Research Article

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Bioanalysis

Microsampling in pediatric studies: pharmacokinetic sampling for baricitinib (Olumiant™) in global pediatric studies

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Background: Managing blood volumes in pediatric studies is challenging and should be minimized where possible. **Results:** A sensitive liquid chromatography with tandem mass spectrometry (LC–MS/MS) method was validated and implemented across two phase III global pediatric trials. Two 10-μl aliquots of blood were collected at each time point using the Mitra® device. Concordance between plasma and dried blood was established from older pediatric patients. Incurred sample reanalysis was performed in both studies using the second Mitra tip and acceptance was greater than 83%. **Conclusion:** The use of microsampling to generate pharmacokinetic data in 2–18-year-old pediatric patients was successfully implemented. Positive feedback was received from clinical sites about the microsampling technique assisting with enrollment of pediatric patients.

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Baricitinib (Olumiant™) is an orally administered, small-molecule, Janus-associated kinase (JAK) inhibitor developed by Eli Lilly and Company and Incyte Corporation. Baricitinib has received regulatory authorization in several countries for the treatment of adults with moderately to severely active rheumatoid arthritis, moderate-to-severe atopic dermatitis (AD), COVID-19 and severe alopecia areata [1]. Baricitinib is also being studied in children and adolescents.

Understanding the pharmacokinetics of drugs is essential and is typically performed via the collection of a series of blood samples. The process of collecting these blood samples (via serial venipunctures), the volumes of blood collected (using standard vacutainer tubes), generation of either plasma or serum (requiring centrifugation) for analysis, and maintaining these samples frozen from the time of collection until analysis (including during shipping) have remained the status quo over several decades, with little to no technological advancements. On the other hand, the analytical techniques have continuously undergone technological advancement and innovation, resulting in highly sensitive instruments that require very small sample volumes for quantification [2].

Over the past decade, there has been a growing desire within the pharmaceutical industry to refine and improve the blood-collection processes, including the adoption of microsampling techniques that can enable the collection of a blood sample without needing a phlebotomist or visiting a clinic [3–5]. Currently, several microsampling techniques and devices are available to collect blood via a finger stick or subcutaneously from the upper arm to provide liquid blood, plasma, serum or dried blood [6,7]. The availability of such capabilities has a significant impact on patient enrollment in clinical trials (e.g., reduction of pain and discomfort of serial venipunctures and the convenience of potentially reducing visits to the clinic). Techniques that generate a dried blood sample have the additional benefit of being able to reach a section of the global population that was otherwise excluded from clinical trials due to lack of resources (centrifuges, freezers, dry ice for shipping, etc.) and making clinical trials more accessible [6,8].

The adoption of dried blood spot (DBS) sampling, a technique that was introduced by Guthrie over 50 years ago to support drug discovery [9], was demonstrated in the early 2000s [10]; however, its broader implementation was slow, likely due to its vulnerability to biases based on hematocrit [11–14]. The challenges with hematocrit and

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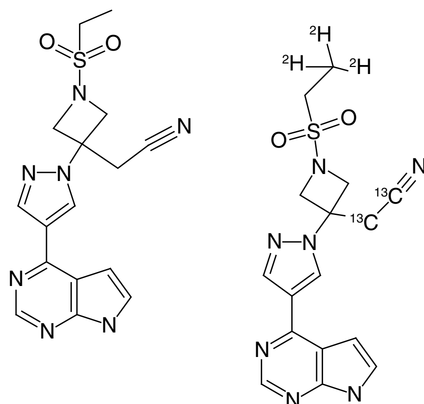

Figure 1. Structure of (left) baricitinib (LY3009104) and its (right) stable isotope-labeled internal standard (LSN3032651).

differences in blood volumes was subsequently resolved by the introduction of collection techniques that enabled the collection of a fixed volume of blood [15].

Pediatric patients are unique – they are not ‘small adults’ – and they range from preterm newborns to term newborns (0–27 days), infants and toddlers (28 days to 23 months), children (2 to 11 years) and adolescents (12 to 16–18 years) [16]. Children go through a continuum of physiological development changes from birth until they reach adulthood. Therefore, the conduct of appropriate pediatric trials for drugs administered to children is a requirement enforced by global regulatory agencies [17]. However, conducting trials in pediatric patients involves ethical challenges (around assent, consent, and balancing risk and benefit), economic challenges (cost of executing a successful pediatric study: including developing new pediatric-friendly formulations and multiple age-appropriate formulations) and pharmacometric challenges (understanding adsorption, distribution, metabolism and excretion in children, relative to adults) [18]. The latter requires numerous visits to the clinic for trial procedures, including the collection of numerous blood samples.

The volume of blood that can be safely drawn from a pediatric patient is based on the child’s weight and is a percentage of the total blood volume (TBV) typically enforced by local guidelines. In most situations, blood volumes are limited to a maximum of 1% of TBV for a single-day blood draw and a maximum of 3% TBV over a period of 4 weeks [19]. Therefore, in pediatric patients and particularly in small pediatric patients, the total daily blood volumes to be collected in clinical studies must be carefully managed.

Pharmacokinetics based on plasma has been the ‘gold standard’, and using a different matrix such as dried blood requires understanding the relationship between the concentration of the drug in plasma versus blood. This can be accomplished by generating concordance data that can then be used to transform blood data to plasma equivalents and vice versa [20,21].

