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Original Article

AN LC-ESI-MS/MS METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTIFICATION OF INFIGRATINIB IN BIOLOGICAL MATRICES

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ABSTRACT

Objective: The study was aimed to develop a precise and simple liquid chromatographic electrospray ionization tandem mass spectrometric (LC-ESI-MSMS) technique is essential for the quantification of Infigratinib in biological matrices.

Methods: Chromatographic resolution was attained with PhenominexC_{18} (50 mm×2.6 mm, 3 µm) stationary column and a mobile solvent composition of 0.1% HCOOH, methyl alcohol and acetonitrile in the proportion of 10:10:80. Chromatograms were resolved by an isocratic separation with a flowing rate of 0.50 ml/min at 40 °C.

Results: Quantitation was executed by monitoring the transitions of *m/z.* 560.19/189.13 for Infigratinib and 494.5→394.5 for Imatinib internal standard in multiple reaction monitoring. The standard curve regression line was y = 0.0016x+0.0062 and the correction coefficient (r^2) was 0.9994. The % CV outcomes for matrix effect at Lower-QC and Higher-QC were 4.95% and 3.61% respectively. The percentage average recoveries for Infigratinib in Higher-QC (900ng/ml), MQC (600ng/ml) and Lower-QC (3ng/ml) were 93.27%, 94.69% and 97.24% respectively. The intra and interday precisions of analytical procedure was estimated by assessing the %CV outcomes and were in between 1.88 to 5.93% for the QC samples.

Conclusion: The developed procedure can be useful for the assessment of Infigratinib in biological matrices in quality control, forensic and bioavailability studies.

Keywords: Infigratinib, Cholangiocarcinoma, LC-MS/MS, Validation, Linearity

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INTRODUCTION

Infigratinib, also known by its chemical name 3-(2, 6-dichloro-3,5-dimethoxyphenyl)-1-[6-[4-(4-ethylpiperazin-1-yl) anilino] pyrimidin-4-yl]-1-methylurea, is a cancer drug. The chemical formula is having an empirical formula of $C_{26}H_{31}Cl_2N_7O_3$. Pan-fibroblasts growth factor receptors (FGFR) kinase inhibition may be achieved with the use of infigratinib. Infigratinib inhibits the FGFR path, which is mutated in malignancies like cholangiocarcinoma. In doing so, it is able to prevent the development of tumors. Cholangiocarcinoma is the most frequent kind of primary cancer that affects the biliary system, and it is also the second most common kind of primary cancers that affects liver. Because it is an ATP-competitive inhibitors of all 4 different FGFR receptors subtype, infitratinib is considered to be a pan-FGFR inhibitor [1-5].

FGFRs are tyrosine kinase receptors that regulate proliferation of cells, migration, differentiation, angiogenesis and survival. After attaching to external signals, such as FGFRs merges to phosphorylate downstream molecules and activate the Ras-MAPK pathway. FGFR signaling pathway disruptions cause uncontrolled expansion and cell proliferation, including malignant cells, in several malignancies. FGFR receptor fusions, amplifications, and mutations, are linked to urothelial, prostate, ovarian, liver and breast, cancers. Recent investigations suggest that up to 45% of intrahepatic cholangiocarcinoma patients have gene rearrangements that produced FGFR2 fusion proteins. Tumors with FGFR mutations may encourage malignant cell growth and survival via constitutive signaling. In cancer cell line with stimulating FGFRs amplification, fusions, or mutations, infigratinib blocks FGFR signalling and suppresses cell propagation. It is reversible, non competitive inhibitors of all 4 subtypes of FGFR. Infigratinib binds best to FGFR1, FGFR2, and FGFR3 among the four subtypes [6, 7]. Literature survey on Infigratinib drug reveals that two quantification approaches for the assessment of Infigratinib in sample plasma were reported. So, there is need of an LC-ESI-MSMS analytical procedure for estimation of Infigratinib in biological matrix.

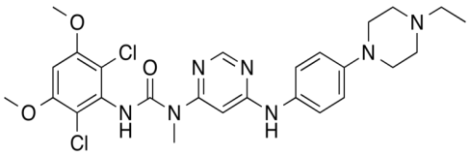

Fig. 1: Infigratinib chemical structure

MATERIALS AND METHODS

Chemicals and reagents

Infigratinib and Imatinib (IS) were gift sample from Solu Clinipharm Pvt. Ltd, Maharastra, India. LC grade CAN (acetonitrile) and methyl alcohol were acquired from J. T. Bakers, Hyderabad. Water utilized for total research work from water purification (Milli-Q) systems. Formic acid of analytical grade was obtained from Merk Pvt. Ltd., Mumbai, India.

