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Research Article

DEVELOPMENT AND VALIDATION OF AN LC-MS/MS ASSAY FOR THE QUANTIFICATION OF FEDRATINIB IN HUMAN PLASMA AND DRIED BLOOD SPOT MATRICES

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ABSTRACT

Objectives: The objective of the study was to develop and validate a highly sensitive and robust LC-MS/MS method for the quantification of fedratinib (FRB), a selective Janus kinase 2 inhibitor used in the treatment of myelofibrosis, in both human plasma and dried blood spot (DBS) samples as per ICH M10 guidelines.

Methods: Quantification was performed using a liquid chromatography-tandem mass spectrometry system equipped with electrospray ionization and multiple reaction monitoring. DBS samples were prepared using Whatman 903 cards, with FRB extracted efficiently to enable low-volume, stable, and decentralized sampling. Calibration was established over the range of 36–3,600 ng/mL.

Results: The method demonstrated excellent selectivity, precision, and accuracy, with recoveries of 63.1% (plasma) and 77.1% (DBS) for FRB, and 60.9% (plasma), and 87.4% (DBS) for the internal standard. Stability assessments confirmed robustness under bench-top, auto sampler, and long-term conditions. Comparative analysis of plasma and DBS matrices showed strong agreement: Passing-Bablok regression indicated a slope close to 1 with negligible intercept, and Bland-Altman analysis revealed a mean bias of approximately −2%, well within the bioanalytical acceptance limits (<15%).

Conclusion: DBS demonstrated equivalence with plasma for FRB quantification across lower-quality control (QC), middle-QC, and high-QC levels, confirming its suitability as a reliable alternative to plasma for pharmacokinetic and bioequivalence studies. This validated method provides a practical and sensitive tool for FRB determination, particularly advantageous in decentralized and resource-limited settings.

Keywords: Fedratinib, Dried blood spot, Bioanalytical method validation, Bioanalysis, Clinical study, Liquid chromatography-mass spectrometry/mass spectrometry, Myelofibrosis.

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INTRODUCTION

Fedratinib (FRB), a selective Janus kinase 2 inhibitor, has emerged as a pivotal therapeutic option in the treatment of myelofibrosis, a rare bone marrow malignancy characterized by progressive fibrosis and ineffective hematopoiesis [1]. The accurate quantification of FRB in biological matrices is critical for pharmacokinetic (PK), bioavailability, and bioequivalence studies, especially considering its narrow therapeutic index and dose-limiting toxicities such as Wernicke's encephalopathy [2].

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) remains the gold standard for bioanalytical quantification due to its high sensitivity, specificity, and reproducibility [3]. Plasma-based analysis has long been the cornerstone of PK profiling; however, the challenges associated with sample collection, transport, and storage in multicentric, or resource-limited settings have encouraged the exploration of alternative matrices [4].

In this context, dried blood spot (DBS) sampling has gained significant attention. DBS offers distinct advantages such as minimal invasiveness, reduced blood volume requirement, simplified logistics, and enhanced sample stability [5]. However, methodological challenges-such as hematocrit effects, non-homogeneous distribution, and variable extraction efficiency-demand thorough validation to ensure comparability with traditional plasma-based methods [6,7].

Therefore, this study aims to develop and validate a robust, selective, and reproducible LC-MS/MS assay for the quantification of FRB in human plasma and compare it with the DBS method using the ICH M10 bioanalytical method validation guidelines [7,12]. The comparative assessment will not only determine the feasibility of adopting DBS as a reliable alternative but also potentially streamline sampling processes in clinical and field-based studies involving FRB.

To the best of our knowledge, this is the first validated LC-MS/MS bioanalytical method developed for the quantification of FRB in DBS and its direct comparison with plasma matrix. This study establishes DBS as a viable alternative for FRB quantification, enabling simplified sample collection and storage for clinical and PK studies.

The objectives of the present study are:

- To develop and optimize a sensitive and specific LC-MS/MS method for the quantification of FRB in human plasma and DBS matrices
- To validate the developed method in accordance with the ICH M10 guidelines, assessing parameters such as selectivity, linearity, accuracy, precision, matrix effect, and stability
- To compare plasma-based quantification with the DBS method and evaluate correlation using statistical tools such as Bland-Altman plots and Passing-Bablok regression.

METHODS

Chemicals and reagents

FRB reference standard (purity: >98%) and internal standard curcumin were obtained from Simson Pharma Ltd., Mumbai. High-performance liquid chromatography-grade methanol, acetonitrile, formic acid, and water were sourced from Sigma-Aldrich, Mumbai. Blank human

plasma and whole blood dipotassium ethylenediaminetetraacetic acid (K_2EDTA) were procured from Bloodline Blood Bank, Thane, Whatman 903 DBS Protein Saver cards were obtained from Neelam Enterprises, Chennai.