Although microsampling has been used to support clinical and nonclinical pharmacokinetics (PK) studies supporting all phases of drug development [6,22], broader adoption has been slow and received with mixed reviews. Its adoption for pediatric studies has been very limited [23–25]. This report describes the bioanalytical experiments used to validate a sensitive liquid chromatography with tandem mass spectrometry (LC–MS/MS) assay to quantify baricitinib in pediatric blood samples collected using the Mitra® volumetric absorptive microsampling (VAM) device along with the demonstration of concordance (between plasma and blood) and its successful adoption to support two phase III pediatric trials.

Experimental section

Reference standards

Baricitinib (also known as LY3009104) and its stable isotope-labelled internal standard (IS) LSN3032651 (Figure 1) were obtained from the Eli Lilly and Company molecule inventory (IN, USA).

Equipment & chemicals

Mitra VAMs (10 μl) were purchased from Neoteryx (CA, USA). Silica gel pack desiccant sachets (5 g) were purchased from Desiccare Inc. (MS, USA). Stainless Steel Grinding Balls 5/32″ were purchased from OPS Diagnostics (NJ, USA). Standard laboratory chemicals (high-performance liquid chromatography [HPLC]-grade

reagents) and laboratory equipment (balances, pipettors, vials, centrifuges, etc.) were used. K2EDTA whole blood was collected from employees at Altasciences Company Inc. (QC, Canada) who had volunteered to be donors.

Stock solutions & reagent solutions

Stock standard solutions of baricitinib and the IS were prepared at 200 μg/ml in acetonitrile (ACN):H_2O (1:1, v:v). A series of intermediate solutions were prepared using the same diluent, as needed to conduct all the method-validation experiments. All standard solutions were stored at 4°C nominal in polypropylene containers. The extraction solution was MeOH:water (90:10 v:v) containing 4 mM K2EDTA, to compensate for the lack of EDTA in patient samples. The IS working solution was 0.50 ng/ml IS prepared in the extraction solution. The reconstitution solution was MeOH:water (45:55, v:v) containing 2 mM K2EDTA.

Preparation of calibration, quality control & stability samples

Calibration and quality control (QC) samples were prepared by spiking a fixed volume (20 μl from a corresponding spiking solution) to 1 ml of human blood. Calibration standards were prepared at 0.2, 0.4, 1.0. 2.5, 5, 15, 30, 80, 140, 170 and 200 ng/ml. Validation QCs were prepared at 0.2, 0.6, 100, 150 and 200 ng/ml, and a dilution QC at 400 ng/ml. Fresh blood was used, within 48 h of collection, and was mixed well prior to aliquoting. The respective spiked whole-blood calibration and QC samples were then absorbed onto the 10-μl Mitra VAM tips, returned to its original container and allowed to air dry for approximately 30 min. A total of 30 min was selected to accommodate a worst-case scenario between the time a patient sample was collected and placed inside the airtight bag containing desiccant. The containers were then closed and sealed in the airtight bag containing 5 g of desiccant and stored at room temperature. One set of stability QC Mitra VAM tips were also stored refrigerated.

Mitra VAM sample extraction

Mitra tips were popped off and placed in 1-ml 96 round-well plates and one 5/32″ stainless steel grinding ball was added into each well containing a Mitra VAM sample. IS (200 μl of the 0.50 ng/ml working solution) was added to all samples except blank samples, to which 200 μl of extraction solution was added. The plates were capped with a 96-well blue cap mat (Thermo Fisher Scientific, IL, USA) and an aluminum plate (custom made) was placed on top of the blue cap mat to ensure it could be secured with tape. The plate was gently shaken for approximately 30 min at 25°C on a microplate shaker and extracted at 1750 strokes/min for 10 min clamped securely on a 2010 Geno/Grinder (SPEX SamplePrep, NJ, USA) Model 2010). The well plate was cooled, with the aluminum plate on top, in a water/ice bath for 15 min and centrifuged at 4612 × *g* at 22°C for 5 min. Water was added (75 μl) to a clean 1-ml round-bottomed 96-well plate and 75 μl of the supernatant was transferred. The plate was centrifuged for 5 min, sealed with a blue cap mat and stored at 4°C nominal until analysis.

Chromatographic conditions

An Agilent 1100 HPLC system (CA, USA) consisting of a liquid chromatography pump, autosampler and a column oven was used with an InertSustain AQ-C18, 50 × 2.1 mm, 3-μm HPLC column (GL Science, CA, USA). Mobile phase A was 20 mM ammonium formate and B was ACN. An injection seat backflush containing 1 mM oxalic acid, 1 mM K2EDTA and 0.1% acetic acid in ACN:H_2O 50:50 (v:v) was incorporated using an additional binary pump and a divert valve. Analytes were separated using a linear gradient, flowing at 0.8 ml/min and held at 30% mobile phase B for 0.4 min, then immediately increased to 80% and held until 1.8 min. The flow was increased to 1.2 ml/min at 1.01 min and to 1.4 ml/min at 1.80 min. At 1.81 min, mobile phase B was reduced to 30% and the flow rate was reduced to 0.8 ml/min at 2.8 min. The injection volume was 5 μl, the column temperature was 30°C and the autosampler temperature was set at 4°C.