Equipment

The Applied Biosystem Sciex-API4000 Tandem mass spectrometer was combined with the Shimadzu LC20ADvp (Shimadzu, Tkyo Japan) high-performance liquid chromatography (HPLC) system that included an auto-sampler. Japan's Shimadzu LC20AD was the device module that served as for the solvent method of delivery. The Analyst program, which was developed by Applied Biosystems and comes in version 01.04.02, was used to combine all of the chromatographic data.

Preparation of quality and calibration standard solutions

A 100µg/ml of Infigratinib and Imatinib (IS) stock solutions executed by solubilizing in exact quantity of mobile phase. Quality and calibration controls were processed with plasma blank samples from these standards stock Infigratinib solution. Eight calibration levels of different concentrations were processed by spike method to the plasma blank with Infigratinib standard solutions to made the concentration levels of 1, 5, 35, 150, 350, 600, 900, and 1200ng/ml.

Low-quality (Lower-QC), medium quality (Medium-QC) and higher quality (Higher-QC) levels were executed by spike method to plasma blanks with Infigratinib to made solutions of 3, 6 and 9 ng/ml respectively. From Imatinib stock solution of 350 ng/ml its working solution was processed with mobile phase. Processed solutions were kept at −20 °C till time estimation.

Chromatographic conditions

Chromatographic resolution was attained with PhenominexC_{18} (50 mm×2.6 mm, 3 µm) stationary column and a mobile solvent composition of 0.1% HCOOH, methyl alcohol and acetonitrile in the proportion of 10:10:80. Chromatograms were resolved by an isocratic separation with a flowing rates of 0.50 ml/min at 40 °C. Auto-sampler and column oven temperature were adjusted to 5 °C and 4 °C correspondingly.

Mass spectroscopic settings

Mass settings were set to source temperatures 450 °C; nebulizer gas 20psi (N_2); heater gas 30 psi (N_2); curtain gas 25psi (Nitrogen); entrance potentials 15V; CAD gas 8(N_2); declustering potential 80V source flow rate 0.5 ml/min with no splitting and 15V collision energy (CE) for both Infigratinib and Imatinib, collision cell exit potentials-15V and dwell time 200 ms for the Infigratinib and collision cells exit potentials-14 V for Imatinib. A turbo-ion-spray interface ran in positive (+) mode of ionization was utilized for the quantitative detection. Parent and product ion detection was employed in MRM (multiple reaction monitoring) manner, with transitions pairs of *m/z*-560.19/189.13 for Infigratinib and 494.5→394.5 for Imatinib internal standard.

Protocol for sample preparation

Each spiked plasma samples of 50µl mixed with 250µl methyl alcohol having 0.1% of HCOOH to precipitate the proteins present in the mixture. The resultant mixture was subjected for the vortex mixing for 10 min. Then these sample solutions were centrifuge for 20 min at 4.0 °C. Then 150µl of supernatant liquid was relocated to polypropylene tubes, from which an aliquot 5µl of samples were infused into LC-MSMS system. The final concentration of these Imatinib should be 350ng/ml in each sample preparation [14, 15].

Method validation

The accuracy, lower limit of quantification (LLOQ), precision, recovery, linearity, selectivity, and matrix effects of the quantitative determination of infigratinib in plasma were tested in accordance with the Guidelines for bio-analytical technique validation in pharmaceutical development. This was done in order to validate the quantitative determination of infigratinib in plasma [16-18].

RESULTS AND DISCUSSION

Method validation

The chromatographs achieved during the validation process were satisfactory and resultant blank, blank+IS, and LLOQC chromatographs were given in (fig. 2 and fig. 3).

Specificity

In order to obtain a clean separation of infigratinib from endogenous impurities and other impurities found in plasma, a solution with a concentration of 1.0 ng/ml was injected into the chromatographic system under conditions that had been tuned for HPLC. Assessing the plasma interference allowed for an estimation of the method's level of specificity [19, 20]. It is recommended that the peak area of the interference peak be less than 20 percent of the peak area of the LLOQ and less than 5 percent of the mean peak area of the IS. There should not be a deviation of more than twenty percent between the nominal concentration (NC) and the LLOQ concentration.

