keeping spiked QC samples at room temperature for 23 hours and 42 minutes. At 2°C–8°C, the half-life of a wet extract was 2 days, 20 hours, and 23 minutes. The shelf life of dry extracts of spike controls was evaluated over a period of 2 days, 20 hours, and 2 minutes at −28°C ± 5°C. All readings fell within acceptable parameters, as shown in Table 8.

Pharmacokinetic studies

One-way analysis of variance and the Tukey–Kramer multiple comparison test were used in Graph Pad InStat software (version 3.00, Graph Pad Software, San Diego, CA). Statistical significance was determined by $p < 0.05$. Rabbit plasma concentration-time curves following a single oral dosage of Infigratinib tablets are shown in Figure 7. Infigratinib oral pharmacokinetics in rabbits are presented in Tables 9 and 10. The C_{max}, T_{max}, and $T_{1/2}$ of infigratinib were 87.25 ± 1.43 ng/ml, 6.0 ± 0.03 hours, and 15.24 ± 0.53 hours, respectively. An

Table 10. Average pharmacokinetic parameters of infigratinib.

C_{max} (ng/ml)	87.25 ± 1.43
AUC_{0-t} (ng. h/ml)	264.53 ± 3.54
AUC_{0-inf} (ng. h/ml)	291.74 ± 3.67
T_{max} (hour)	6.0 ± 0.03
$T_{1/2}$ (hour)	15.24 ± 0.53

$n = 6$.

important measure in assessing medication bioavailability from the dosage form is AUC, which indicates the total integrated area under the blood concentration-time profile and the total quantity of drug reaching the systemic circulation following oral delivery. The $AUC_{0-\infty}$ infinity for infigratinib was 291.74 ± 3.67 ng h/ml.

CONCLUSION

A precise and linear LC-MSMS technique was developed for the estimation of infigratinib in human K2EDTA plasma. Chromatographic isolation of infigratinib and dasatinib was attained on Orosil, 3 μm, C18, 150 × 4.6 mm stationary phase with a 0.8 ml/minute movable phase flow rate. The method was rectilinear in a concentration range of 1–1,640 ng/ml. Validation showed an $r^2 = 0.9997$ and an equation of $y = 0.0015x − 0.0063$. The average accuracies of back-assessed concentrations for all QCs were between 96.34 and 100.76. At MQC, HQC, and LQC concentrations, Infigratinib had 98.14%, 96.36%, and 97.21% mean recoveries, respectively. The developed method was successfully applied for the pharmacokinetic study of infigratinib in healthy rabbits. The $C_{max,}$ $T_{max,}$ and $T_{1/2,}$ of the infigratinib were 87.25 ± 1.43 ng/ml, 6.0 ± 0.03 hours, and 15.24 ± 0.53 hours, respectively. $AUC_{0-\infty}$ infinity for infigratinib was 291.74 ± 3.67 ng h/ml.

AUTHOR CONTRIBUTIONS

Kunala Anusha made substantial contributions to conception and design, acquisition, analysis, and interpretation of data and took part in drafting the article. Gummadi Sowjanya was involved in drafting and critically revising the manuscript for important intellectual content. Both the authors agreed to submit to the current journal, gave final approval of the version to be published and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

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

ETHICAL APPROVALS

The ethical number was obtained from the institutional ethical committee (1447/PO/Re/S/11/CPCSEA-69/A) to conduct the pharmacokinetic experiments on healthy rabbits.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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