Mass spectrometric conditions

An API 5000 Triple Quadrupole mass spectrometer and Analyst® Software was used (Applied BioSystems, CA, USA). Full scan and selected reaction monitoring acquisitions were performed using positive ion atmospheric pressure electrospray ionization. Nitrogen (ultra-high pure) was used as the nebulizer gas, curtain gas, collision gas and turboionspray gas. The IonSpray voltage was approximately 4500 V and the source temperature was approximately 700°C. Quantification was performed using the following transitions: 372.1 → 251.2 for baricitinib (dwell time 150 ms) and 377.2 → 253.2 for the IS (dwell time 75 ms). A collision energy of 42 eV was used for both analytes.

Validation experiments

Accuracy & precision

Three batches were analyzed to evaluate accuracy and precision, as well as to define the calibration range, linearity and regression. Dilution integrity was evaluated by performing a 5× dilution of the 400 ng/ml dilution QC.

Selectivity/specificity

Six independent lots of blood prepared on Mitra VAM tips were extracted (with and without IS) and evaluated for interferences. Any peaks corresponding to baricitinib were compared to the response from the lower limit of quantification (LLOQ) standard. In addition, specificity was also evaluated in the presence of 20 commonly administered drugs: acetaminophen, acetylsalicylic acid, caffeine, ibuprofen, nicotine, cotinine, dextromethorphan, pseudoephedrine, cyproterone acetate, drospirenone, ethinyl estradiol, 3-keto-desogestrel, levonorgestrel, norelgestromin, norethindrone, dimenhydrinate, diphenhydramine, estradiol, medroxyprogesterone acetate and progesterone. These were tested by spiking into blank blood (with and without IS) and to low and high QC samples.

Matrix effect

Seven independent lots of blood prepared on Mitra VAM tips, including one lipemic (triglycerides >300 mg/dl), were extracted and spiked with baricitinib and IS and compared with pure solutions of baricitinib and IS. Three replicates of each lot were assayed at the low QC (LQC) and high QC (HQC). The ratio of the peak area in the presence of matrix to the peak area in the absence of matrix was calculated. Matrix factor was calculated as the ratio of the peak response in the presence of matrix extracts to the mean peak response in the absence of matrix extracts.

Recovery

Percent extraction yield from Mitra VAM tips was determined using replicates of six each at the LQC, mid QC (MQC) and HQC. Peak area ratios from spiked and extracted Mitra VAM samples were compared to extracted and spiked blank matrix Mitra VAM samples.

Carryover

Three blank matrix extracts (without IS) were injected following upper limit of quantification (ULOQ) standards and carryover was calculated as a percent of the LLOQ peak response.

Hematocrit assessment

Blood with hematocrit of 28.8 and 48.7% was prepared by adding plasma and red blood cells (harvested following centrifugation of human blood), respectively, and used to represent the range possibly encountered during pediatric clinical studies [26]. Hematocrit was determined using a standard hematocrit meter (HemataStat II™system, EKF Diagnostics, IN, USA). LLOQ, LQC, MQC and HQC samples were prepared on Mitra VAMs (n = 6 each) representing the two hematocrits and analyzed against ‘normal’ adult blood.

Stability experiments

Sample collection stability

Whole-blood samples were spiked at LQC and HQC and left at room temperature (22°C). Blood was then collected on to the Mitra VAMs immediately postspike and analyzed after 8 h to assess a worst-case scenario between blood collection and complete drying.

Processed sample stability

An aliquot was transferred out from the original extraction plate (containing the Mitra VAM tips) on to a second 96-well plate and diluted for injection on to the LC–MS/MS. Therefore, stability of the original extract as well as the final extract used for analysis was performed. The original extraction plate containing LQC and HQC (n = 6 each) was analyzed, and the original plate was stored at 4°C nominal (sealed with a Pierce-it-Lite heat-sealing foil). Following storage, the extracts were reanalyzed against a freshly prepared calibration curve. A final extract plate containing LQC and HQC (n = 6 each) was analyzed and stored at 4°C nominal. Following storage, the plate was reanalyzed against a freshly prepared calibration curve.

Short-term matrix stability

LQC and HQC Mitra VAM samples (in replicates of six) were stored at 47.5°C nominal at 40% relative humidity with and without desiccant as well in a freezer at -20°C nominal, with and without desiccant, to mimic short-term extremes that may be encountered during shipping.

Long-term matrix stability

LQC and HQC Mitra VAM samples (in replicates of six) were stored away from direct light at room temperature (22°C nominal) and refrigerated (4°C nominal) in sealed plastic bags containing desiccant for an extended period (sufficient to complete the analysis of clinical trials). All stability experiments were conducted using freshly prepared calibration standards and QCs or with QCs that were within established stability.

Clinical studies

This method was implemented in two phase III pediatric clinical trials to collect blood for pharmacokinetic analyses. The first study, "A study of baricitinib in participants from 2 years to less than 18 years old with juvenile idiopathic arthritis" is listed in clinicaltrials.gov as NCT03773978. The second study, "A study of baricitinib (LY3009104) in children and adolescents with atopic dermatitis" is listed in clinicaltrials.gov as NCT03952559 [27,28]. Both studies enrolled pediatric patients aged 2 years to less than 18 years old.

