



An Advanced Biological Matrix Extraction method development and Validation for Dacomotinib in Human Plasma (K₂ EDTA plasma) by using LC-MS/MS

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Abstract:

In the current investigation, a validated LC-MS/MS assay was created in order to measure Dacomitinib in K₂EDTA human plasma. Dacomitinib was resolved chromatographically using a C18 column and an isocratic mobile phase. With a mean recovery of 82.08% and a linear calibration curve spanning from 0.503 ng/ml to 250.010 ng/ml, the validated procedure meets all of the acceptance criteria by using QuEChERS extraction method. Dacomotinib D10 is the internal standard, and its corresponding mean recovery is 77.45%. With a retention time of 1.35 min for dacomotinib and dacomotinib D10 and a run time of 4 mins, column oven temperature of 30° C at a flow rate of 0.800 ml/min, and autosampler temperature of 5° C, the method is fully validated and is successful, sensitive, simple, and robust and apply for routine analysis. Dacomitinib's obtained r^2 is 0.9992, and the LLOQ is 0.506 ng/ml.

Keywords: Dacomotinib, QuEChERS, LC-MS/MS

1.Introduction:

Lung cancer is the most frequent cause of cancer-related death, and non-small cell lung cancer (NSCLC), which has an incidence of about 90%, is especially dangerous. Recent years have seen an increase in the importance of the EGFR signalling pathway as a therapeutic target for NSCLC. Cancers with EGFR are successfully treated with anti-tyrosine kinase inhibitors (TKIs) that suppress EGFR. Mutations, with a distinctive window for treatment First-line TKIs for EGFR (such as Good early responses are seen with erlotinib and gefitinib against these mutations. Unfortunately, their therapeutic efficacies are reduced by acquired resistance in around 60% of patients and side effects that develop throughout therapy. Due to this, researchers created irreversible, second-generation EGFR TKIs, such as dacomitinib (DMB) and avitinib.

Dacomitinib, an irreversible inhibitor of the epidermal growth factor receptor, is used to treat metastatic NSCLC. Dacomitinib accurately and persistently binds to the human epidermal growth factor receptor (EGFR), inhibiting the proliferation of tumour cells that express this receptor and inducing death. In the treatment of metastatic NSCLC with EGFR mutations L858R or del19, dacomitinib was found to be more advanced than drugs with equal efficacy, such as afatinib, gefitinib, or erlotinib. Dacomitinib was found to be superior than afatinib, cisplatin, and carboplatin in individuals with a variety of EGFR mutations. Dry skin, a sour taste in the mouth, weight loss, decreased appetite, and swelling or infection around the nails are some of the side effects of dacomitinib.

The current study's goals were to construct a validated LC-MS/MS technique to quantify DMB in human plasma as a separate biological matrix from the medication and to create and assess criteria for dacomitinib.

2. Experimental

2.1. Reagents and chemicals

Aceonitrile, water, methanol, formic acid, magnesium sulphate, and sodium chloride were all acquired from SD Fine Chemicals in Mumbai, India, and were all of HPLC or analytical grade. From Clearsynth Labs, standards Dacomitinib and Dacomitinib-D10 were received.

2.2 Instrumentation

Agilent 1200 series and API 3200 Tandem mass spectrometers made up the LC-MS/MS system (AB Sciex). Chromatographic separation was accomplished using a Thermo Hypersil BDS, C18, column that was 100 mm by 4.6 mm by 5 m. CONDITIONS of LC-MS/MS

Chromatographic conditions

Mobile Phase : acetonitrile(80%) :10mm Ammonium formate (20%)
Diluent : 50% Acetonitrile
Column :Analytical column (Discovery[®] C18 10 cm X 4.6 mm, 5 μ m)
Column Oven Temperature 30⁰C
Auto Sampler Temperature 5^oc
Run time :4.00 min
Retention Time :Analyte 1.35 \pm 0.5 min and ISTD 1.35 \pm 0.5 min.
Mass spectrometer parameters:
Dacomitinib : Q1/Q3- 470.200/385.100 Mass (amu)
Dacomitinibd10 : Q1/Q3- 480.200/385.100 Mass (amu)
Ionization mode : Turbo Ion spray (TIS/ESI)
Polarity : Positive
Acquisition Mode : Multiple Reaction Monitoring

2.3 Stock solution preparation:

To obtain a known concentration of 200.000 g/ml, weigh approximately 1.000 mg of dacomitinib, dissolve in 5 ml of methanol, and keep in the refrigerator. To reach a known concentration of 200.000 g/ml, dissolve approximately 1.000 mg of dacomitinib D10 in 5 ml of methanol. Store in the refrigerator.