Instrumentation, equipment, and analytical conditions

Instrument and equipment

Biological samples often retain a considerable proportion of matrix components even after preliminary purification steps. To ensure selective and sensitive quantification of the analyte, LC-MS/MS was employed using electrospray ionization (ESI) in multiple reaction monitoring (MRM) mode. This technique was selected for its high specificity and analytical sensitivity.

The chromatographic analysis was conducted using the Thermo Ultimate 3,000 system (Thermo Fisher Scientific), coupled with a TSQ ENDURA triple quadrupole mass spectrometer (Thermo Fisher Scientific). Data acquisition and processing were carried out using LCquan 3.0 software.

Sample preparation was carried out using a nitrogen evaporator (N_2 Fastvap, PEI Analytics), a centrifuge (Electrotecnik model, REMI), and a vortex mixer (Praveen Scientific Corporation) [8].

Analytical conditions

For separation, mobile phase A consisted of 1 mL of formic acid in 1,000 mL of LCMS grade water and mobile phase B consisted of methanol. The flow rate was set at 0.5 mL/min with a total run time of 3.0 min. The injection volume was 10 µL.

Mass spectrometric detection was carried out in the positive ESI+ mode with MRM, as mentioned in Table 1.

Sample preparation

Analyte stock solutions, calibration standards, and quality controls (QCs)

10 mg of analyte (FRB) was weighed on semi-microbalance and was dissolved in 10 mL of methanol which yielded into 997.200 µg/mL solution considering the purity of analyte, it was further diluted by transferring 7.2 mL into 100 mL methanol (diluent) to prepare intermediate dilution. Further aqueous linearity dilutions were prepared of varying concentrations, assisting to obtain desired plasma concentration.

Table 1: Time segments-2 scan parameter

Compound name	Start time	End time	Prec. ion (m/z)	Prod. ion (m/z)	Collision energy (V)
Curcumin	0	3	369.11	177.02	45
Curcumin	0	3	369.11	253.04	45
Curcumin	0	3	369.11	285.02	45
Fedratinib	0	3	525.20	369.91	55
Fedratinib	0	3	525.20	371.80	55
Fedratinib	0	3	525.20	397.90	55

Table 2: Targeted CC and QC concentrations

Level	Criteria	Concentrations (ng/mL)
CC-1	LLOQ	36
CC-2	×2 of LLOQ	72
CC-3	~3–4% of ULOQ	144
CC-4	~6–8% of ULOQ	216
CC-5	~10% of ULOQ	360
CC-6	~20% of ULOQ	720
CC-7	~40% of ULOQ	1440
CC-8	~60% of ULOQ	2160
CC-9	ULOQ	3600
LLOQ QC	100% of LLOQ	36
LQC	×3 of LLOQ	108
MQC	50% of ULOQ	1800
HQC	80% of ULOQ	2880

CC: Calibration curve, QC: Quality control, LLOQ: Lowest limit of quantification, ULOQ: Upper limit of quantification, HQC: High-quality control, MQC: Middle-quality control, LQC: Lower-quality control

For the calibration curve, 0.25 mL from each linearity dilution was further diluted to 1.0 mL with blank plasma to obtain concentrations ranging from 36 ng/mL to 3,600 ng/mL, corresponding to the lower limit of quantification (LLOQ) and the upper limit of quantification (ULOQ), respectively, as mentioned in Table 2. QC samples were prepared at four levels: LLOQ QC (36 ng/mL), low QC (LQC, 108 ng/mL), mid QC (MQC, 1,800 ng/mL), and high QC (HQC, 2,880 ng/mL), similar procedure was followed to prepare linearity and QC samples, using blank whole blood as matrix.

For the preparation of the internal standard (Curcumin), an accurately weighed quantity of curcumin was transferred into a volumetric flask to prepare a primary stock solution. The weight was calculated based on the molecular weight and solubility of curcumin to achieve the desired concentration. The analyte was dissolved in an appropriate diluent and made up to the mark to obtain a stock solution of 1,000 µg/mL. From this primary stock, serial dilutions were performed using the same diluent to obtain a working solution of 50 µg/mL, which was used for routine spiking into plasma and whole blood samples during calibration curve and QC sample preparation.

Curcumin was used as an internal standard as deuterated standard of FRB was commercially unavailable during research and curcumin exhibited comparable chromatographic behavior, stable ionization in positive ESI mode, and reproducible recovery during extraction [18,19].

Method development, and extraction procedure

Chromatographic optimization was performed through systematic trials, considering polarity and pKa of the analyte [16,17]. Given the polar nature of FRB, different C18 columns and various mobile phase compositions ranging from non-polar to low-polar solvents were evaluated under different trials.

Plasma samples (200 µL) were aliquoted into tubes and spiked with 50 µL of internal standard (Curcumin, 50 µg/mL) followed by vortex mixing. Protein precipitation was induced by adding 1.5 mL of methanol, after which the samples were vortexed and centrifuged at 4,000 rpm for 5 min. From this solution, 1 mL of the clear supernatant was transferred to a clean vial using micropipette and evaporated to dryness under nitrogen at 50°C for 15 min. The residue was reconstituted in 500 µL of mobile phase, vortexed, and subjected to LC-MS/MS analysis under optimized chromatographic conditions.

