RESEARCH ARTICLE

Development of Stability Indicating Method for the Quantification of Futibatinib in K2EDTA Human Plasma by LC-MS/MS

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ABSTRACT

A sensitive, accurate, and linear LC-MS/MS method for the quantification of futibatinib in human plasma K2 ethylene diamine tetraacetic acid (EDTA) was developed. A Phenomenex C18, 150×4.60 mm, 2.1 µ column, 0.1% HCCOH, ACN and methyl alcohol (13/67/20, v/v/v) mobile solvent system at 0.8 mL/min was employed for the isolation of components. 10 µL. of volumes were employed to isolate the peak responsess within 2.0 minutes at 40 ± 5ºC of oven temperature. Analyte retention was 1.261 minutes and ISTD of 1.292 minutes. Throughout the process of validation, each of the four calibration curves exhibited linear behavior for standards with concentrations ranging from 0.16 to 3250 ng/mL. Validation showed an r2 = 0.9997. At MQC, HQC, and LQC concentrations, futibatinib had 98.49, 99.35, and 98.47% mean recovery. Every QC level had a mean recovery of 90.51% and a %CV of 3.06. All of the control solutions had back-calculated concentration values that were accurate between 94.61 and 98.86% of the time. The range of %CV of back-calculated values for all quality control samples were in the range of 0.6 and 4.63, which is within the acceptable range of 15%. The developed method can be applied successfully for the quantification of the Futibatinib in biological matrices.

Keywords: Futibatinib, Fibroblast growth factor receptors, Liquid Chromatography with tandem mass spectrometry, Validation, Linearity, Stability.

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INTRODUCTION

An inhibitor of the fibroblast growth factor receptors (FGFR), which is a group of receptor tyrosine kinases that are essential to the processes of cell proliferation, differentiation, migration, and survival, futibatinib is a drug that blocks these processes. Because FGFR genetic abnormalities and dysregulation of FGFRs signalling paths are seen in certain cancer, like urothelial malignancies, and cholangiocarcinoma.[1,2] FGFR was examined in oncology as a potential therapeutic target. To treat various forms of intrahepatic cholangiocarcinoma, the Food and Drug Administration (FDA) granted first approval for the use of futibatinib, a new FGFR inhibitor, in September of 2022. The conditional marketing authorization for futibatinib (Figure 1) for the treatment of cholangiocarcinoma[3] was granted by the European Commission on July 4, 2023.

With IC_{50} values lower than 4 nM, futibatinib is a highly irreversible and selective inhibitor of the FGFR 1 to 4 proteins. This is achieved by making a strong bond with cysteine which is available in the pocket of ATP-binding. This lets it link to the FGFR kinase region. Once futibatinib binds to FGFR, it stops FGFR from being phosphorylated and stops communication pathways that come after it. Some of these pathways are the RAS-dependent mitogen-activated protein kinase (MAPK), PI3KCA/Akt/mTOR, PLC, and JAK/STAT. Futuribatinib has been shown to finally lower the amount of live cells in cancer cell lines that have FGFR changes, like FGFR rearrangements or fusions , mutations, or amplifications.[4,5]

In the research done on futibatinib, one of the analytical processes that was reported on was LC/MS/MS.[6] A method using LC-MS/MS with a short retention period was developed for the estimation of futibatinib. The investigation of biological materials necessitates the use of LC-MS/MS technology because of its potential utility in pharmacokinetic, pharmacodynamic, and forensic research.

MATERIALS AND METHODS

Materials

Sidmak Laboratories India Pvt Ltd. was the supplier for both futibatinib and dasatinib. Merck in Mumbai, India, supplied us with methyl alcohol, acetonitrile, and GR-grade formic acid and ammonia, all of which we used in our experiments. During the course of the inquiry, LC-water was produced by filtering purified water via a MilliQ-system (made in the United States).