Complete PK profiles were required from the open-label PK lead-in cohorts in both studies to evaluate PK and safety profiles and confirm the dose for each age group (atopic dermatitis [AD]: 10 to <18 years, 6 to <10 years and 2 to <6 years; and juvenile idiopathic arthritis [JIA]: 12 to <18 years, 9 to <12 years, 6 to <9 years and 2 to <6 years). PK sampling was designed to minimize the time spent at the clinic and to collect one to three Mitra VAM microsamples during a single visit. The patients were provided tools to capture the times at which the drug was taken each day and Mitra VAM PK samples were collected as follows in both studies during three scheduled clinic visits:

- Day 1: patients take their first dose in the clinic and Mitra VAM PK samples are collected at 15 min and 1 h postdose;
- Day 4: patients take their dose at home and Mitra VAM PK samples are collected at 2 and 4 h postdose at the clinic;
- Day 11 (AD) and day 14 (JIA): three Mitra VAM PK samples are collected at the clinic, one prior to the daily dose and two samples subsequently at 30 min and 6 h post-dose during the clinic visit.

Mitra VAM samples were collected using the Mitra cartridge device that contains two 10-μl microsamplers packaged as a kit containing labels, a lancet, a foil zip-lock bag with desiccant to return the cartridge, etc. Two 10-μl samples were collected via a finger stick, using both Mitra VAMs tips in the cartridge, at each designated sampling per the respective clinical study protocol.

A training video was prepared (approximately 4 min) and posted on the study training website. Online meetings were hosted by the study team prior to enrolling patients with each clinical site to provide additional clarity and answer any questions, and a newsletter was sent to the sites subsequently reminding patients how to access the training video and how to collect the samples correctly. The key points included ensuring that the date and time of the baricitnib daily dose was recorded accurately using the tool provided, ensuring the Mitra VAM tips were completely covered with blood, applying the label to both the Mitra VAM cartridge and foil pouch, ensuring the desiccant was inside the foil pouch, and ensuring clear, correct and complete documentation of sample collection time/date and subject identification.

Concordance

A representative number of matching venous and Mitra VAM samples were collected in both studies to evaluate and establish concordance between traditional PK sampling (data generated using a validated plasma LC–MS/MS method) and the blood collected using the Mitra VAM device via a finger stick (using the validated method described in this manuscript). The matching venous blood samples were collected, in addition to the scheduled Mitra VAM blood samples, during scheduled clinic visits. Due to blood volume limitations, venous sampling was limited to older age-group patients.

Table 1. Accuracy and precision.

	Within-run accuracy (%)	Within-run precision (%)	Between-run accuracy (%)	Between-run precision (%)
LLOQ 0.2 ng/ml	101.9–112.2	4.4–10.7	110.70	9.00
Validation QCs	99.4–112.2	2.7–10.7	100.6–110.7	3.9–7.9
Dilution QC (400 ng/ml, 5× dilution)	97.9	3.6	NA	NA
Specificity in the presence of commonly used comedications (LQC and HQC)	98.3–106.5	4.9–7.9	NA	NA
Hematocrit 28.8% blood (LLOQ, LQC, MQC and HQC)	97.7–102.8	2.3–5.2	NA	NA
Hematocrit 48.7% blood (LLOQ, LQC, MQC and HQC)	96.5–115.3	5.1–7.0	NA	NA

HQC: High-quality control; LLOQ: Lower limit of quantification; LQC: Low-quality control; MQC: Mid-quality control; NA: Not available; QC: Quality control.

Matching concentration data – baricitinib concentrations in plasma derived from venous blood versus baricitinib concentrations in dried blood from Mitra VAM samples – were plotted and regressed to determine the blood:plasma ratio (which is the slope from this regression) that would be applied when transforming dried blood data to plasma data equivalents or vice versa. Clinics were instructed to collect the matching concordance samples within 5 min of each other and to document the time of collection.

Sample analysis & incurred sample reanalysis

Instructions were provided that the sealed foil bag containing the Mitra VAM samples were not to be opened until it reached the bioanalytical laboratory, to maintain the Mitra VAM in a sealed environment containing desiccant. Once samples were received at the laboratory and scheduled for analyses, they were carefully inspected to ensure the tips were completely covered with blood.

Incurred sample reanalysis (ISR) was performed in both studies using the second Mitra VAM tip. ISR samples were selected to represent the maximum concentrations as well as samples from the elimination phase of the exposure profile.

The validation and the sample analyses described above were conducted at Altasciences Company Inc.

The assay validation and study sample analyses were conducted following US FDA 2018 guidance for industry on bioanalytical method validation and EMA 2012 guidelines on bioanalytical method validation.

Results

Validation experiments

Accuracy & precision

The assay was validated ranging from 0.2 ng/ml (LLOQ) to 200 ng/ml (ULOQ) to quantify baricitinib from Mitra VAM samples (Table 1). A weighted ($1/x^2$) linear least-squares regression was used. A fivefold dilution was also validated to quantify any samples above the ULOQ.

Selectivity

There were no endogenous interferences or interference peaks detected in the control blood from six independent lots tested (no peaks detected >20.0% of the LLOQ response or >5.0% of the IS response). Mitra VAM samples prepared at the LQC and HQC and spiked with a cocktail of 20 commonly administered comedications also had no impact on the quantification of baricitinib (Table 1).

Matrix factor

The IS-normalized matrix factor across the six independent lots was 0.98 and 1.01 at the LQC and HQC, respectively. The matrix factor for the lipemic blood was 1.00 and 1.02 at the LQC and HQC, respectively. The overall relative standard deviation across all lots (six independent lots and one lipemic lot) was 2.7 and 1.8% at the LQC and HQC, respectively. These results indicated no significant matrix-related impact on the quantification of baricitinib and the IS.