DBS extraction procedure and hematocrit effect

During DBS development technique, couple of DBS papers from multiple sources was evaluated. The procured Whatman 903 protein saver card was finalized.

To mitigate hematocrit-related variability and the inconsistencies caused by disc punching, an advanced approach was adopted wherein the entire volumetrically applied blood spot was analyzed, rather than relying on fixed-size partial punches. The entire DBS can be punched out or cut out after the application of a defined volume of blood or the blood can be spotted onto prepunched discs [7-11].

Linearity and QC concentrations spiked with FRB in whole blood samples were prepared, and further extraction procedure was carried out.

- 200 µL of pre-spiked whole blood was aliquoted on DBS card, followed by air drying for 1–2 h at room temperature. To avoid hematocrit, effect the complete spot was cut and placed in the vial
- 1.5 mL methanol was added to the vial containing DBS sample followed by addition of 50 µL internal standard. These samples were vortexed and centrifuged at 4,000 RPM for 5 min

- 1 mL of upper layer was transferred to vial and evaporated at 50°C for 15 min
- This was then reconstituted with 500 µL of mobile phase and vortex to mix followed by injecting supernatant in LC-MS/MS.

Method validation for plasma and DBS procedures
The developed method was validated as per ICH M10 guidelines [12].

Selectivity, specificity, and autosampler carry over test (ASCOT)
Selectivity was assessed using different human plasma lots and blood lots. One blank sample from each lot; likewise, multiple blank samples were processed a long with one set of freshly spiked, prepared CC standards, and batch evaluation QCs separately for plasma method validation and DBS method validation, as per their extraction procedures.

Specificity was assessed by evaluating the potential interference from commonly co-administered medications. Blank plasma samples were spiked with the test analyte along with drugs at their reported Cmax concentrations, including caffeine (10 µg/mL), ibuprofen (15 µg/mL), diclofenac (100 µg/mL), paracetamol (10 µg/mL), ondansetron (40 ng/mL), and ranitidine (800 ng/mL). The absence of significant interference at the retention time of the analyte and internal standard confirmed the method's specificity.

ASCOT was performed by injecting DBS processed samples in the sequence as extracted blank, extracted ULOQ, extracted blank, extracted LLOQ, extracted blank, extracted ULOQ, extracted blank, and at last extracted LLOQ blood samples.

Linearity
Calibration curves were constructed in the range of 36 ng/mL to 3,600 ng/mL.

Accuracy and precision
Assessed at LLOQ, low-, medium-, and high-QC levels (36.000 ng/mL, 108.000 ng/mL, 1800.000 ng/mL, and 2880.000 ng/mL).

Matrix effect
Matrix effects were evaluated by preparing low- and high-QC (108 ng/mL and 2880 ng/mL) samples. FRB was spiked into different lots of human plasma containing K_2EDTA as an anticoagulant to generate these controls and for DBS method validation FRB was spiked in different blood lots. These samples were processed using the established plasma extraction method and DBS technique, respectively, and analyzed alongside freshly prepared CC standards and batch qualifying QC samples. For all matrix lots, QC samples accuracy was required to fall within 85–115% and the coefficient of variation to be maintained at ±15%.

Recovery
Recovery was determined by comparing FRB response at lower-QC (LQC), middle-QC (MQC), and high-quality control (HQC) with detector response obtained from recovery samples with extracted spiked QC samples. Precision for mean % recovery obtained from each QC levels shall be ≤15% for both FRB and curcumin.

Stability
For plasma and DBS, bench top (BT), long-term (LT), autosampler, and freeze-thaw (only for plasma) stability are critical parameters. For each stability assessment, six separate aliquots of LQC and HQC samples (stability samples) were aliquoted in plasma for plasma method validation and spotted on to DBS paper for DBS method validation. The BT stability samples were stored at room temperature on the bench for 8 h, whereas LT stability samples were kept at −70°C for 28 days in deep freezer and for DBS kept in a desiccator at room temperature for 28 days. After stability duration, stability samples along with one set of freshly spiked CC standards and freshly spiked batch qualifying QC's were processed and analyzed. The mean back-calculated values of the stability samples were compared to their nominal values, and percent change was required to be no more than 15%.

RESULTS AND DISCUSSION

Method development
Chromatographic conditions were optimized to achieve adequate retention, sharp peak shape, and minimal matrix interference. Thermo Hypersil GOLD 100×2.1 mm, 1.9 µ column with mobile phase A consisted of 1 mL of formic acid in 1,000 mL of LCMS grade water and mobile phase B consisted of methanol was selected as the final condition due to its ability to produce well-resolved and stable chromatographic peaks. The selected mobile phase composition of 90:10 v/v, methanol: 0.1%, formic acid in water enabled efficient ionization, and resolution of FRB and IS within 2 min.

The optimized mass parameters are presented in Table 3.