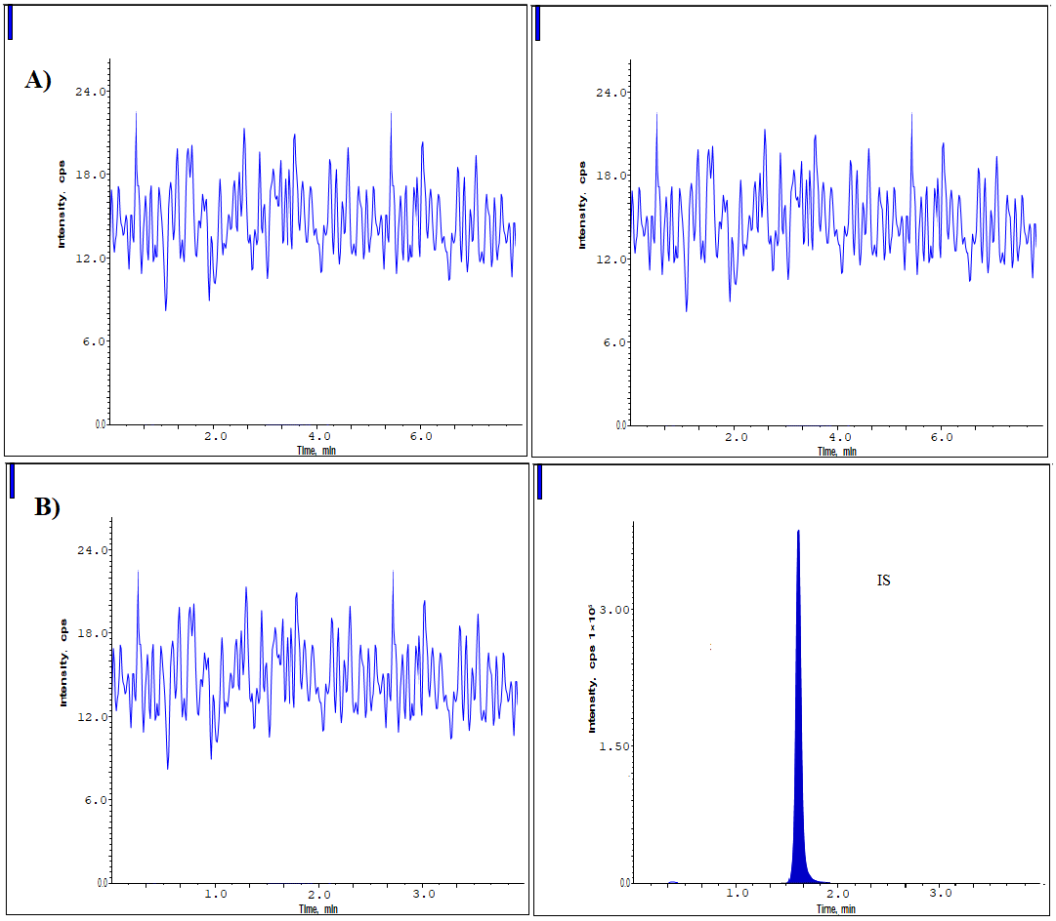

Fig. 2: Chromatographs of plasma blank (a), plasma blank with Imatinib (b)

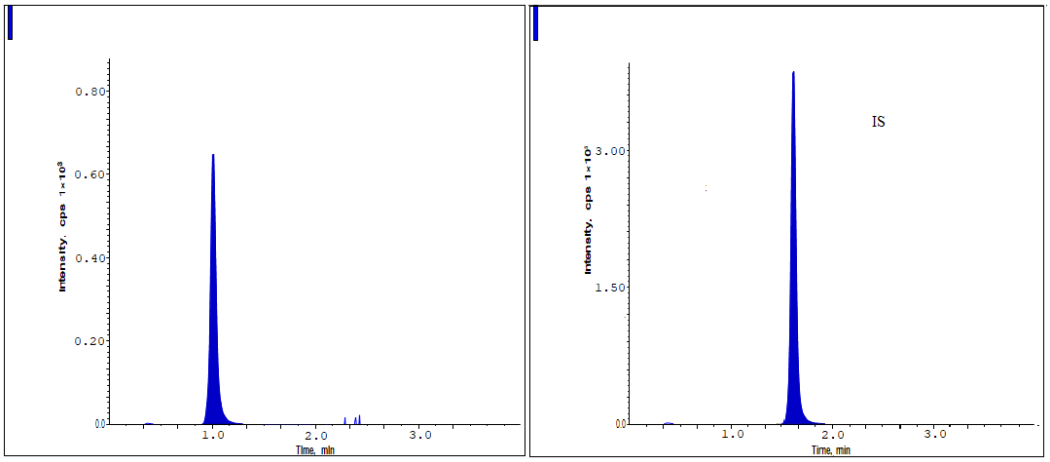

Fig. 3: Representative chromatographs of plasma spiked with Infigratinib at LLOQC and Imatinib

