

Article

Development of an LC-MS/MS Method for Simultaneous Quantification of Ibrutinib and Ritonavir in Plasma and Dried Plasma Spots

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Abstract

The instability of ibrutinib in plasma at room temperature necessitates temperature-controlled sample transport, complicating therapeutic drug monitoring (TDM) and multicenter studies. Dried plasma spots (DPSs) offer a potential advantage by enabling sample storage and transport under ambient conditions. The aim of this study was to develop and validate an LC-MS/MS method for the simultaneous quantification of ibrutinib and ritonavir in plasma and DPSs. Following validation of an extended plasma calibration range, DPS method development included card selection and subsequent validation in accordance with ICH M10 and EMA guidelines. The analytical method demonstrated robust performance in plasma for both analytes. For DPS sampling, ritonavir fulfilled all validation criteria. In contrast, ibrutinib showed significant instability in DPS samples at room temperature after 48 h and did not meet the predefined acceptance criteria under these conditions. Refrigerated storage improved stability within the acceptance criteria for at least one week. The developed method is suitable for simultaneous quantification of ibrutinib and ritonavir in plasma and for ritonavir in DPSs. While DPS sampling may offer logistical advantages, its successful application is dependent on analyte stability. For ibrutinib, the requirement for refrigerated storage limits the anticipated benefits, and further research is needed to optimize dried sampling strategies.

Keywords: ibrutinib; dried plasma spots; therapeutic drug monitoring; LC-MS/MS; ritonavir



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1. Introduction

Ibrutinib is a selective and covalent Bruton's tyrosine kinase (BTK) inhibitor used in treatment of chronic lymphocytic leukemia (CLL), lymphoplasmacytic lymphoma (LPL), mantle cell lymphoma (MCL) and marginal zone lymphoma (MZL) [1]. The predominant metabolic pathway is mediated by CYP3A4, making it a promising candidate for pharmacokinetic boosting [2]. Although therapeutic drug monitoring (TDM) of ibrutinib is not standard practice [3], TDM guidance can be of added value in drug–drug interaction management and in boosted regimens [2].

A major practical limitation for the TDM of ibrutinib is the instability of ibrutinib in liquid plasma at room temperature [4–6], necessitating sample transport on dry ice via courier services. This approach is cumbersome, associated with high costs and a substantial

environmental burden [7]. The use of dried plasma spots (DPSs) may provide a promising solution to these logistical challenges [8]. DPS samples may facilitate simplified storage and transport, potentially allowing shipment via regular (medical) mail [9,10], resulting in the simplification of logistics and a considerable reduction in both costs and CO₂ emissions.

Anticipating an upcoming multicenter clinical trial in which ritonavir will be used as a CYP3A4 inhibitor to boost ibrutinib plasma levels, the liquid chromatography–tandem mass spectrometry (LC-MS/MS) method should include ritonavir quantification. With the pharmacokinetic equivalence of boosted ibrutinib and standard ibrutinib as the primary endpoint of the study, accurate plasma-level quantification is essential. While both therapeutic agents have been separately quantified from DPS before [10,11], to our knowledge, no validated method currently exists for their simultaneous quantification. Ibrutinib quantification in DPS has been reported over a concentration range of 2–400 ng/mL as part of a multi-TKI assay [10] and ritonavir quantification is described from 50 to 5000 ng/mL, using dog plasma samples [11]. Low-dose ritonavir is expected to exert lower plasma levels than those reported previously; therefore, a dedicated method covering the clinically relevant concentration ranges for both analytes is required. Following successful completion of the clinical study, broad implementation of ibrutinib–ritonavir combination therapy is expected, making a combined laboratory method important for routine TDM. In this study, we developed and validated a quantitative LC-MS/MS method to simultaneously determine ibrutinib and ritonavir concentrations from plasma and DPSs. Through this work, we aim to contribute to more sustainable and cost-effective strategies for sample storage and transport in therapeutic drug monitoring. At the same time, we facilitate broader implementation of ibrutinib TDM in clinical practice.

2. Materials and Methods

2.1. Chemicals and Reagents

The following reagents and corresponding suppliers were used: ibrutinib, [²H₅] ibrutinib (Alsachim, Illkirch-Graffenstaden, France), ritonavir (Cayman Chemical, Ann Arbor, MI, USA) and [¹³C,²H₃] ritonavir (Alsachim, Illkirch-Graffenstaden, France), dimethyl sulfoxide (Merck, Schiphol-Rijk, The Netherlands), methanol (BioSolve, Valkenswaard, The Netherlands), and ammonium formate (Thermo Fisher Scientific, Waltham, MA, USA). Blank human K3EDTA gender pooled plasma was used (BioIVT, Burgess Hill, UK).