Method validation
Selectivity
Compared to LLOQ (36 ng/mL) of FRB during the selectivity assessment, no interference was observed at the RT of FRB and curcumin in plasma and whole blood.

In addition, no interference was detected at RT and m/z of FRB and curcumin in specificity samples. Based on these results, the plasma and DBS method was found to be selective across multiple human plasma and blood lots and specific for human plasma and blood, even in the presence of concomitant drugs.

In ASCOT assessment, following the first and second injections of extracted ULOQ samples, responses of the extracted blank samples show zero interference, which was within the acceptance criteria. Hence, no autosampler carry over observed for FRB and curcumin with human plasma and blood bioanalytical method, so method and system are considered as free from carryover.

Linearity
Calibration curves demonstrated good linearity with r^2>0.99. As shown in Figs. 1 and 2, representative chromatograms for CC1 (LLOQ) and CC9 (ULOQ) are shown in Figs. 3-6.

Precision and accuracy: Precision and accuracy
In all PA batches, both blank and zero samples were free from any interference at the FRB's RT. Moreover, CCs of non-zero calibration standards, as shown in Tables 4 and 5, did not exceed ±15% of their nominal concentrations and these standards met criteria with a determination coefficients r^2>0.99, as shown in Figs. 1 and 2. As represented in Table 6, within-run and between-run precision for QCs ranged from 1.90% to 13.03% whereas within-run and between-run accuracy ranged from 93.11% to 110.91% for plasma method.

Table 3: Mass parameters (TSQ Endura)

Parameter	Setting/Value
Ionization source and polarity	H-ESI
Ion transfer tube temp (°C)	400°C
Vaporizer temp (°C)	400°C
Sheath gas flow (Arb)	40.00 Arb
Aux gas flow (Arb)	15.00 Arb
Sweep gas flow (Arb)	0.00 Arb
Mode	SRM
Cycle time (sec)	1.0 s
Q1 resolution (FWHM)	0.7
Q3 resolution (FWHM)	0.7
CID gas (mTorr)	1.5 mTorr
Chromatographic peak width (sec)	10 s
Use chromatographic filter	Yes

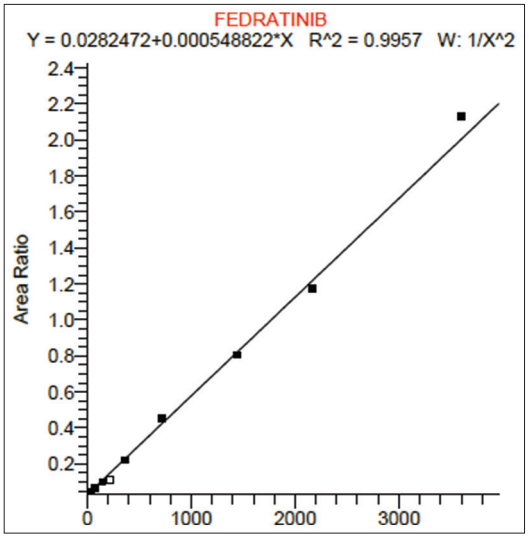

Fig. 1: Calibration curve (concentration versus area ration) of fedratinib in human plasma showing linearity (r^2=0.9957)

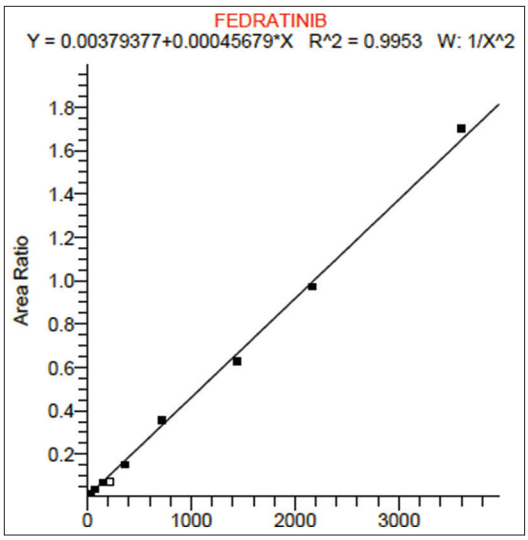

Fig. 2: Calibration curve (concentration versus area ration) of fedratinib in dried blood spot matrix showing linearity (r^2=0.9953)

Similarly, for DBS method, within-run and between-run precision for QCs ranged from 2.61% to 13.56% whereas within-run and between-run accuracy ranged from 87.66% to 107.61%, as shown in Table 7.

In completed batch, 67% of overall QCs with 50% at each level meet acceptance criteria.

DISCUSSION

Although the LLOQ accuracy (89.46%) and precision (13.56% CV) for DBS were within the generally accepted bioanalytical limits (80–120% and ≤20% CV), the marginal performance at this concentration is acknowledged. This limitation, however, did not affect the reliability of quantification for samples within the working range, as evidenced by consistent calibration and QC recoveries.