Recovery

The recovery of baricitinib was consistent across the LQC, MQC and HQC, and averaged 98.4, 94.8 and 98.3%, respectively. The recovery of the IS averaged 91.7%.

Table 2. Stability of baricitinib on Mitra® volumetric absorptive microsampling.

Stability experiment	Duration	Low QC (%bias)	High QC (%bias)
Whole blood at 22°C	8 h	1.9	1.1
Extract on the primary extraction plate, at 4°C	138.3 h	9.4	-6.2
Final extract for analysis, at 4°C	119.9 h	7.2	2.1
VAMs at 22°C for without desiccant	96.1 h	5.1	1.6
VAMs at -20°C for with desiccant	96.6 h	7.1	6.2
VAMs at -20°C for hours without desiccant	96.5 h	6.3	4.4
VAMs at 47.5°C, 40% relative humidity, with desiccant	97.5 h	12.4	5.4
VAMs at 47.5°C, 40% relative humidity, without desiccant	97.5 h	14.1	3.7
VAMs at room temperature with desiccant	710 days	6.2	-0.6
VAMs refrigerated with desiccant	374 days	-1.2	-1.4

QC: Quality control;; VAM: Volumetric absorptive microsampling.

Carryover

Based on the analysis of blanks samples following the ULOQ standard, there was no significant carryover. Any peaks detected corresponding to baricitinib were <10.0% of the response of the LLOQ calibration standard. No peaks were detected corresponding to the IS.

Hematocrit

The accuracy and precision data from hematocrit 28.8 and 48.7% blood are summarized in Table 1 and are in agreement with the validation data.

Stability experiments

A summary of all the stability experiments and the results are detailed in Table 2. The stability experiments were designed to cover worst-case scenarios typically encountered during shipping of samples, such as Mitra VAM samples being shipped frozen or being exposed to high temperatures during shipping, or being shipped without the desiccant. Results show that samples were stable up to 96 h even under these extreme conditions. Long-term stability data show that the Mitra VAM samples can be stored for 710 days at room temperature in the sealed foil bags containing desiccant. The original extraction plate and the final extract used for analysis were stable for 138.3 and 119.9 h, respectively, sufficient to enable reanalysis and reinjection of samples if needed.

Sample analysis

Mitra VAM samples were shipped at ambient temperature to the bioanalytical laboratory via a central laboratory (Figure 2). Upon inspection at the laboratory, 15 Mitra VAM samples from the JIA study and 16 Mitra VAM samples from the pediatric AD study were unsuitable for analysis, most of them due to incomplete sampling (Table 3 & Figure 3). There were a few samples not analyzed due to other reasons: for example, one sample had the first tip incompletely sampled and the second tip was missing, one sample had both tips 'over-sampled', and one sample had the tips inverted and blood applied to the bottom of the tip (Figure 3). Overall sample analysis was successful across both studies and the performance of the batch QCs are summarized in Table 4. All samples had concentrations within the validated assay range, therefore none of the samples required reanalysis following dilution. There were no carryover issues encountered during the analysis.

Incurred sample reanalysis

ISR was performed in both studies, required by regulatory agencies [29], as a means to establish confidence in the reliability and reproducibility of the validated method and the concentrations reported. Across both studies, a total of 38 samples were reanalyzed for ISR using the second Mitra VAM tip. Twenty of the 38 samples were within 10% and six samples were outside the ISR limit of 20%. Overall, the ISR passing rates for the JIA and pediatric AD studies were 83.3 and 84.6%, respectively. Two of the ISR samples were home-collected samples. One of them had an original concentration of 2.18 ng/ml and ISR concentration of 2.97 ng/m' resulting in a bias of >20% and not meeting ISR acceptance, while the other sample was acceptable. A Bland Altman plot representing the initial analysis and the ISR data is shown in Figure 4.

Figure 2. Clinical trial samples. (A) Mitra® volumetric absorptive microsampling (VAM) samples shipped ambient and received at the bioanalytical lab. **(B)** Mitra VAM cartridge packaged and secured inside the foil zip-lock bag. **(C)** Sample identification verified once the bag is opened at the bioanalytical laboratory. **(D)** Mitra VAM cartridge showing two tips with sampled patient's blood.

Table 3. Summary of study samples and incurred sample reanalysis.

	Juvenile idiopathic arthritis	Pediatric atopic dermatitis
Number of Mitra® VAM samples	196	235
Number of samples not analyzed†	15	16
Number of samples above ULOQ and requiring dilution	0	0
Number of ISR samples	12	26
Number of ISR samples >20%	2	4
ISR passing rate	83.3%	84.6%

†Due to incomplete sampling.
ISR: Incurred sample reanalysis; ULOQ: Upper limit of quantification; VAM: Volumetric absorptive microsampling.

Establishing concordance between plasma & DBS

A total of 15 concordance samples (Mitra VAM dried blood samples and the matching venous blood/plasma samples) were available from each study (total n = 30). The slope of the regression for the pediatric AD and JIA studies were 1.32 and 1.29, respectively. Since there was no difference between the slopes of the regression lines between the two studies, the data were combined and are depicted in Figure 5. The slope of the regression line was 1.3, which depicts the Mitra VAM blood:plasma ratios in pediatric patients across both clinical studies.

Pharmacokinetics

The slope of the regression line described above was used to transform Mitra VAM data to plasma equivalents. The concentration versus time profiles for baricitinib from two pediatric patients, based on Mitra VAM data transformed to plasma equivalents, following the administration of a 2-mg and a 4-mg dose, is shown in Figure 6.