2.2. Preparation of Stock Solutions and Working Solutions

Stock solutions for ibrutinib and ritonavir were prepared separately in dimethyl sulfoxide (DMSO), obtaining drug concentrations of 100 mg/L and stored at −80 °C until further use. Stock solutions were serially diluted using methanol (MeOH). Blank plasma was spiked to obtain a calibration curve over the following ranges. Ibrutinib: 1–400 µg/L (1.00, 2.00, 5.00, 40.0, 80.0, 160, 320 and 400 µg/L) and ritonavir: 10–1000 µg/L (10.0, 20.0, 50.0, 100, 200, 400, 800, 1000 µg/L). Quality control (QC) samples were prepared at the lower limit of quantification (LLOQ), low, medium and high levels. Final QC concentrations were 1, 2, 160 and 320 µg/L for ibrutinib and 10, 20, 400 and 800 µg/L for ritonavir. Stock solutions for internal standards (ISs) were prepared in DMSO and diluted in MeOH for the following IS working solutions: [²H₅] ibrutinib 5 µg/L and [¹³C,²H₃] ritonavir 10 µg/L. QCs and calibrator standards were prepared from independent stocks.

2.3. Validation of the Extended Plasma Range

The plasma method used in this study was based on a previously validated LC-MS/MS method for ibrutinib [12] and ritonavir (data on file). The validated ibrutinib assay served as the primary basis for method development. In the present study, both analytes were

combined into a single analytical method to enable simultaneous quantification of ibrutinib and ritonavir, and the calibration range was extended to cover the intended concentration range. Extraction volumes were adjusted to obtain appropriate concentrations from the DPS samples. The modified method was subsequently validated according to the relevant criteria of the ICH M10 guidelines for bioanalytical method validation [13].

Sample Preparation

For plasma analysis, 500 μL of IS solution was added to 100 μL of EDTA plasma, as previously established in the analytical validation procedure [12]. The mixture was vortexed for 1 min and centrifuged for 5 min at 9500 RCF, all at room temperature. The sample was injected into the LC-MS/MS system.

2.4. DPS Method Development

2.4.1. Card Selection

After validation of the extended plasma method, the assay was adapted for use with DPS. Two types of filter paper cards were tested and evaluated in parallel: Whatman Protein Saver 903 (Cytiva, Little Chalfont, UK) cards and Whatman DMPK-C (Qiagen, Hilden, Germany) cards. In comparison to Whatman 903 cards, DMPK-C cards demonstrated higher precision, lower variability, and improved analytical consistency and were therefore selected for further method validation.

2.4.2. DPS Sample Preparation

A 20 μL aliquot of calibrator standards and QCs was spotted onto the filter paper and allowed to dry at room temperature for at least 3 h. After drying, an 8 mm disk was punched from the center of each spot and transferred into a tube. Analytes were extracted by adding 250 μL IS solution. The mixture was vortexed, sonicated for 15 min and vortexed again. A total of 150 μL of the sample was transferred to a vial with insert and injected into the LC-MS/MS system.

2.5. LC-MS/MS Method Conditions

2.5.1. Chromatography

Chromatographic separation was carried out using an LC system combined with a C18 column (AccucoreTM, 2.6 μm 50 $\times$ 2.1 mm, Thermo Fisher Scientific, Waltham, MA, USA). The temperature of the autosampler was set at 10 $^{\circ}\text{C}$, the temperature of the column oven was set at 60 $^{\circ}\text{C}$. Two mobile phases were used in the elution gradient, mobile phase A (0.02 mol/L ammonium formate buffer, pH 3.5) and mobile phase B (acetonitrile). The elution gradient was programmed as follows: 0.000–1.150 min, 30% B; 1.150–1.450 min, 95% B; 1.500 min, 30% B. The flow rate was maintained at 1.000 mL/min throughout the run. Autosampler (Vanquish, Thermo Fisher Scientific, Waltham, MA, USA) volume was set at 5 μL for plasma and DPS.