Linearity

In order to determine whether or not the approach was linear, a calibration curve was constructed by comparing spiking results to peak area proportions of Infigratinib to IS. Standard values for calibration that range from 1 to 1200 ng/ml. All three calibration curves were linear across the operating range, and a calibration at eight points was employed for quantitative analysis using linear regression (fig. 4) [21, 22]. The standard curve regression equation was found to be y = 0.0016x+0.0062 and with correction coefficient (r^2) of 0.9994. The linear curve findings were shown in the table 1.

Table 1: Linearity standard solutions for infigratinib

LS-ID	Concentration (ng/ml)	Average response	IS response	Analyte/IS response
LS-1	1	643	402154	0.001599
LS-2	5	3215	402741	0.007983
LS-3	35	26505	402561	0.065841
LS-4	150	96957	402285	0.241016
LS-5	350	237051	402402	0.58909
LS-6	600	387884	402247	0.964293
LS-7	900	561747	402645	1.395142
LS-8	1200	771648	402393	1.917648

CS: Calibration standard.

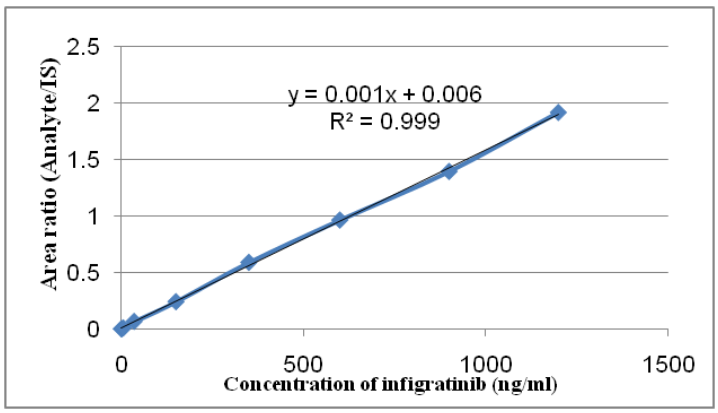

Fig. 4: Calibration curve of Infigratinib

Intra and interday precisions

Both intra and inter days accuracy and precisions were examined. By randomly injecting QC standards (3, 600, and 900ng/ml) and LLOQ (1.0ng/ml) in five duplicates each day, intraday accuracy and precision were examined. The examination of each quality control standard (3, 600, and 900 ng/ml) and LLOQC standard (1.0ng/ml) once on each of five distinct days was used to estimate the interday accuracy and precision. By examining the % CV data, it was possible to determine the analytical method's intraday and interday accuracy. The obtained results for the QC samples ranged from 1.88 to 5.93%. The outcomes were all within the 15% precision range. The outcomes were compiled in table 2.

Recovery

Recoveries of analytes were executed by equating the area of peak for Infigratinib (extracted samples) with response peaks of control Infigratinib level. The % average recovery for Infigratinib in High-QC (900 ng/ml), MQC (600 ng/ml) and Low-QC(3 ng/ml) were 93.27%, 94.69% and 97.24% respectively (table 3).

Table 2: Infigratinib intra and inter day precision data

QC	Infigratinib (µg/ml)			
Intra batch	**Average**	**SD**	**%CV**	**% Bias**
LLOQC	0.96	0.02	2.07	-3.44
Low-QC	3.138	0.12	3.82	4.61
Median-QC	586.74	34.82	5.93	-2.21
High-QC	953.60	44.38	4.65	5.96
LLOQ	1.036	0.024	2.31	3.60
Low-QC	2.89	0.129	4.46	-3.67
Median-QC	614.65	20.94	3.41	2.441
High-QC	903.14	39.27	4.35	0.35
LLOQ	0.96	0.018	1.88	-4.44
LLOQ	3.08	0.13	4.22	2.67
Low-QC	579.45	33.89	5.85	-3.425
Median-QC	959.12	42.25	4.41	6.56
Inter batch	Average	SD	%CV	% Bias
LLOQ	1.036	0.035	3.37	3.604
Low-QC	2.89	0.098	3.397	-3.66
Median-QC	614.65	25.27	4.117	2.46
High-QC	933.14	42.67	4.577	3.68