Discussion

This manuscript describes the successful validation of a quantitative LC–MS/MS methodology for the analysis of baricitinib in microsampled dried blood and the adoption and implementation of Mitra VAMs to collect PK

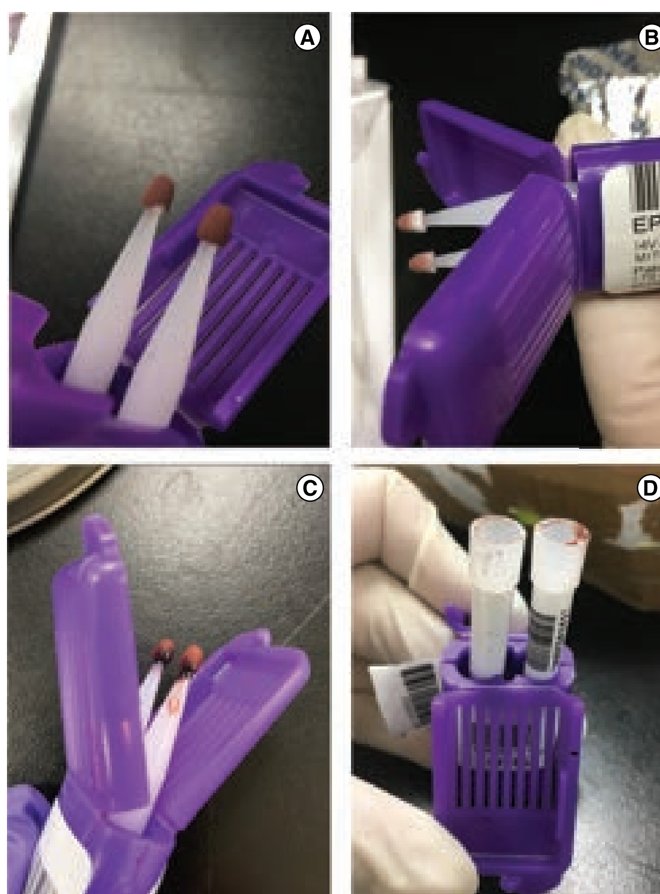

Figure 3. Examples of Mitra® volumetric absorptive microsampling cartridges with tips that were unsuitable for analysis. (A) One tip was under-sampled (the one on the left; the one on the right was used for analysis). **(B)** Both tips were undersampled. **(C)** Both tips were oversampled. **(D)** Blood was collected on the wrong end of the device.

Table 4. Assay performance during sample analysis from the juvenile idiopathic arthritis and pediatric atopic dermatitis studies.

	QC1 0.60 ng/ml	QC2 100.00 ng/ml	QC3 150.00 ng/ml
Juvenile idiopathic arthritis study			
Batches/runs (n)	7	7	7
Mean	0.58	100.50	146.76
SD	0.04	6.11	6.82
%CV	7.17	6.08	4.65
%bias	-3.33	0.50	4.83
n	28	28	28
Pediatric atopic dermatitis study			
Batches/runs (n)	11	11	11
Mean	0.59	101.50	146.60
SD	0.04	4.92	4.88
%CV	6.8	4.8	3.3
%bias	-1.7	1.5	-2.3
n	44	44	44

For precision, the %CV should be ≤15.0%. For accuracy, the %bias should be ±15.0%.
CV: Coefficient of variation; QC: Quality control; SD: Standard deviation.

samples across two phase III pediatric trials. The blood sampling technique described here is less invasive and collects only 20 μl of blood compared with venipuncture, which requires 1–2 ml of blood.