2.5.2. Mass Spectrometry

Data acquisition, peak integration, and quantitative data analysis were performed using Xcalibur version 4.7 software (Thermo Fisher Scientific, Waltham, MA, USA). The MS/MS system included a TSQ Quantiva Triple Quadrupole, Vanquish Autosampler, Vanquish Horizon Binary Pump, Vanquish Column Compartment, and Vanquish Charger (Thermo Fisher Scientific, Waltham, MA, USA). H-ESI ion source type was utilized with positive polarity and static spray voltage (positive ion 1500 V). The sheath, auxiliary, and sweep gas pressures were 60, 20, and 0 arbitrary units. The ion transfer tube temperature was set at 140 $^{\circ}\text{C}$ and vaporizer temperature was set at 350 $^{\circ}\text{C}$. Analytes and their internal standards were detected using selected reaction monitoring (SRM). Details of SRM con-

ditions are listed in Table 1. The cycle time was 0.05 s, with Q1 and Q3 resolutions set at 0.7 Full Width at Half Maximum (FWHM). Collision-induced dissociation (CID) gas was 1.5 mTorr.

Table 1. Selected reaction monitoring conditions.

Compound	Retention Time (min)	RT Window (min)	Precursor (m/z)	Product (m/z)	Collision Energy (V)	Min Dwell Time (ms)
Ibrutinib	0.6	0.3	441.3	304.1	30	9.88
[² H ₅] Ibrutinib	0.6	0.3	446.3	309.1	30	9.88
Ritonavir	0.8	0.3	721.2	197	39	9.88
[¹³ C, ² H ₃] Ritonavir	0.8	0.3	725.3	201	38	9.88

2.6. Method Validation

Validation was performed according to the ICH M10 guidelines for both the extended plasma method and the DPS method [13].

2.6.1. Linearity

The concentrations of the calibration standards, back-calculated on LLOQ, should be within $\pm 20\%$ of the nominal value at LLOQ and within $\pm 15\%$ of the nominal value for all other calibration points based on ICH M10 guidelines. At least 75% of all calibrator standards should comply with these criteria. Linearity of the calibration curve was assessed on three different calibration runs at three different days. The weighing factor for the peak height ratio analyte/IS was set at $1/x$.

2.6.2. Dilution Integrity

Dilution integrity was determined by preparing a sample with a concentration two-fold higher than the HLQ for ibrutinib and ritonavir. Plasma samples were diluted with blank plasma to obtain a concentration within the linear range. For the DPS, a spot with a concentration two-fold higher than the HLQ was diluted $10\times$ after extraction with blank extract. Diluted samples should be within $\pm 15\%$ of the nominal concentration.

2.6.3. Accuracy and Precision

Within- and between-run accuracy and precision were determined using QCs at the LLOQ, low, medium and high levels. Five replicates of each QC were determined on three different days. QCs should be within $\pm 15\%$ of the nominal concentration.

2.6.4. Selectivity and Carry-Over

For assessment of selectivity, six blank samples were analyzed. Blank samples were spiked with the analyte and IS at LLOQ. Responses of potential interfering substances should not be more than 20% of the analyte response at LLOQ and not more than 5% of the IS response at LLOQ. Carry-over was assessed by injecting blank samples following high concentration (HLQ) samples. Analyte response in the blank samples should not exceed 20% of the analyte response at LLOQ and 5% of the IS at LLOQ.

2.6.5. Matrix Effect and Recovery

Matrix effects were evaluated using a post-extraction addition approach and IS-normalized matrix factors were calculated. The matrix effect of the DMPK-C paper and plasma was assessed at low, medium and high QCs using six independent batches of blank plasma. The response was compared to that of the analyte with extraction liquid. Recovery was determined by comparing pre-extraction spiked samples with post-extraction

spiked blank matrix samples at equivalent concentrations. The analyte/internal standard peak area ratio of the post-extraction spiked samples was defined as 100% recovery. The acceptance limit for the coefficient of variation (CV) is 15%.