Recovery

Batch qualifying QC samples met acceptance criteria, with precision for mean % recovery a teach QC level being ≤15% for both FRB and ISTD. The mean recovery was determined 63.1% for FRB and 60.9 % for ISTD, respectively, in human plasma method.

Similarly, for DBS method, the mean recovery was determined 77.12% for FRB and 87.40% for ISTD, respectively.

Matrix effect

Ion suppression/enhancement was within acceptable limits, with %CV <15%. Consequently, the bioanalytical method for human plasma and blood was determined to be free from significant matrix effects.

Stability

FRB was stable under all tested conditions (results provided in Tables 8 and 9).

Percent change of LQC and HQC samples was within the acceptance criteria of ±15%, while comparing the mean of back-calculated values of stability samples against nominal values after BT stability of 8 h and LT storage of 28 days (Tables 8 and 9).

From stability, evaluation confirmed that FRB stable on the bench at room temperature for 8 h as well as stable in a desiccator for 28 days, which will be sufficient to complete bioanalysis after sample collection in therapeutic drug monitoring (TDM) or clinical studies.

DBS versus plasma comparison

By plotting the difference between the two methods against their mean, the mean difference provides an estimate of bias, and the

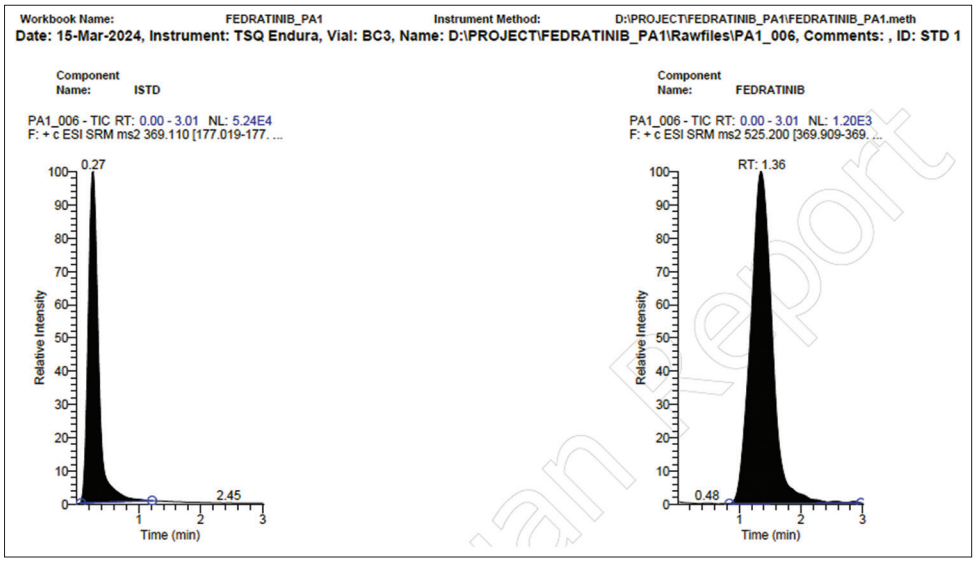

Fig. 3: Representative chromatogram of plasma extracted lower limit of quantification (36 ng/mL), ISTD, and fedratinib

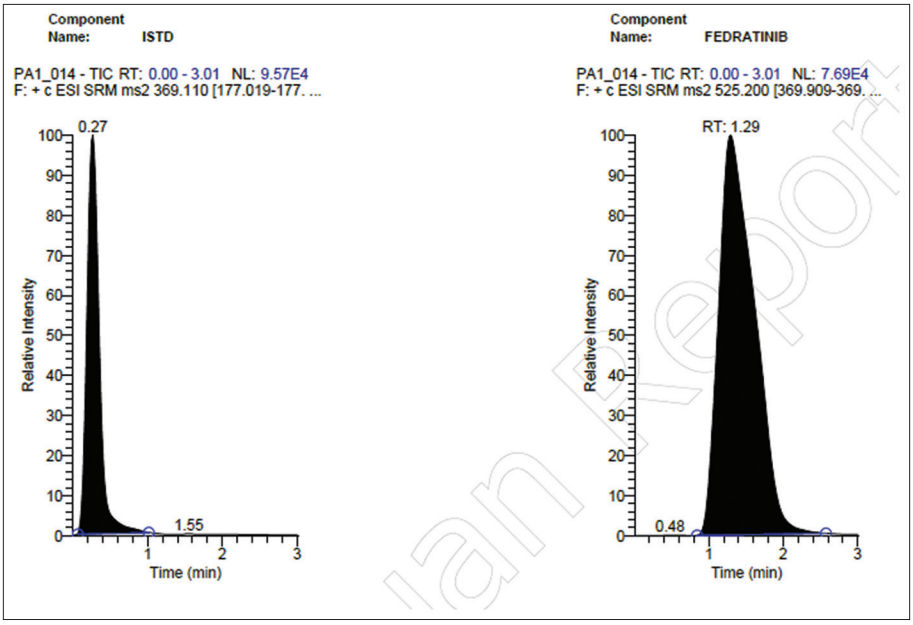

Fig. 4: Representative chromatogram of plasma extracted upper limit of quantification (3600 ng/mL), ISTD, and fedratinib