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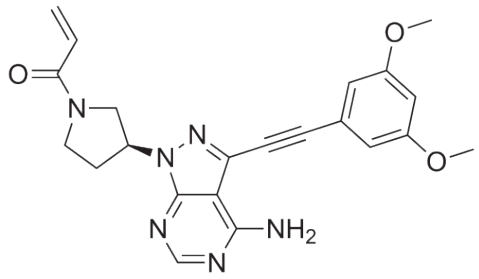

Figure 1: Chemical structure of futibatinib

Instrument

An LC-MS/MS instrument of Quattros X.E Premier combined with LC2695 separation module was employed for the present work. The software version of Mass Lynx V 4.1was utilized for the processing of chromatograms and data generation during the research work.

Preparation of Internal Standard Solution

Dasatinib reference component of 1-mg weight was introduced to a 1.0-mL volumetric flask, where it was dissolved before being brought to the correct volume with ACN. With calibrated pipettes, 0.075 mL of the ISTD stock solutions, which has a concentration of 1-mg/mL, was transferred to a volumetric flask holding 100.0 mL, and the remaining space in the flask was filled with ACN having a concentration of 750 ng/mL. After a thorough mixing, labeling, and marking of the mixture, keep the temperature between 2 and 8°C.

Processing of Calibration Solutions

10.0 mg futibatinib standard is weighed and relocated to a flask of 10.0 mL. Make up to the volume by dissolving in methyl alcohol. Label and store at 2 to 8°C. Using the serial dilution approach, produce solution concentrations ranging from 0.16 to 3250 ng/mL in mobile phase. Using human K2 Ethylenediaminetetraacetic acid plasma, prepare spiked calibration standards in the same concentration range.

Processing of Quality Control Standards

10.0 mg futibatinib was weighed and relocated into a 10 mL flask using a calibrated balance. Methanol was used to make up the volume once the ingredients were dissolved. LQC (0.48 ng/mL), MQC (1480 ng/mL), and HQCs (2250 ng/mL) spiking solutions were created using QC stock solution.

Extraction of Sample

After being stored in the deep freezer, the required plasma samples were brought into ambient temperature, at the exception of the STD blank, 50 µL of working solutions at a concentration of 750 ng/mL were introduced in batch sequence to empty tubing that had been prelabeled. In ISTD tubes, 200 µL of plasma from step-1 was vortexed for five seconds. Five seconds should be spent agitating each tube containing 100 µL of extraction buffer. Each vial is given 2.50 mL of ethinyl acetate, and it is spun at 5000 rpm for 25 minutes. Centrifuge all of the vials for ten minutes at 4500 rpm and 5.0°C. After moving 2.0 mL of the top layer to pre-labeled evaporation tubes, the liquid is then evaporated at a temperature of 40 ± 5°C in the presence of nitrogen until it is dry. All of the tubes containing the reconstitution solution should be shaken vigorously for one full minute. Inject a total volume of 10.0 µL of the reconstituted solution into the LC-MS/MS apparatus using auto sampler vials that have previously been labeled.

Optimized Chromatographic Conditions

A Phenomenex, 150 × 4.60 mm, 2.1 µ; C18-column, 0.1% HCCOH, ACN and methyl alcohol (13/67/20, v/v/v) mobile solvent system at 0.8 mL/min was executed for the isolation of components. 10 µL. of volumes were employed to isolate the peaks within 2.0 minutes at temperature of the oven monitored at 40 ± 5ºC. Analyte retention was 1.261 minutes and ISTD of 1.292 minutes.

Mass Equipment Parameters

Table 1 represents the parameters for mass system utilizing an electro spray ionization (ESI) source and multiple reactions monitoring (MRM). Futibatinib's MRM transitions were m/z 419.31/124.07 and the internal standard's at 488.0/401.