2.6.6. Stability

The stability of ibrutinib and ritonavir in DPSs was evaluated under refrigerated (2–8 °C) and room temperature (RT) storage conditions. Samples were analyzed at predefined timepoints of 24, 48, 72, 96, and 168 h for both card types and additionally 2, 3, and 4 weeks for DMPK-C card samples. DPS cards were dried at room temperature for 3 (± 30 min) h. After drying, samples were placed in an airtight zip-lock bag with desiccant at room temperature or in a refrigerator until the predefined timepoint. At each timepoint, samples were transferred to -20 °C until batch analysis. Additional investigated RT storage conditions were without protective packaging or desiccant and storage in a zip-lock bag with desiccant and additional aluminum packaging to prevent UV-degradation. The mean concentration of each QC level should be within $\pm 15\%$ of the nominal concentration (T_0), calculated on freshly spiked calibration standards. Autosampler stability was assessed by reinjecting low and high QCs after 3 days at 10 °C. No additional plasma stability experiments were performed, as the stability of ibrutinib and ritonavir in plasma has already been established in previous method validations [12].

3. Results

3.1. DPS Method Development and Optimization

Card Comparison

Samples derived from 8 mm spots from 20 μ L plasma on the Whatman 903 cards showed inconsistent analyte concentrations in relation to the specified amount. Bias ranged from -15% to $+12\%$, compared to a range from -6% to $+4\%$ on DMPK-C cards. Analysis of full spot samples from Whatman 903 cards showed deviations ranging from -9% to $+2\%$. Supplementary Table S1 shows the obtained results. DMPK-C cards were selected for further validation.

3.2. Method Validation

Chromatograms of blank matrix samples and samples at LLOQ are presented in Figure 1. No interfering substances were found at the retention times of ibrutinib (0.61 min) and ritonavir (0.77 min).

3.2.1. Linearity, Accuracy and Precision

For ibrutinib, both the plasma and the DPS method showed linear response over the range of 1–400 μ g/L. For ritonavir, both the plasma and the DPS method showed linear response over the range of 10–1000 μ g/L. The regression coefficients (R^2) of all the standard curves exceeded 0.99 (Supplementary Figure S1). All back-calculated calibration standards were within $\pm 15\%$ of the nominal concentrations (Table 2); the test therefore meets the ICH M10 criteria for method validation. Table 3 shows the accuracy and precision for ibrutinib and ritonavir from plasma and dried plasma spots. All values are within 15% of the nominal concentration and therefore comply with the ICH M10 requirements.