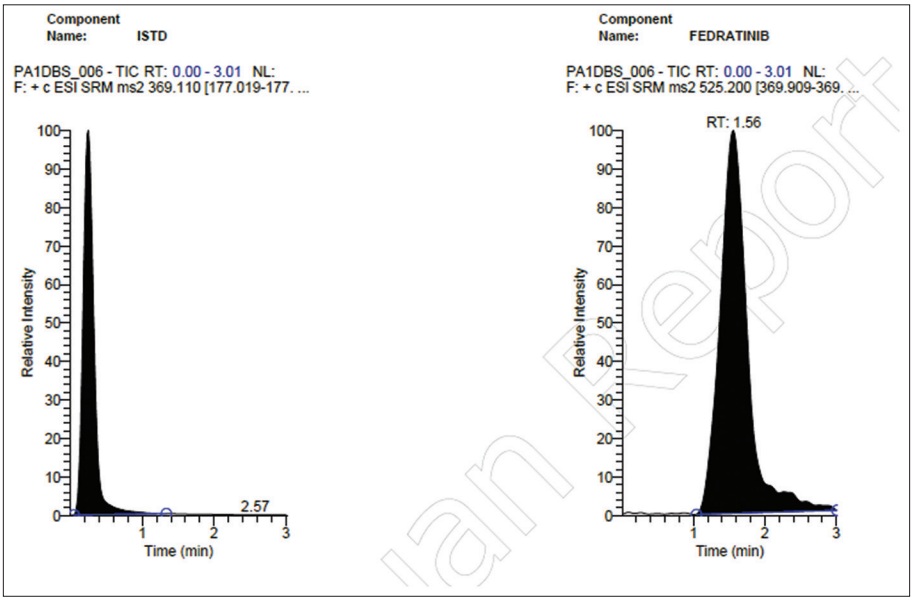

Fig. 5: Representative chromatogram of dried blood spot extracted lowest limit of quantification (36 ng/mL), ISTD, and fedratinib

Table 4: CC data for precision and accuracy batches (Plasma method)

CC ID	Nominal concentration (ng/mL)	Mean (ng/mL)	SD (±)	CV (%)	Mean % nominal
CC1	36	37.0701	1.360943312	3.67	102.97
CC2	72	65.3451	4.829182696	7.39	90.76
CC3	144	142.7494	12.95371183	9.07	99.13
CC4	216	230.9732	6.499571032	2.81	106.93
CC5	360	364.4217	8.337782417	2.29	101.23
CC6	720	727.2860	42.02052119	5.78	101.01
CC7	1440	1423.5579	8.422189282	0.59	98.86
CC8	2160	2142.0041	41.87510393	1.95	99.17
CC9	3600	3681.103039	133.21085	3.62	102.25

Values are expressed as mean±SD. % CV ≤15% for all CC levels except LLOQ (≤20%). LLOQ: Lowest limit of quantification, SD: Standard deviation

Table 5: CC data for precision and accuracy batches (DBS method)

CC ID	Nominal concentration (ng/mL)	Mean (ng/mL)	S D (±)	CV (%)	Mean % nominal
CC1	36	39.6790	4.5282	11.41	110.22
CC2	72	68.6365	7.6582	11.16	95.33
CC3	144	143.2156	3.2642	2.28	99.46
CC4	216	197.2346	0.9687	0.49	91.31
CC5	360	358.5117	27.1097	7.56	99.59
CC6	720	734.3254	34.9225	4.76	101.99
CC7	1440	1418.0457	42.2065	2.98	98.48
CC8	2160	2147.4849	21.83631309	1.02	99.42
CC9	3600	3647.3945	61.06919582	1.67	101.32

Values are expressed as mean±SD. % CV ≤15% for all CC levels except LLOQ (≤20%). LLOQ: Lowest limit of quantification, SD: Standard deviation