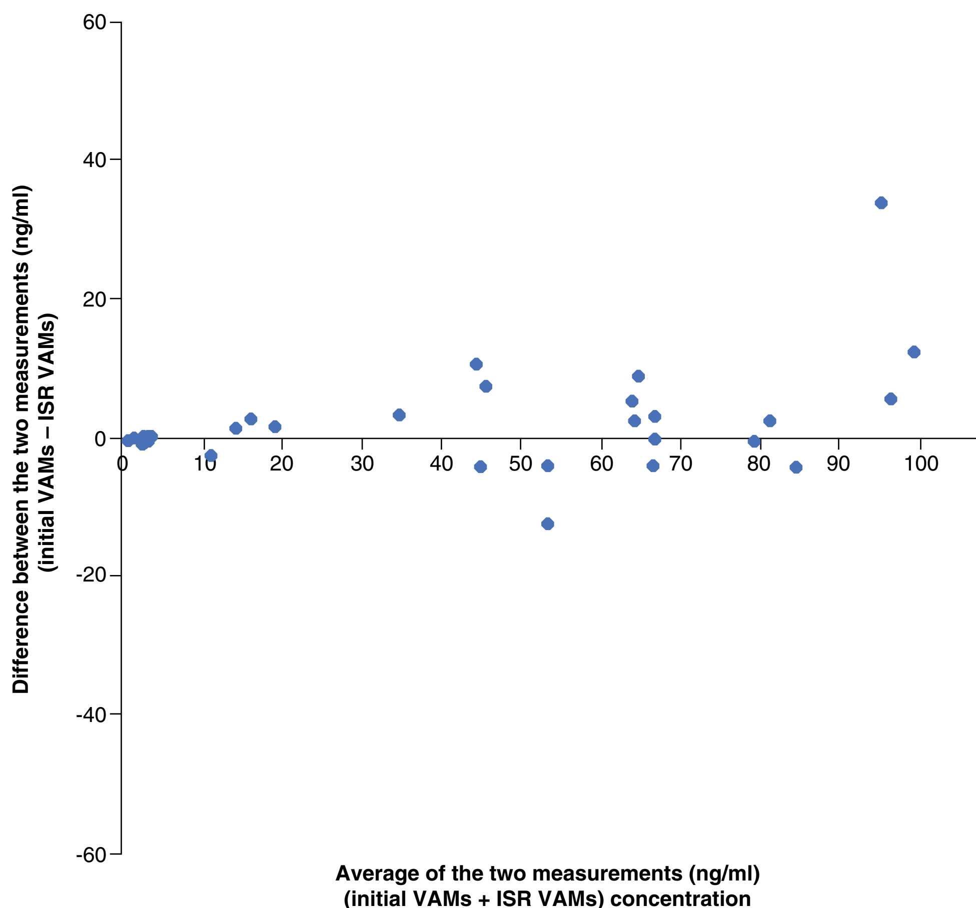

Figure 4. Bland Altman plot representing the initial analysis and in incurred sample reanalysis data.
ISR: Incurred sample reanalysis; VAM: Volumetric absorptive microsampling.

Enrolling pediatric patients and completing pediatric trials are challenging [30]. Two key factors, besides the risk–benefit of being involved in 'testing' a new therapy, are the inconvenience (numerous and long visits to the clinic/hospital) and the trial procedures (invasive, including numerous blood draws). The choice to adopt microsampling for collecting blood for PK helped to minimize overall daily blood volume needs, overcome blood volume limitations (especially in the youngest patients), and made the blood draws less traumatic (compared to performing a venipuncture in young children). In addition, the study was designed to collect only a few PK time point samples (typically two) during a clinic visit, therefore minimizing the time spent at the clinic and away from home or school, as opposed to having to spend an extended period at the clinic/hospital (possibly days) to collect a complete PK profile.

The validation experiments were carefully designed and executed to meet the regulatory guidelines for bioanalytical methods, as well as to accommodate a broad range of situations and conditions that could be encountered during clinical trials. Extreme hematocrit values have been reported to cause a bias when performing traditional DBS sampling where a fixed area of a DBS is used for analysis [14]; however, Mitra VAM tips are designed to absorb a fixed volume of blood irrespective of the hematocrit [15]. The evaluation of hematocrit was included to cover a range of hematocrit values that could be observed in children ranging from 2 to 18 years of age [26] and the results show no impact due to hematocrit (Figure 7). The use of impact-assisted extraction with stainless steel beads and the Geno/Grinder may have also contributed, as it resulted in almost complete extraction recovery of baricitinib from the Mitra VAM tips.

The evaluation of stability under higher temperatures and humidity as well as when frozen was included to mitigate any situations where samples may have been exposed to these conditions, especially during shipment. There was one situation very early in the study where central laboratories had opened the sealed foil zip-lock