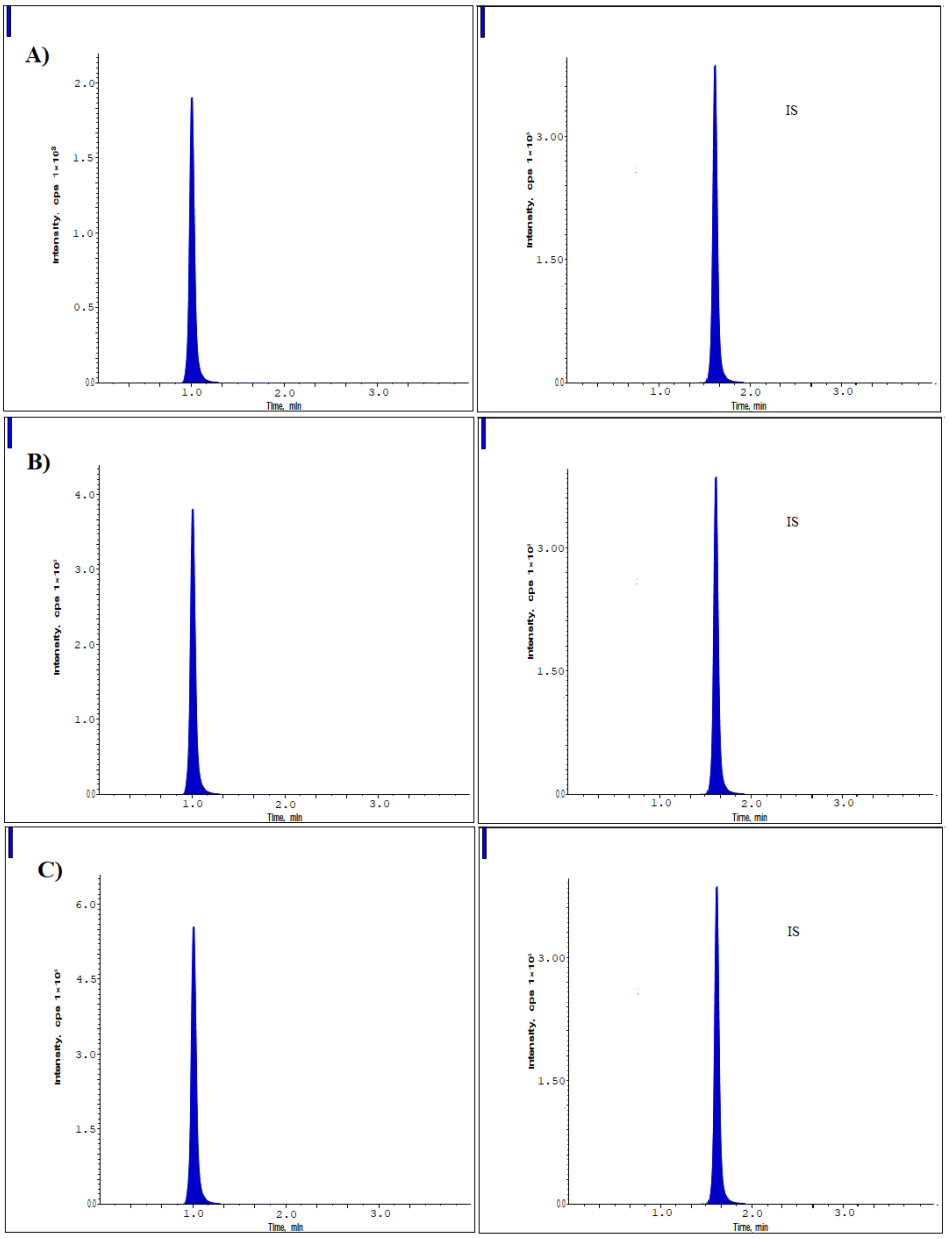

Fig. 5: Infigratinib chromatograms for A) Lower-QC B) Medium-QC and C) Higher-QC

Table 3: Analytes recovery

Concentration levels	X	Y	% Recovered	% Mean recoveries	% RSD
LQC	1929	1875	97.24	95.07	1.73
MQC	385800	365314	94.69		
HQC	578700	539753	93.27		
IS	402345	395867	98.39		

X, recoveries of mean unextracted sample; Y, recoveries of mean extracted sample.