Method Validation

The established method was verified according to FDA (2001) and EMA (2011a) guidelines.[7-11]

RESULT AND DISCUSSIONS

Method Validation

System suitability

The sample was subjected to processing that included six consecutive injections of an aqueous standard combination at the MQC (Figure 2) concentration. The appropriateness of the system was examined on a daily basis during the method validation.[12-15] The results showed that the analyte and ISTD both had retention times with %CV values less than 0.28. The coefficient of variation of the peak area ratio (Analyte area/ ISTD area) was less than 0.18% CV. The findings are outlined in Table 2.

Auto sampler carryover effect

The auto sampler's carryover effect was evaluated by injecting mobile solvent, lower limit of quantification (LLoQ) and upper limit of quantification (ULoQ) solutions into an unextracted

Table 1: Parameters of mass instrument

ES– Source parameters	*Values*
Source Temp (°C)	150
Capillary	3.00 kV
Extractor	1.00 V
Cone Flows	100 ± 5 L/h
De solvation Flow (L/h)	800 ± 10
Collision cell Pressure(mbar)	$3.5e^{-3}$ - $4.5e^{-3}$
Cone voltage (V)	21
De solvation Temp (°C)	350
Dwell	0.200
Collision Energy	18

Table 2: Futibatinib system suitability

System suitability					
Drug	*Futibatinib istd dasatinib*				
Sample name	*Name of file*	*Area of drug*	*Drug RT(min)*	*IS response*	*IS RT*
AQ. MQC	813	3188364	1.261	3145957	1.292
AQ. MQC	814	3098021	1.262	3145005	1.290
AQ. MQC	815	3188327	1.262	3146024	1.293
AQ. MQC	816	3198456	1.261	3145658	1.291
AQ. MQC	817	3188651	1.260	3144952	1.292
AQ. MQC	818	3078327	1.262	3145254	1.292
MEAN			1.26		1.29
SD			0.0007		0.0009
%CV			0.05		0.07

sample, as well as blank, ULoQ, and LLoQ solutions into an extracted sample. Resulting from the research, it was clear that there was no residual impact.[16-18]

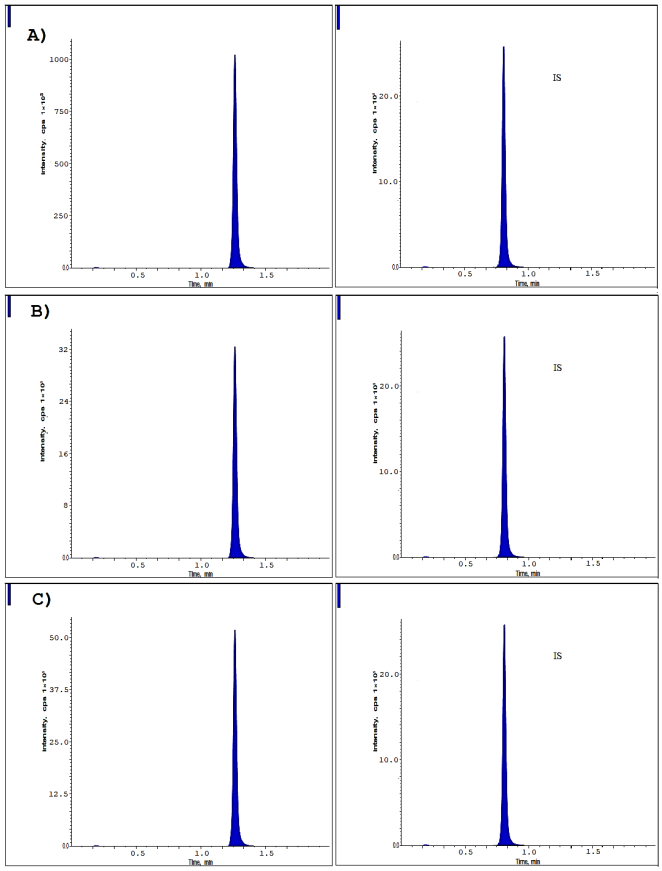

Figure 2: Representative Chromatograms of A) LQC, B) MQC and C) HQC.