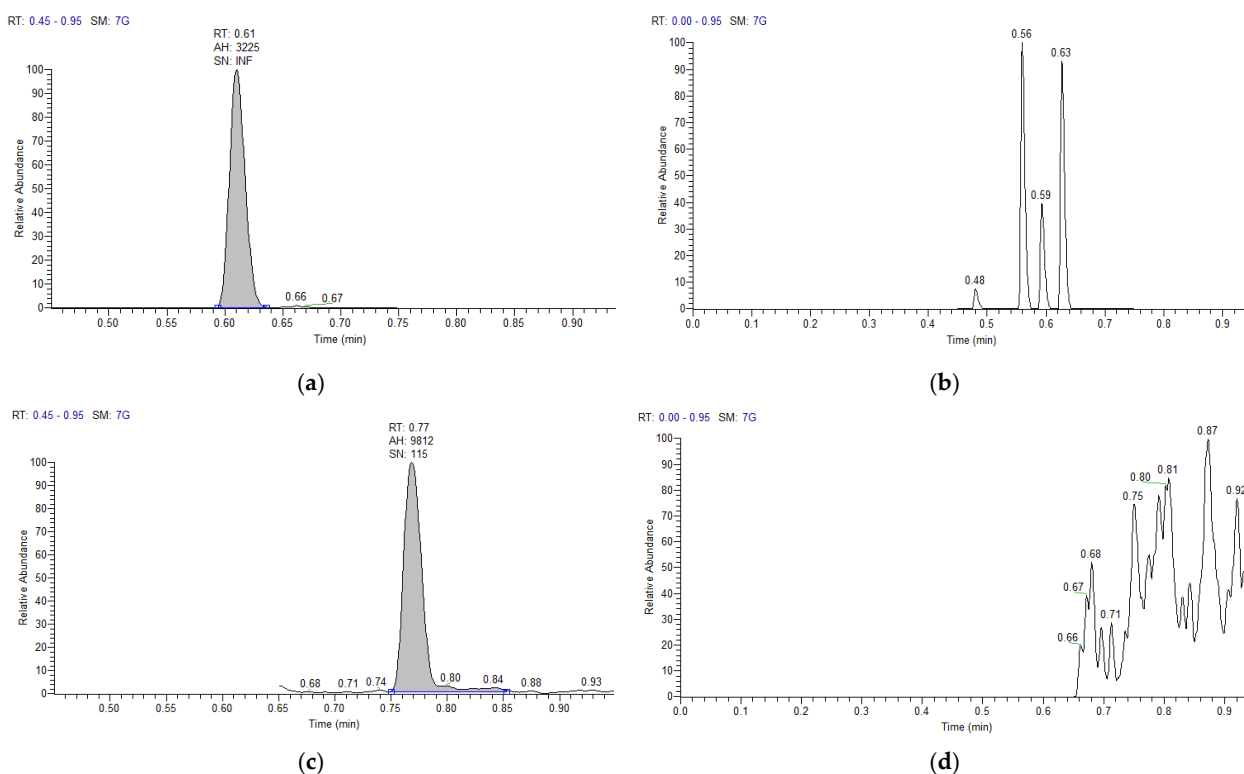


Figure 1. Chromatograms for ibrutinib and ritonavir from DPS. (a) Ibrutinib at LLOQ, showing a distinct peak at a retention time of 0.61 min and an infinite signal-to-noise (SN) ratio. (b) A blank matrix around the ibrutinib retention time. (c) Ritonavir at LLOQ, showing a distinct peak at a retention time of 0.77 min. and a SN signal of 115. (d) A blank matrix around the ritonavir retention time.

Table 2. Linearity range of ibrutinib and ritonavir calibration standards from plasma and DPS.

Analyte	Concentration Calibrator (µg/L)	Plasma			DPS		
		Bias (%)			Bias (%)		
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Ibrutinib	1	−0.1	−0.6	0.0	4.0	0.0	−5.4
	2	−4.5	−5.0	−0.5	−7.5	−9.0	−6.5
	5	−5.6	−7.4	−6.6	−4.2	−5.2	−5.6
	40	1.5	3.0	2.0	−3.0	−4.3	−7.0
	80	−0.3	0.7	3.5	−1.4	−8.9	−7.3
	160	0.0	0.6	0.0	3.1	−8.1	−4.4
	320	−1.3	−0.9	0.6	5.6	−0.3	−0.6
Ritonavir	400	−1.0	−0.5	0.0	−1.0	1.0	1.8
	10	−1.9	−0.4	−1.9	2.0	0.0	−2.2
	20	−2.5	−2.0	−4.5	−11.5	−5.5	−9.5
	50	−4.8	−5.2	−2.8	−8.4	−4.6	−9.0
	100	−0.4	1.0	1.0	−12.4	−4.9	−10.4
	200	1.5	2.0	3.0	−10.5	−14.0	−10.5
	400	2.3	1.5	1.5	−6.0	−11.5	−7.3
	800	0.4	−1.8	1.0	1.1	1.9	−2.4
	1000	−0.3	2.0	1.0	−1.9	2.0	2.0

Table 3. Within- and between-run accuracy and precision of ibrutinib and ritonavir QC levels in plasma and DPS (CV = coefficient of variation).

Analyte	QC	Plasma					DPS	
		Concentration (µg/L)	Bias (%)	Within-Run (CV%)	Between-Run (CV%)	Bias (%)	Within-Run (CV%)	Between-Run (CV%)
Ibrutinib	LLOQ	1	0.5	2.5	0.6	1.6	5.8	1.8
	Low	2	−2.2	1.5	0.2	0.1	5.3	0.0
	Medium	160	1.8	1.2	0.8	−2.4	2.3	3.7
	High	320	1	1.9	0.0	−2.4	2.1	2.6
	Dilution	800	1.6	1.0	0.0	−3.6	2.3	2.4
Ritonavir	LLOQ	10	−0.5	1.8	2.7	0.7	5.2	1.1
	Low	20	−2.6	1.3	0.9	−7	5.0	3.3
	Medium	400	1.8	1.5	1.4	−5.6	3.6	1.1
	High	800	1.4	1.7	0.0	−3.7	2.7	0.0
	Dilution	2000	2.2	1.1	0.6	−7	3.4	0.6

3.2.2. Selectivity and Carry-Over

No interfering peaks exceeding 20% of the analyte response at LLOQ or 5% of the IS response were observed in blank samples, demonstrating adequate selectivity. No peaks exceeding 20% of the analyte response at LLOQ or 5% of the IS response were detected, indicating negligible carry-over. All results met the predefined acceptance criteria.