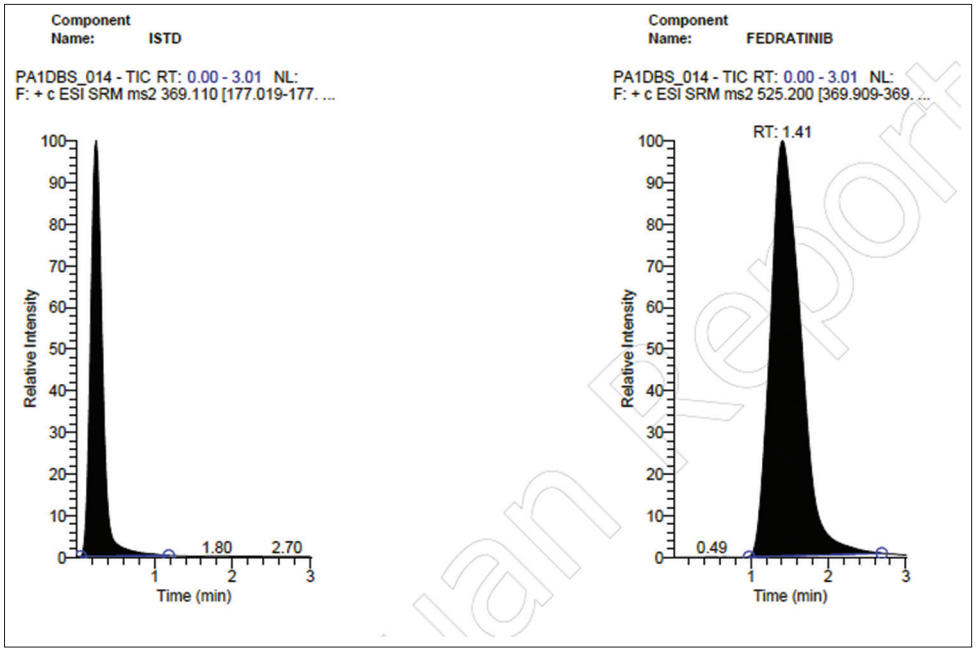

Fig. 6: Representative chromatogram of dried blood spot extracted upper limit of quantification (3600 ng/mL), ISTD, and fedratinib

Table 6: Within- and between-run precision and accuracy for plasma method

QC level	Within-run	CV%	Nominal (%)	Between-run	CV%	Nominal (%)
	Mean CC (ng/mL)			Mean CC (ng/mL) n=10		
LLOQQC (36.0 ng/mL)	34.67±4.51	13.03	96.33	33.51±3.10	9.26	93.11
LQC (108.0 ng/mL)	109.83±12.62	11.49	101.70	107.39±8.08	7.53	99.44
MQC (1800.0 ng/mL)	1936.69±56.04	2.89	107.59	1834.57±80.38	4.38	101.92
HQC (2880.0 ng/mL)	3194.34±60.85	1.90	110.91	2979.49±160.55	5.39	103.45

Values are expressed as mean±SD (n=6). %CV ≤15% for all QC levels except LLOQ (≤20%). LLOQ: Lowest limit of quantification, SD: Standard deviation

Table 7: Within- and between-run precision and accuracy for DBS method

QC level	Within-run (%)			Between-run		
	Mean CC (ng/mL)	CV	Nominal	Mean CC (ng/mL) n=10	CV%	Nominal (%)
LLOQQC (36.0 ng/mL)	32.21±2.22	6.89	89.46	33.74±4.57	13.56	93.73
LQC (108.0 ng/mL)	94.67±7.45	7.87	87.66	97.88±6.98	7.13	90.63
MQC (1800.0 ng/mL)	1907.33±49.76	2.61	105.96	1828.40±63.75	3.49	101.58
HQC (2880.0 ng/mL)	3099.17±96.91	3.13	107.61	2942.50±125.63	4.27	102.17

Values are expressed as mean±SD (n=6). %CV ≤15% for all QC levels except LLOQ (≤20%). LLOQ: Lowest limit of quantification, SD: Standard deviation

Table 8: Stability assessment of FRB in DBS samples

Parameters	Bench top stability (8 h)		Autosampler stability		Long-term stability	
Nominal Conc. (ng/mL)	LQC (108.0 ng/mL)	HQC (2880.0 ng/mL)	LQC (108.0 ng/mL)	HQC (2880.0 ng/mL)	LQC (108.0 ng/mL)	HQC (2880.0 ng/mL)
Mean CC	94.98±6.23	2861.20±7.49	97.96±8.94	2858.84±6.44	97.54±7.34	2868.08±4.45
CV%	6.56	0.26	9.13	0.23	7.53	0.16
Percentage of Nominal	87.94	99.35	90.70	99.27	90.31	99.59

Values are expressed as mean±SD (n=5). %CV ≤15% for all QC levels except LLOQ (≤20%). LLOQ: Lowest limit of quantification, SD: Standard deviation, QC: Quality control, LQC: Lower-quality control, HQC: High-quality control

standard deviation of the differences represents the extent of random fluctuations between the methods [13,20].

Passing-Bablok method is a new regression procedure is described which is robust, non-parametric, and particularly useful for method comparison studies, where measurement errors are present in both methods [14,15].

In Bland-Altman analysis, comparative evaluation between DBS and plasma (n=12) demonstrated an excellent correlation. The Bland-Altman analysis yielded mean bias within acceptable limits, although the 95% limits of agreement (−93.74–128.87) were wide. Considering FRB's therapeutic concentration range (36–3,600 ng/mL), this variation remains clinically acceptable, supporting the suitability of DBS as a surrogate sampling technique.