Biological matrix screening and specificity

The precision of an LC-MS/MS method was shown by looking at regular plasma samples. Ten different lots of plasma were inspected at to figure out the specificity. Seven of the ten samples were supposed to have anticoagulant plasma in them, one had hemolytic plasma in it, one had lipidemic plasma in it, and one had anticoagulant plasma in it (heparin). All the human plasma samples that were tested did not have any major issues with the drug's holding times or ISTD (Figure 3).

Sensitivity

Sensitivity of 0.16 ng/mL for futibatinib was found by evaluating 6 LLoQs. Precision and accuracy for Futibatinib were determined to be 2.29 and 95.35% at the LLoQ level, correspondingly.

Matrix effect

The effect of the matrix on LC-MS/MS was evaluated using six batches of plasma that had been tested chromatographically. At each step, plasma was administered in duplicate with futibatinib concentrations matching the LQC and HQC16, 18. Back estimated concentration %RSD was 1.06 for high QC solutions and 3.07 for low QC solutions across all batches, respectively. HQC samples of all lots had a back-calculated value of 98.81%, whereas LQC samples had a value of 95.78% (Table 3).

Calibration curve

A 1/x2 weighted least square regression study of calibration graphs from a 8-point linear curve verified the method's linearity.[17] During testing, the four standard curves were straight for standards with amounts ranging from 0.16 to 3250 ng/mL. Figure 4 shows an example calibration curve from the first precision and accuracy batch. Validation showed an $r^2 = 0.9997$ (Table 4).

Precision

Throughout the validation procedure, the LCMS/MS method's precision was evaluated using the %CV at varying levels of LQC, MQC, LLoQ, and HQC. All quality control samples showed a coefficient of variation (CV) of back-calculated

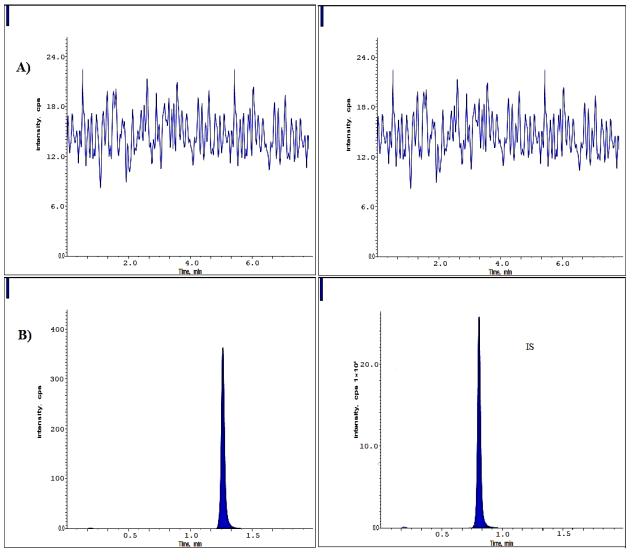

Figure 3: Chromatogram of blank A) and LLOQ B) solution.

Table 3: Matrix effect for analyte

Effect of matrix for analyte			
Analyte Futibatinib ISTD Dasatinib			
S.No.	*Plasma Lot*	*HQC*	*LQC*
		Actual Concentrations (ng/mL)	
		2250	0.48
		2236.19	0.51
1	F-701	2231.78	0.49
		2227.06	0.45
		2249.88	0.44
2	F-702	2227.02	0.44
		2194.36	0.46
		2157.52	0.43
3	F-704	2236.25	0.45
		2203.76	0.42
		2240.70	0.51
4	F-705	2245.58	0.44
		2240.90	0.45
		2204.02	0.45
5	(F-721)-Lipemic	2231.78	0.43
		2250.14	0.43
		2236.13	0.44
6	(F-722) - Hemolyzed	2194.70	0.42
		2212.96	0.53
	n	18	18
	Mean	2223.37	0.45
	SD	23.62	0.03
	%CV	1.06	4.07
	%Mean Accuracy	98.81	95.78