3.2.3. Matrix Effect and Recovery

Matrix effects and recovery for ibrutinib and ritonavir in plasma and DPS are summarized in Table 4. In plasma, IS-normalized matrix effects ranged from 1.7% to 5.5% for ibrutinib and from 1.8% to −0.9% for ritonavir. In DPS, IS-normalized matrix effects ranged from 3.8% to 1.8% for ibrutinib and from −0.4% to 3.1% for ritonavir.

Table 4. Matrix effect and recovery, normalized for internal standard, of ibrutinib and ritonavir from plasma and DPSs.

Matrix	Analyte	QC Level	Matrix Effect (IS Normalized) (%)	Matrix Effect (IS Normalized) (CV%)	Recovery (IS Normalized) (%)
Plasma	Ibrutinib	Low	5.1	4.5	99.6
		Med	1.7	1.4	103.2
		High	5.5	9.2	99.0
	Ritonavir	Low	−1.1	1.8	98.4
		Med	−1.8	2.1	100.0
		High	−0.9	9.0	97.8
DPS	Ibrutinib	Low	−3.8	5.7	91.0
		Med	1.8	3.3	92.3
		High	−1.1	9.2	88.8
	Ritonavir	Low	3.1	7.1	89.1
		Med	−0.1	1.4	94.4
		High	−0.4	1.1	94.2

The coefficient of variation for matrix effects was below 15% for all analytes and QC levels, indicating acceptable reproducibility across different matrix batches (Table 4). The recovery of ibrutinib in DPS ranged from 88.8% to 92.3%, and the recovery of ritonavir in DPS ranged from 89.1% to 94.4%. The recovery of both analytes in DPSs was lower than in plasma (99.0–103.2% for ibrutinib, 97.8–110.0% for ritonavir). The recovery was consistent across all concentration levels, indicating reproducible extraction of both analytes from the DPS matrix. All values met the acceptance criteria according to ICH M10 guidelines.

3.2.4. Stability

In DPS on DMPK-C cards, degradation of ibrutinib exceeded 15% within 48 h (Table 5). In Whatman 903 cards, degradation exceeded 20% after 24 h. Refrigerated conditions demonstrated acceptable stability (<15% degradation) of ibrutinib for up to 168 h (1 week). Results are presented in Table 5. On DMPK-C cards, further degradation of ibrutinib was observed up to 4 weeks (Table S2). Ritonavir was stable (<15% deviation) at all storage conditions: RT up to 4 weeks, refrigerator up to 1 week. The ICH M10 requirements were met with the ibrutinib method when DMPK-C cards were stored in the fridge. The ritonavir method met the ICH M10 requirements at room temperature as well (Supplementary Table S3). UV-protection and the absence of a zip-lock bag and desiccant showed no improvement of stability to within acceptable criteria in benchtop conditions in comparison to storage at room temperature, with a zip-lock bag and desiccant (Table S4). All samples remained stable in the autosampler for at least three days.

Table 5. Stability of ibrutinib in DPS samples prepared on DMPK-C and Whatman 903 cards under different storage conditions, expressed as the percentage difference in the analyte/IS ratio relative to T_0 . $n = 3$ for investigated QC-levels. RT = room temperature (20 °C).

RT, zip-lock bag + desiccant (DMPK-C)											
Time QC-level Deviation (%) CV (%)	0 h		24 h		48 h		96 h		168 h		
	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH	
			−9.8	−13.8	−15.3	−17.7	−18.2	−24.4	−20.4	−26.0	
	1.8	1.8	2.9	1.0	3.2	2.0	1.4	3.7	1.3	3.1	
Refrigerator, zip-lock bag + desiccant (DMPK-C)											
Time QC-level Deviation (%) CV (%)	0 h		24 h		48 h		72 h		168 h		
	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH	
			0.7	2.0	0.2	−3.2	−1.5	−3.1	1.1	−5.9	
	4.1	3.2	4.3	2.1	8.5	3.3	1.3	3.3	2.8	0.9	
RT, zip-lock bag + desiccant (Whatman 903)											
Time QC-level Deviation (%) CV (%)	0 h		24 h		48 h		72 h		168 h		
	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH	LOW	HIGH	
			−21.5	−28.4	−29.8	−38.8	−37.8	−45.3	−53.9	−57.0	
	3.2	0.8	5.7	1.5	2.8	0.7	10.1	0.4	8.0	2.6	