2.4 Preparation of internal standard dilution:

To obtain a known concentration of 100.000 ng/ml, dilute 0.025 ml of dacomitinib D10 stock solution to 50 ml with 50% acetonitrile. Store in the refrigerator.

2.5Preparation of .CC spiking solutions:

From the analyte stock solution in the range of 25.104 ng/ml to 12520.800 ng/ml, prepare the CC spiking solutions.

Building QC spiking solutions From the analyte stock solution, prepare the QC spiking solutions. MQC1: 4041.297ng/ml, MQC2: 755.723ng/ml, HQC: 9599.280ng/ml LLOQ: 25.290ng/ml and LQC: 73.305ng/ml.

2.6.1 sample setup (extraction procedure)

In tubes with polypropylene labels, place 100 mg of QuEChERS salt (a 4:1 mixture of MgSO₄ and NaCl), then add 50 L of ISTD dilution (100.000 ng/mL) (except blanks). 50 mL of 50% acetonitrile should be added to the blank samples (diluent). Pipette 200 L of sample (CC standard, QC sample, Blank, etc.) into each tube, then give it a quick vortex. Acetonitrile should be added, then vortex for 5 minutes. All samples should be centrifuged at 15000 RPM

for 5 minutes at 5°C. The supernatant should then be fully dried by being exposed to a spray of nitrogen at 40°C. The dried residue samples must be immediately vortexed after being reconstituted with 0.200 mL of mobile phase and placed into the proper, marked auto sampler vials.

Method Development:

The present work objective was to obtain an efficient and simple develop and validate the analyte by using QuEChERS extraction procedure for the analyte with ISTD. Tuning parameters of MS/MS instrument mentioned below to get reliable method develop and to applicable to pharmacokinetic studies.

System depend parameters:

Compound depend parameters:

ESI Source Parameter Settings

CUR 30.00

CAD 6.0

IS 5500.00

TEMP(°C) 450.00

GS1 50.00

GS2 50.00

Ihe ON

Parameter Analyte ISTD

DP(Declustering potential) 80.00 80.00

EP(Entrance potential) 10.00 10.00

CE(Collision energy) 40.00 40.00

CXP(Collision cell exit potential) 15.00 15.00

Dwell Time (m sec) 200.00 200.00

Method validation

The established LC-MS/MS assay was validated for Carryover, Selectivity, Matrix factor, Linearity, Sensitivity, Accuracy and Precision, Recovery, Dilution integrity, Run size evaluation, ReInjection reproducibility, Ruggedness and Stability, in accordance with the US Food and Drug Administration (FDA) guidelines. The validation parameters of the LC-MS/MS assay, developed to assess DMB, were previously described in more detail. The calibration curve equations ($y = ax + b$) and the statistical method of least squares were used to calculate these parameters. The linear fit was verified using the r^2 value.

The following sequence of samples were injected to test for carryover: extracted blank plasma, ULOQ sample, double extracted blank plasma, LLOQ, and the same order for aqueous samples.

Selectivity:

The purpose of this test is to evaluate the selectivity of the bioanalytical method for the analyte(s) and ISTD(s) of interest from the naturally occurring variance of endogenous matrix components among individuals for various independent matrix lots. Ten distinct plasma lots (Screened), two Lipemic (L) and two Haemolyzed (H) blanks, as well as the LLOQ standard for the relevant lots, were extracted.

Matrix factor:

Ten lots of blank biological matrix, including two Lipemic (L) samples and two Haemolyzed (H) samples, were extracted in triplicate for each lot, spiked with the aqueous dilutions at LQC and HQC levels, and then compared to aqueous samples of the same concentration. 10 replicate injections of LQC and HQC aqueous samples were added to the post-extracted LQC and HQC samples.

Linearity:

Calibration standards were put into plasma samples before K2EDTA was used as an anticoagulant. The 10-point calibration curve for dacomitinib was found to be linear from 0.503 ng/mL to 250.010 ng/mL. A linear least squares regression with a 1/x² weight factor was used to evaluate the goodness-of-fit using three replicates of the calibration standard concentration..

Precision and Accuracy:

For this assessment, matrix samples from A & P runs with various Dacomitinib doses were analysed. The calibration curve (LLOQ to ULOQ) and six replicates of each of the quality control samples HQC, MQC1, MQC2, LQC, and LLOQQC were required for each run, and three Precision & Accuracy runs were processed and examined using a freshly spiked calibration curve. The assay's quantitative precision and accuracy were assessed both within and between runs.