Table 4: Futibatinib linearity

Concen (ng/mL)	*Analyte/IS*	*analyte response*	*IS response*
0.16	0.00011	346	3145957
32	0.0220492	69345	3145005
195	0.1341271	421967	3146024
410	0.2805025	882365	3145658
860	0.5911105	1859014	3144952
1480	1.0343247	3253214	3145254
2250	1.5342178	4823659	3144051
3250	2.2848195	7186532	3145339

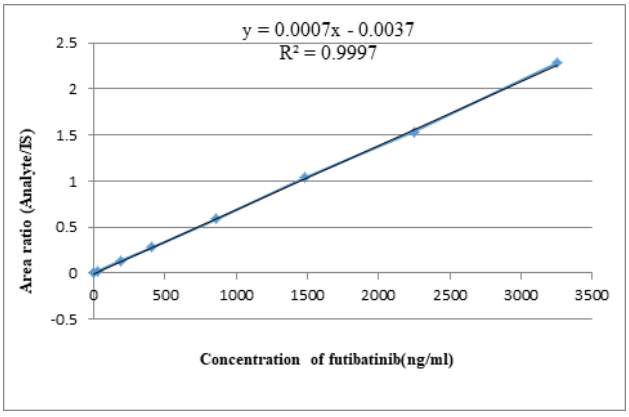

Figure 4: Futibatinib linearity.

Table 5: Futibatinib dara for accuracy and precision.

Precision and accuracy				
Analyte	*Futibatinib*		*ISTD*	*Dasatinib*
	HQC	*MQC*	*LQC*	*LLOQQC*
		I		
Mean	2217.56	1459.74	0.45	0.15
SD	16.73	22.61	0.01	0.01
%CV	0.75	1.54	1.03	2.85
%Mean Accuracy	98.55	98.63	95.22	94.61
		II		
Mean	2206.01	1455.89	0.46	0.15
SD	13.80	23.15	0.02	0.002
%CV	0.62	1.59	4.63	1.42
%Mean Accuracy	98.04	98.37	96.80	95.95
		III		
Mean	2224.43	1437.50	0.45	0.15
SD	17.34	10.76	0.01	0.003
%CV	0.78	0.74	4.34	2.14
%Mean Accuracy	98.86	97.13	95.48	95.56
Inter day accuracy and precision				
n	18	18	18	18
Mean	2217.33	1451.05	0.46	0.15
SD	17.65	21.96	0.02	0.003
%CV	0.79	1.513	3.79	2.28
%Mean Accuracy	98.49	98.04	95.83	95.37

concentrations between 0.62 and 4.63, well within the allowable range of 15%. The percent CV of the back-calculated concentration levels for all LLoQ samples were shown 2.28 (Table 5), which is within the allowable range of ±20%.

Accuracy

The precision of the test was calculated by comparing the estimated average readings of quality controls to their corresponding nominal results. For all control solutions, the maean accuracy of back-calculated concentrations were between 94.61 and 98.86[15] (Table 5).

Recovery

When comparing extracted and un-extracted plasma quality control solutions at MQC, HQC, and LQC concentrations[13], the average recoveries in percent were determined. Mean recoveries for Futibatinib were from 98.49 to 99.35 percent at MQC, 99.35 to 98.47%vat HQC, and 98.47% at LQC. At every QC grade, the mean recovery was 90.51 and the CV was 3.06 (Table 6).