4. Discussion

The development and validation of an LC-MS/MS method for the simultaneous quantification of ibrutinib and ritonavir in plasma and dried plasma spots across clinically relevant concentration ranges was successfully performed in accordance with ICH M10 [13]. Using DMPK-C cards, all validation criteria were met for ritonavir and stability at room temperature was shown up to 4 weeks. The recovery of both analytes remained consistently above 88% across all QC-levels, demonstrating sufficient extraction from the dried matrix. For ibrutinib, analytical performance met the predefined acceptance criteria; however, stability in the DPS samples stored at room temperature was insufficient beyond 48 h. Consequently, routine shipment under ambient conditions was not feasible. Refrigerated storage maintained ibrutinib concentrations within the predefined acceptance criteria for 1 week and therefore represents a potential alternative for sample transport. Figure 2 provides a schematic diagram illustrating the analytical workflow.

The 8 mm punched samples from the DMPK-C cards showed more consistent results in terms of accuracy, precision and stability compared to the 8 mm punches from the Whatman 903 Protein Saver Cards. Although full-spot extraction improved accuracy and aligned with findings from the previous literature [10], this approach was considered less practical as it requires cutting out entire spots rather than using a punching device. Despite results remaining within the $\pm 15\%$ range, initial deviations from the nominal concentrations were

discouraging for further development using Whatman 903 Protein Saver cards. Differences in card performance may be related to differences in physicochemical properties, such as pore size, card thickness and absorption characteristics [14,15].

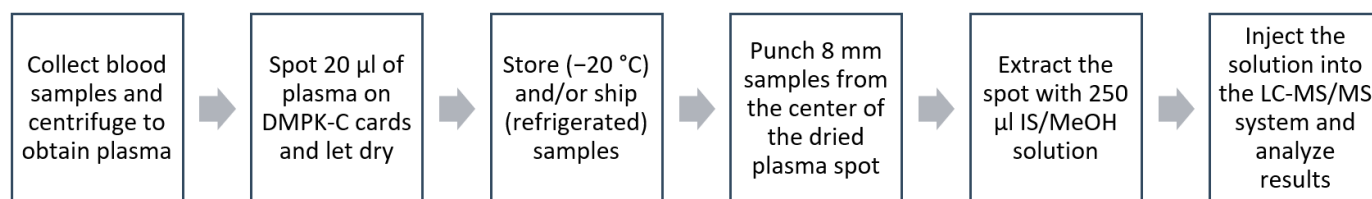


Figure 2. Schematic overview of the analytical workflow.

For ritonavir, all validation parameters met the ICH M10 requirements, indicating that analysis of ritonavir from the DPS samples is feasible. However, ritonavir had no a priori limitations regarding stability, as plasma samples can be shipped at room temperature as well; therefore, DPSs offer limited additional logistical benefit for this compound. To the best of our knowledge, the determination of ritonavir from dried plasma spots was only previously described for dog plasma [11]. The present study extends this application to human plasma.

The instability of ibrutinib in plasma at room temperature was a key motivation for evaluating DPSs. Previous studies have reported acceptable stability of ibrutinib in dried matrices, 2 weeks at room temperature in volumetric absorptive microsampling (VAMS) [16], 8 weeks at room temperature in dried blood spots (DBSs) [17] and 12 weeks at room temperature in DPSs [10]. The latter especially is considerably discrepant with the present study, as degradation exceeding 15% is observed within 48 h. Potential explanations include differences in sampling devices (Whatman 903 versus DMPK-C cards), sample preparation procedures, storage conditions or analytical methodology.

The observed degradation of ibrutinib exceeding 15% within 48 h at room temperature indicates that routine shipment without temperature control is not feasible. Although shipment at room temperature may still be feasible when delivery within 24 h is guaranteed, this would considerably restrict the practical applicability of DPS sampling. Refrigerated storage conditions substantially improved stability, with degradation remaining within the predefined acceptance criteria for at least one week. While room-temperature shipment was not feasible, DPS sampling may still simplify logistics because samples can be transported under refrigerated conditions without requiring dry ice.