Recovery

For both the analyte (Dacomitinib) and the ISTD, the recovery of the analyte at various concentration levels, such as HQC, MQC, and LQC (six replicates of extracted QCs), was compared to the six replicates of post-extracted HQC, MQC, and LQC (at equivalent concentrations) (Dacomitinib D10)

Stability

To evaluate the stability of samples under anticipated conditions when handling, storing, processing, and analysing subject samples, stability exercises were carried out. The short-term and long-term stability of analyte and ISTD stock solutions were examined at

room temperature and at 5 °C, respectively. For benchtop, processed sample stability at room temperature (24 ± 4°C), processed sample stability under refrigeration (2-8°C), and freeze-thaw stability studies, extracted samples stability performed by newly spiked calibration curve standards and quality control samples were utilised.

Results and Discussion:

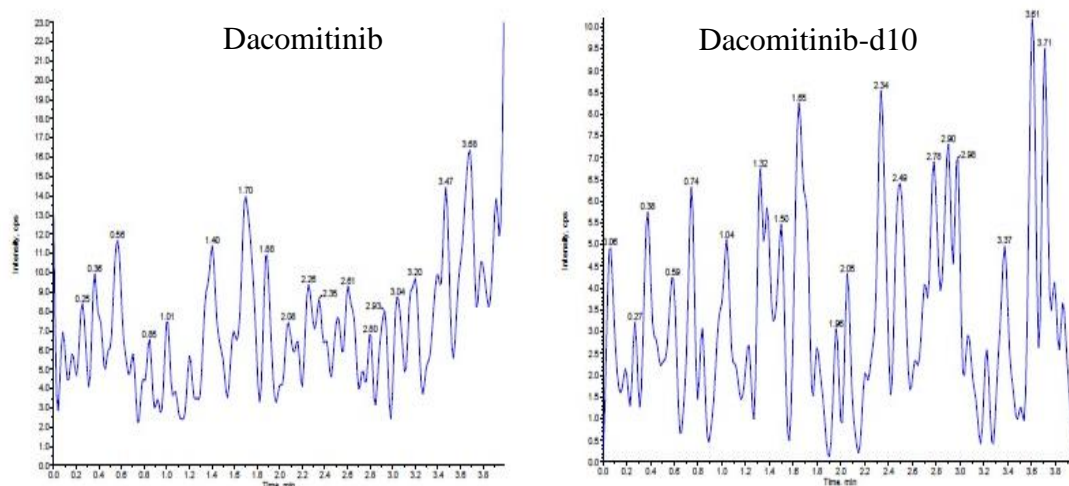
The goal of the current investigation was to create and validate a sensitive and specific LC-MS/MS technique for measuring dacomitinib in human plasma. By enhancing the chromatographic, mass spectrometric, and extraction processes, the study's goal was fulfilled.

In the run for blanks, which were injected in accordance with a ULOQ standard, the results were assessed for carryover. With a further injection of ULOQ standard at the corresponding retention durations for the analyte and the ISTD, it was demonstrated that the reaction was insignificant and that there was no enhanced response in the blank samples.

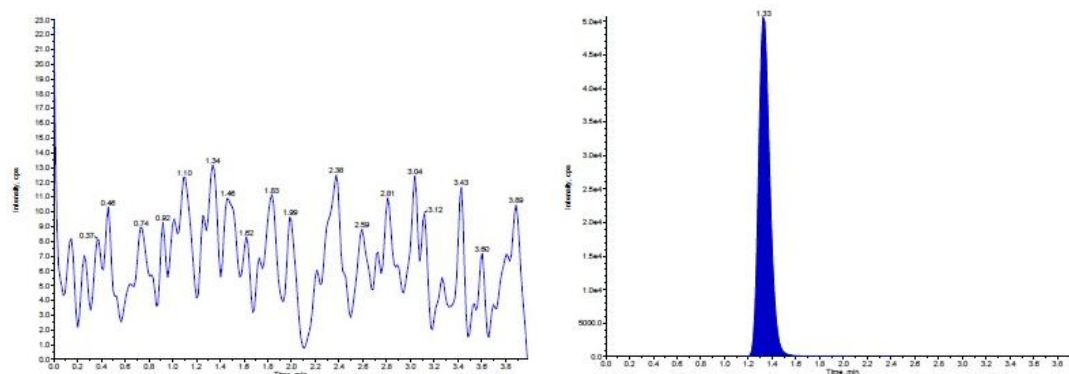
Selectivity

As demonstrated in fig. a&c, all samples were found to be devoid of any appreciable interference from endogenous matrix components, proving the selectivity of the approach at LLOQ in terms of accuracy.