Matrix effect

Following the extraction of six blank plasma samples from six distinct lots in triplicate, the samples were spiked at Low-QC and High-QC levels, and the results were ultimately compared with the same amounts in the alternative infusions. Low-QC samples had a coefficient of variation (CV) of 4.95%, while High-QC samples had a CV of 3.61%. Both of these values fall within the acceptability requirements of 15%. The findings of the matrix factor analysis may be found summarized in table 4.

Table 4: Infigratinib matrix effect

S. No.	LQC			HQC		
	Peak area in absence of matrix	Peak area in presence of matrix	Matrix factor	Peak area in absence of matrix	Peak area in presence of matrix	Matrix factor
1.	1892	1788	94.51	578524	592293	102.38
2.	1881	1748	92.94	577952	552927	95.67
3.	1984	2008	101.24	578921	549917	94.99
4.	1991	1957	98.34	578417	545910	94.38
5.	1923	1787	92.96	579014	589552	101.82
6.	1958	2049	104.67	579239	561167	96.88
Mean			97.44			97.69
±SD			4.82			3.52
% CV			4.95			3.61

Stability studies

The requirements for stability were as follows: post-preparative (24 h at 10 degrees Celsius), short-term (19 h at 25 degrees Celsius), long-term (36 d at-70 degrees Celsius), stock solution stability, and freeze/thaw (3 cycles) stability [23]. The findings of the stability tests showed that there was no substantial degradation of infigratinib that happened during the chromatographic method, extraction, or sample storage of infigratinib plasma samples under various storage settings. This was proved by the fact that there was no significant change in the concentration of infigratinib. The statistics about stability were presented in table 5.

Table 5: The stability data of infigratinib in human plasma under different storage conditions

Storage condition	LQC		MQC		HQC	
	Accuracy (Mean%)	Precision (RSD%)	Accuracy (Mean%)	Precision (RSD%)	Accuracy (Mean%)	Precision (RSD%)
Post preparative (24h at 10 °C)	101.92	4.38	97.69	2.98	92.97	3.28
Short-term (19h at 25 °C)	96.37	5.39	105.97	5.31	93.41	4.58
Long-term (36 d at-70 °C)	95.28	3.28	96.39	4.92	103.25	5.09
Three freeze/thaw (3cycles)	98.17	4.67	95.16	3.67	102.49	2.88
Stock solution (20h at 25 °C)	103.59	5.28	103.77	5.07	96.34	4.77
Stock solution (17day at 2 to 8 °C)	102.36	3.97	96.24	4.08	94.66	5.34

Carry over effect

The carry-overs have been evaluated by calculating the concentration of a blank sample by following the highest calibration standard concentration in six repetitions. The peak response of the blank sample produced after the HQC should not be more than twenty percent higher than the drug response of the LLOQ, nor should it be more than five percent higher than the response of Imatinib. The carrying over of funds was deemed appropriate.

CONCLUSION

A precise and simple liquid chromatographic electrospray ionization tandem mass spectrometric (LC-ESI-MS/MS) technique was developed for the quantification of Infigratinib in biological matrices. In the range of 1.0 to 1200 ng/ml, the drug content is directly related to the peak reaction. Chromatographic separation was done using a PhenominexC18 (50 mm×2.6 mm, 3 µm) column and a mobile solvent made of 0.1% HCOOH, methyl alcohol, and acetonitrile in the ratio of 10:10:80. Chromatograms were separated using an isocratic elution at 40 0C and a flow rate of 0.50 ml/min. Compared to other methods for Infigratinib, the total time for research looks good. The regression equation for the linear curve was found to be y = 0.0016x+0.0062, and the correction coefficient (r2) was 0.9994. At the Low-QC and High-QC levels, the % CV results for the matrix effect were 4.95 and 3.61, respectively. Infigratinib was recovered at 93.27%, 94.69%, and 97.24% in High-QC (900 ng/ml), MQC (600 ng/ml), and Low-QC (3 ng/ml), respectively. By looking at the %CV numbers, it was possible to fig. out how accurate the analysis method was during the day and between days. For the QC samples, the numbers found ranged from 1.88 to 5.93%.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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