Integrity of dilution

By the process of dilution, 1 in 5 and 1 in 10 times to 3×ULOQ, the method's dilution integrity was evaluated. It was found that the accuracy for dilute solutions of 1/5th and 1/10th was 0.74 and 0.05%, correspondingly.[14]

Table 6: Futibatinib recovery results

Recovery - Analyte						
Analyte	*Futibatinib*		*ISTD*		*Dasatinib*	
S. No.	*HQC*		*MQC*		*LQC*	
	Un extracted drug response	*Extracted drug response*	*Un extracted drug response*	*Extracted drug response*	*Un extracted drug response*	*Extracted drug response*
1	4865742	4845632	3202654	3188364	1014	953
2	4865219	4855675	3212547	3098021	1045	932
3	4865328	4845666	3201259	3188327	1047	1124
4	4865002	4765602	3208845	3198456	1037	953
5	4865027	4825647	3203621	3188651	1038	982
6	4865324	4865647	3203695	3078327	1030	966
n	6	6	6	6	6	6
Mean	4865273	4833978	3205436	3156691	1035	985
SD	245.78	32890.71	3951.78	48909.53	10.97	63.96
%CV	0.005	0.68	0.12	1.54	1.06	6.41
%Mean Recovery	99.35		98.47		95.15	
Overall %Mean Recovery	97.66326					
Overall %CV	2.271023					

Table 7: Futibatinib stability studies

Stabilities level	*Concentration level*	*Comparison samples area mean*	*Stability samples area mean*	*% Mean stability*
Short-terms	LQC	990.16	5.72	95.65
	HQC	4816003	0.72	98.98
Long-terms	LQC	992.83	4.31	95.91
	HQC	4788982	1.36	98.43
Freeze thaws at -28 ± 5°C	LQC	964	1.82	93.12
	HQC	4818974	0.79	99.04
Bench top stability	LQC	959	1.41	92.69
	HQC	4833939	0.67	99.35
Auto sampler stability	LQC	982.83	2.86	94.94
	HQC	4834022	0.68	99.35
Wet extract stability RT	LQC	956.66	1.57	92.41
	HQC	4833723	0.67	99.35
Dry extract stability	LQC	997.33	5.13	96.39
	HQC	4833978	0.68	99.35

Stability study

For short-term stability, the analytes and IS were kept at room temperature for 8 hours. For 10 days, 16 hours, and 20 minutes, both HQC and LQCs were tested for stability at temperatures ranging from 2.0 to 8.0°C. The temperature was held at -28 5°C for three freeze-thaw cycles. The 17 hours and 28 minutes of room temperature18 stability of a spiking quality control sample solution was evaluated on a laboratory bench. To ensure the stability of the controls, they were kept in an auto sampler at 5 3°C for 2 days 20 hours and 27 minutes. Wet extract stability was assessed by keeping spiked quality control samples at room temperature for 23 hours and 42 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 5°C. All probes fell within acceptable parameters, as shown in Table 7.

CONCLUSION

A sensitive, accurate, and linear LC-MS/MS method for the quantification of futibatinib in human plasma K2 EDTA was developed. A Phenomenex C18, 150 × 4.60 mm, 2.1 µ column, 0.1% HCCOH, ACN and methyl alcohol (13/67/20, v/v/v) mobile solvent system at 0.8 mL/min was employed for the isolation of components. 10 µL. of volumes were utilized to separate the peaks within 2.0 minutes at 40 ± 5ºC of oven temperature. Analyte retention was 1.261 minutes and ISTD of 1.292 minutes. During validation, all four calibration curves were linear for standards concentrations from 0.16 to 3250 ng/mL. Validation showed an $r^2 = 0.9997$. At MQC, HQC, and LQC concentrations, Futibatinib had 98.49, 99.35, and 98.47 percent mean recovery. Every QC level had a mean recovery of 90.51% and a %CV of 3.06. All of the control solutions had back-calculated concentration values that were accurate between 94.61 and 98.86% of the time. The range of %CV of back-calculated values for all quality control samples was between 0.6 and 4.63, which is within the acceptable range of 15%.

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