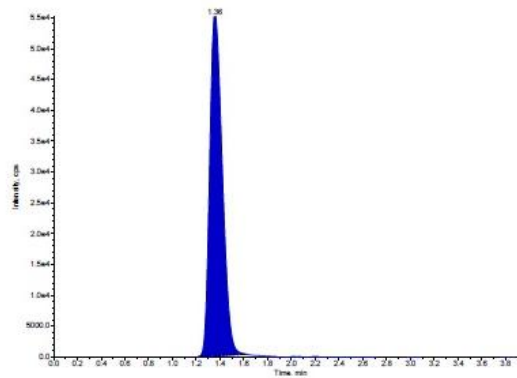
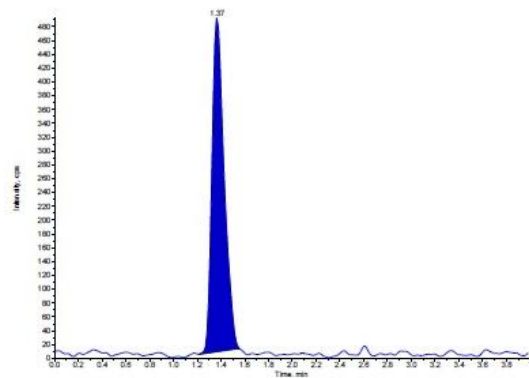
A



B



C



D

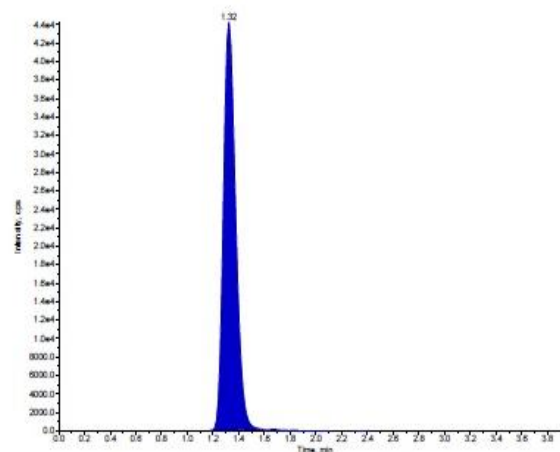
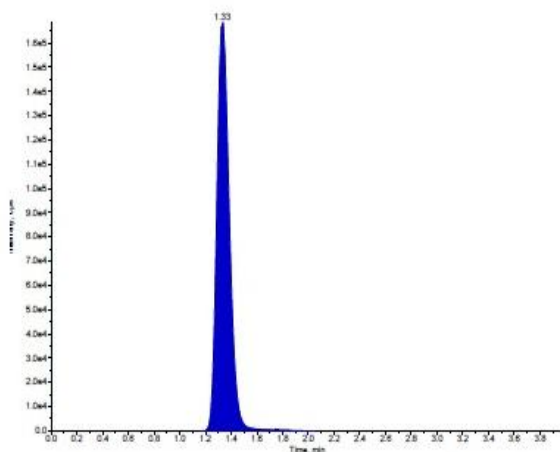


Fig. 2 MRM ion–chromatograms of dacomitinib, dacomitib10 in (A) blank plasma,(B) blank plasma spiked with IS (C)Extracted LLOQ (D) Extracted ULOQ

Matrix factor

In this extraction procedure either ion suppression/enhancement in the signal was found as % C.V 0.86%&1.55% at LQC & HQC level when compared to extracted samples with aqueous samples.

Linearity

It was discovered that every calibration curve was linear between 0.500 and 250.400ng/ml. In all three of the situations given in tables 1 and 2, the mean correlation coefficient was consistently higher than 0.999.