Previous studies have described stability issues for ibrutinib in wet plasma matrices at room temperature [5,6]. Several degradation mechanisms have been proposed, including oxidation, conjugation with glutathione and the intrinsic reactivity of the acrylamide moiety present in the molecule [18,19]. Such mechanisms may potentially persist after sample collection and drying, although the exact cause of the instability observed in the present study remains unclear. Identification of relevant degradation products or metabolites could provide further insight into the degradation pathways and could hence lead to a targeted approach to improve stability. Although acceptable stability has been reported for alternative microsampling strategies, these methods often require additional analytical corrections or specialized sampling devices and were therefore considered less readily applicable in this current study. Future studies could evaluate the use of DBSs combined with hematocrit correction strategies, VAMS, or alternative DPS sampling substrates. Furthermore, additional sample pretreatment steps, such as protein precipitation or the use of stabilizing agents directly after plasma collection, may help to reduce degradation and improve analyte stability.

This study evaluated only two card types, namely Whatman 903 and DMPK-C cards. Other sampling devices or card types may provide improved stability. Despite the limited stability of ibrutinib under ambient conditions, the clinical relevance of the TDM of ibrutinib remains unchanged. Exposure–response relationships have been described, and reliable quantification of ibrutinib may contribute to treatment optimization and improved patient outcomes [3]. DPS sampling remains a promising strategy because of the potential to simplify sampling procedures, reduce transport costs and decrease environmental impact. However, successful implementation is strongly dependent on analyte-specific stability and logistical considerations in clinical practice.

The primary motivation for this study was to address logistical challenges associated with sample transport in an upcoming multicenter clinical trial. Due to the instability of ibrutinib in plasma at room temperature, frozen shipment on dry ice via courier services is currently required, resulting in a costly and logistically demanding procedure with a substantial environmental impact. Although the possibility of shipping DPS samples at room temperature, as previously reported [10], could not be reproduced in the present study, transport under refrigerated conditions still represents a substantial improvement over conventional plasma shipment. The feasibility of using cooled packaging or refrigerated courier services is currently being evaluated. As an alternative strategy, samples may be stored and shipped in batches on dry ice, as immediate TDM results are not required for the intended study setting.

5. Conclusions

The development and validation of an LC-MS/MS method for the simultaneous quantification of ibrutinib and ritonavir in plasma and dried plasma spots across clinically relevant concentration ranges was successfully performed in accordance with ICH M10 guidelines. Ibrutinib showed insufficient stability in DPSs at room temperature beyond 24 h, whereas refrigerated storage improved stability to within acceptable limits for up to 1 week. These findings indicate that DPS may enable sample shipment under refrigerated conditions without the need for dry ice, thereby simplifying sample handling and storage in multicenter studies.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/labmed3030020/s1>, Figure S1: Calibration curves of ibrutinib and ritonavir in plasma and dried plasma spots (DPSs); Table S1: Calculated concentration of ibrutinib and deviation from the nominal value obtained from an 8 mm punch of DMPK-C cards, an 8 mm punch of Whatman 903 cards, and full-spot isolation from Whatman 903 cards; Table S2: Stability of ibrutinib in dried plasma spots (DPSs) prepared on DMPK-C and Whatman 903 cards under different storage conditions (n = 3 per QC level); Table S3: Stability of ritonavir in dried plasma spots (DPSs) prepared on DMPK-C and Whatman 903 cards under different storage conditions (n = 3 per QC level); Table S4: Stability of ibrutinib in dried plasma spots (DPSs) prepared on DMPK-C cards with and without protective packaging and UV protection (n = 3 per QC level). RT = room temperature (20 °C). Values are expressed as the analyte/IS ratio relative to T0. Bias (%) was calculated relative to the nominal concentration at time zero (T0). CV (%) represents the coefficient of variation across replicates.

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Abbreviations

The following abbreviations are used in this manuscript:

BTk	Bruton's tyrosine kinase
CID	Collision-induced dissociation
CLL	Chronic lymphocytic leukemia
CV	Coefficient of variation
CYP3A4	Cytochrome P450 3A4
DBS	Dried blood spot
DPS	Dried plasma spot
FWHM	Full width at half maximum
HLQ	Highest level of quantification
H-ESI	Heated electrospray ionization
ICH	International Council for Harmonisation
IS	Internal standard
LC-MS/MS	Liquid chromatography–tandem mass spectrometry
LLOQ	Lower limit of quantification
LPL	Lymphoplasmacytic lymphoma
MCL	Mantle cell lymphoma
MZL	Marginal zone lymphoma
QC	Quality control
RT	Room temperature
SRM	Selected reaction monitoring
TDM	Therapeutic drug monitoring
VAMS	Volumetric absorptive microsampling

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