Table 9: Stability assessment of FRB in plasma samples

Parameters	Bench top stability		Freeze-thaw stability		Autos ampler stability		Long-term stability	
Nominal Conc. (ng/mL)	LQC (108.0 ng/mL)	HQC (2880.0 ng/mL)	LQC (108.0 ng/mL)	HQC (2880.0 ng/mL)	LQC (108.0 ng/mL)	HQC (2880.0 ng/mL)	LQC (108.0 ng/mL)	HQC (2880.0 ng/mL)
Mean BCC	112.22±2.23	2881.14±23.27	115.54±4.51	2879.28±28.89	100.53±2.75	2861.16±33.33	97.34±3.03	2922.45±26.56
CV%	1.99	0.81	3.90	1.00	2.74	1.16	3.12	0.91
Percentage of Nominal	103.91	100.04	106.99	99.98	93.08	99.35	90.14	101.47

Values are expressed as mean±SD (n=5). %CV ≤15% for all QC levels except LLOQ (≤20%). LLOQ: Lowest limit of quantification, SD: Standard deviation, LQC: Lower-quality control, HQC: High-quality control, FRB: Fedratinib

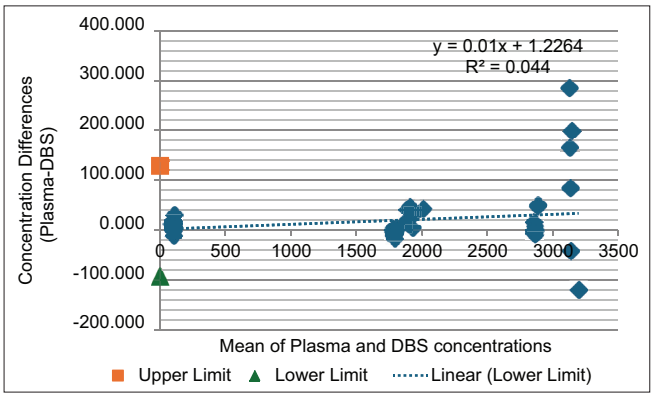

Fig. 7: Bland-Altman plot comparing fedratinib concentrations in both matrices (plasma and dried blood spot)

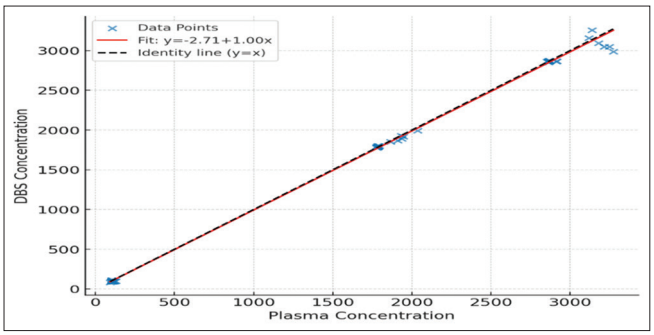

Fig. 8: Passing-Bablok regression comparing plasma versus dried blood spot quantification

Bland-Altman: Mean bias is small (≈−2%), within acceptable bioanalytical limits (<15%). The bias is not clinically significant. Passing-Bablok regression yielded the equation: y=×1.00+2.71, as shown in Fig. 7.

Passing-Bablok: DBS shows a slope ~1 and intercept ~0 → excellent linear agreement with plasma, as shown in Fig. 8.

Overall: DBS can be considered interchangeable with plasma for quantification at LQC, MQC, and HQC levels.

The DBS method demonstrated minimal matrix effects (IS-normalized MF 0.98–1.05), consistent recovery, and stability under ambient and refrigerated conditions. Compared to other kinase inhibitors such as ruxolitinib and tofacitinib, FRB exhibited slightly higher lipophilicity (logP 4.1), which could influence DBS absorption. Nevertheless, results support DBS as a feasible micro sampling alternative [21-23].

CONCLUSION

In this work, a robust and reliable LC-MS/MS method was successfully developed and validated for the quantification of FRB in both plasma and DBS samples. The DBS method demonstrated significant advantages over traditional plasma method, particularly in terms of reduced sample volume requirements, simplified logistics, and enhanced suitability for TDM and clinical studies where sample handling and storage pose challenges. All critical validation parameters, including selectivity, sensitivity, recovery, matrix effect, stability, precision, and accuracy, were found to be within the acceptable limits defined by ICH M10 guidelines, thereby ensuring the reliability of the proposed assay.

A comparative evaluation using Passing-Bablok regression and Bland-Altman analysis further established the interchange ability of plasma and DBS measurements. The regression results showed a strong correlation between the two methods, while the Bland-Altman plot confirmed that the differences observed were within clinically acceptable limits, supporting the agreement of DBS with plasma.

Considering, these findings suggest that the DBS method is not only scientifically sound but also offers practical benefits such as reduced invasiveness, easier transport, and improved patient compliance. This validated method therefore holds promise as a sustainable alternative to plasma analysis for routine quantification of FRB in PK, clinical, and therapeutic monitoring applications.

AUTHORS' CONTRIBUTIONS

All authors are contributed equally.

CONFLICTS OF INTEREST

The authors completed this research work without any influence or financial interests or personal relationships, and the same reported in this paper.

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