Table1: Back calculated concentrations of calibration curve samples for dacomitinib
in human plasma

CC ID	Nominal concentration (ng/ml)									
STDs	0.500	1.000	2.504	12.52	25.040	50.08	100.16	150.24	200.32	250.4
1	0.507	0.984	2.997	12.016	24.954	50.667	101.505	150.473	200.814	246.712
2	0.502	1.032	2.848	12.221	25.609	51.470	101.020	151.691	198.505	245.115
3	0.512	0.987	2.882	12.181	26.011	52.512	101.951	151.046	201.176	245.815
Mean	0.507	1.001	2.909	12.139	25.525	51.550	101.492	151.07	200.165	245.880
S.D	0.005	0.027	0.078	0.109	0.53352	0.925	0.466	0.609	1.449	0.801
Accuracy	101.4	99.5	96.6	100.8	101.9	102.9	101.1	100.7	100.1	98.3
Precision	1.0	2.7	2.7	0.9	2.1	1.8	0.5	0.4	0.7	0.3

Table2: Linearity parameters summary for dacomitinib in human plasma

calibration curve	slope	y-intercept	correlation coefficient(r^2)
1	0.0147	-0.000433	0.9998
2	0.0143	-0.000782	0.9992
3	0.014	0.000301	0.9994

Precision and Accuracy:

The method's intra- and inter-day precision & accuracy ranged from 1.32-4.58 and 1.91-8.28% c.v., 99.18-105.65 and 100.27-103.48, respectively, for varied concentration levels. The outcomes demonstrated the method's accuracy and precision, and the table3 statistics support this claim.

Table 3: Dacomitinib precision and accuracy (intra-batch & inter-batch)

QC level (nominal concentration, ng/ml)	intra batch (n=6; single batch)			inter batch(n=18; 6 from each batch)		
	mean conc. found(ng/ml)	Accuracy (%)	% C.V	mean conc. found	Accuracy (%)	% C.V
HQC (191.526)	193.842	101.21	1.32	198.194	103.48	1.91
MQC-1(80.632)	85.186	105.65	1.78	85.146	105.25	1.48
MQC-2 (15.078)	15.675	103.96	1.51	15.525	102.96	2.01
LQC(1.463)	1.451	99.18	3.29	1.467	100.27	4.08

LLOQQC(0.505)	0.507	100.40	4.58	0.507	100.40	8.28
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Recovery:

The global recovery of dacomitinib at three concentration levels 77.45 and for ISTD was 82.08. the extraction recovery data was shown in the table 4.

Table4: Extraction recovery for dacomitinib

QC level (nominal concentration, ng/ml)	Mean area response (n=6)		Recovery (B/A%)		Global % Recovery	
	A (Post extracted area)	B (Extracted Area)	Analyte	IS	Analyte	IS
HQC (191.526)	1376500	1085599	78.87	82.81	77.45	82.08
MQC-1(80.632)	641742	513085	79.95	85.57		
LQC(1.463)	12468	9166	73.52	79.57		

Stability:

At two concentration levels, such as HQC and LQC, the various stability metrics bench top, processed sample stability, and freeze/thaw were carried out. The findings, which are presented in Table 5, suggest that there were no stability-related difficulties that would have complicated the use of the assay to PK studies. According to USFDA requirements, the results of additional metrics, including robustness, reinjection reproducibility, influence of PID, dilution integrity, prolong P&A batch, and robustness and aqueous solution stability, were found to be within the acceptable range. Stability of Dacomitinib in human plasma at various conditions (n=6).

Table 5.

Stability	Storage condition	Duration	Level	Nominal concentration (ng/ml)	Mean Stability sample (mean \pm SD, ng/ml)	%C.V	Accuracy
Bench top	Room temperature	8 hrs	HQC	191.526	188.057 \pm 1.9	1.1	98.19
			LQC	1.463	1.463 \pm 0.05	4.0	100.00
Processed sample	Refrigerated (2-8°C)	3 days	HQC	191.526	188.981 \pm 3.7	2.0	98.67
			LQC	1.463	1.454 \pm 0.05	3.6	99.38

stability							
Processed sample stability	Room temperature (24 ± 4°C)	28 hrs	HQC	191.526	188.820±2.5	1.4	98.59
			LQC	1.463	1.486±0.03	2.2	101.57
Freeze/ thaw (on water bath)	-70±10°C	5 cylces	HQC	191.526	188.338±2.4	1.3	98.34
			LQC	1.463	1.486±0.07	5.0	101.57

Conclusion

The results of the validation confirm the precision and reproducibility of the analytical device and the assay for dacomitinib in human plasma over the concentration range of 0.503 ng/mL - 250.010 ng/mL. Extraction procedure of QuEChERS shows good recovery which is one of advance SPE extraction. The assay was accurate, and the results demonstrate that there was no interference that could have been foreseen. The evaluated analytes have proven to be stable in stressful scenarios that could occur during the application of this strategy.

Acknowledgment:

Conflicts of interest:

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