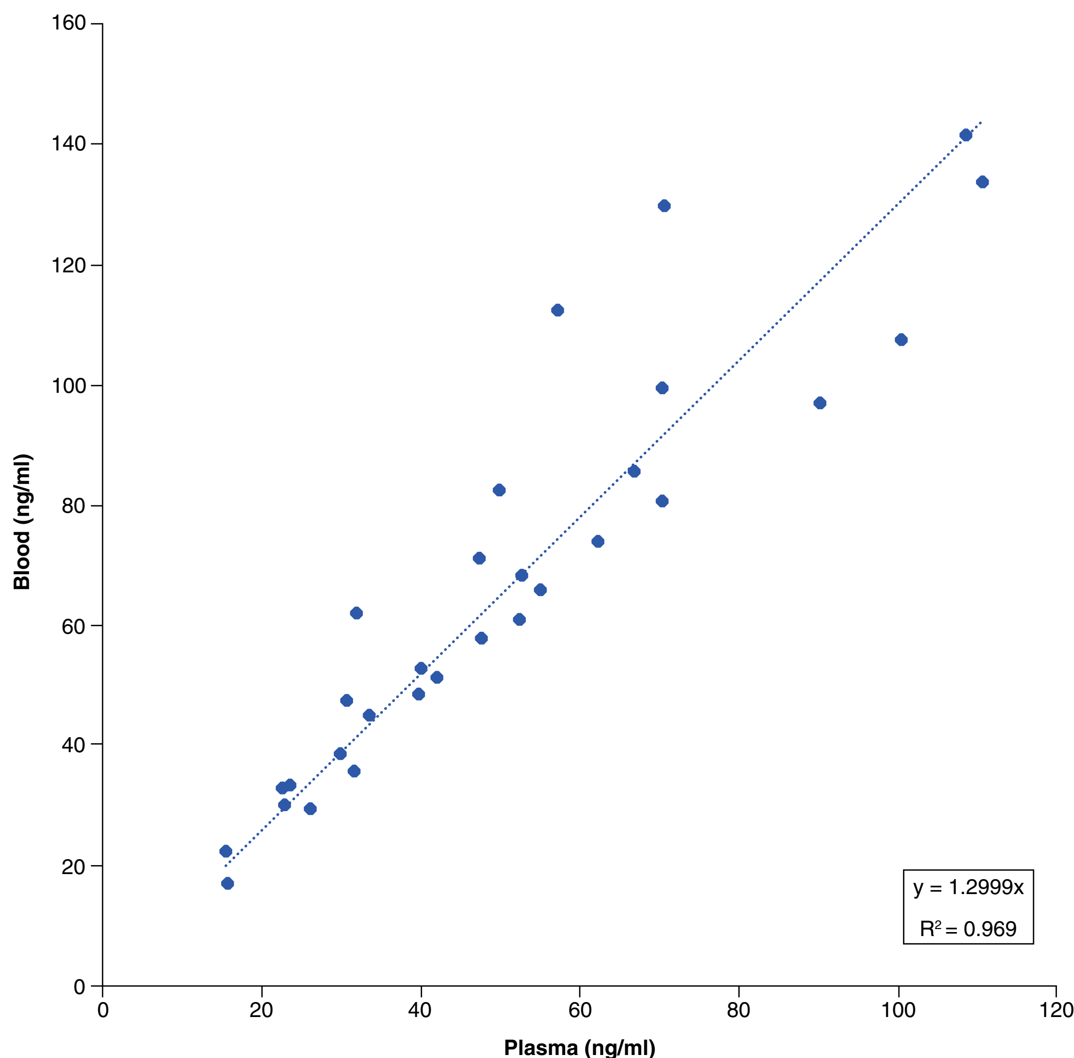

Figure 5. Concordance between baricitinib concentrations in plasma versus blood in pediatric patients from juvenile idiopathic arthritis and pediatric atopic dermatitis clinical trials (n = 30).

bag and taken the Mitra VAM cartridge out accidentally and then shipped it to the bioanalytical laboratory in a regular zip-lock bag. Luckily they had included the desiccant. Instructions were immediately provided to the central laboratories that the foil zip-lock bag (containing the cartridge) should not be opened. Overall, baricitinib was stable on Mitra VAM tips when stored at room temperature (nominal 22°C) for up to 710 days.

Before validation, the assay range was carefully selected to cover the expected range of concentrations across both studies. All the samples had baricitinib concentrations within the validated range and therefore dilution reanalysis was not needed. This was especially important in instances where there was only one Mitra VAM sample (tip) that was available for analysis.

One of the most important aspects of a validated bioanalytical method is to ensure the assay is robust and the data are reproducible. This was demonstrated by conducting ISR in both studies, which showed a greater than 83% passing rate. For the JIA study, there should have been 19 ISR samples analyzed, but only 14 were analzed due to oversight.

Although higher ISR passing rates are generally observed for plasma LC–MS/MS assays, one must consider the fact that ISR is conducted using the second tip (a second sample) and this may contribute to additional inherent variability, which is not an issue for a liquid plasma sample. The successful ISR analyses demonstrate the reliability of using the second Mitra VAM tip (sample) as a representative aliquot of the first tip (sample).

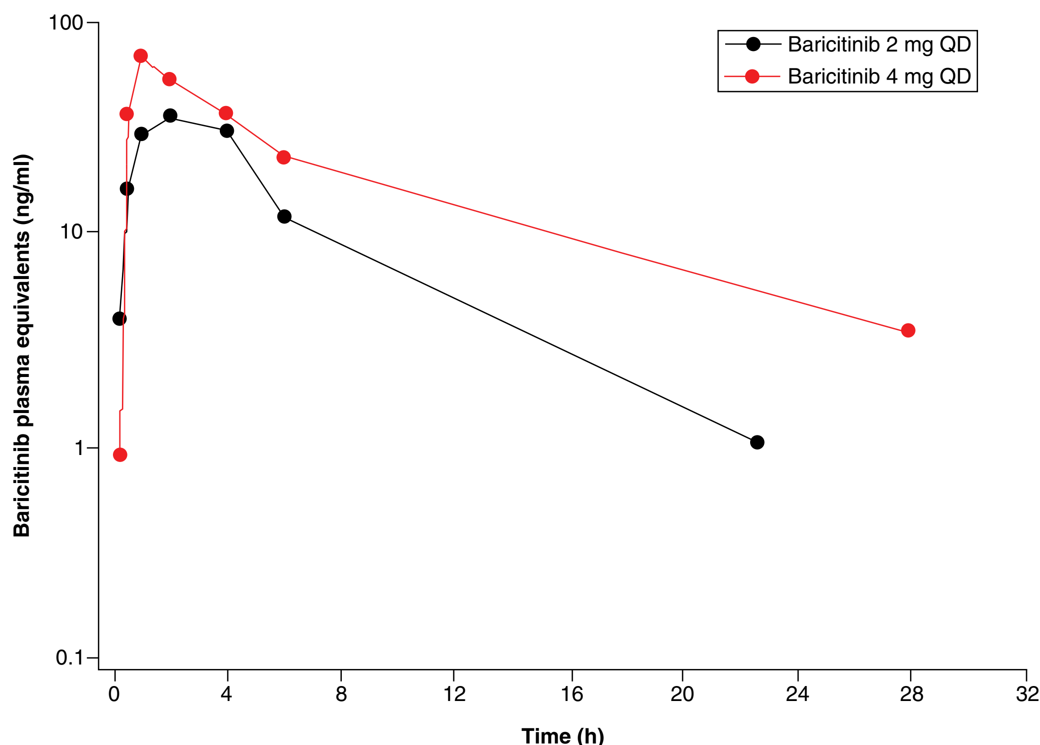

Figure 6. Individual pharmacokinetic profile from two patients, dosed at 2 mg and 4 mg, respectively. The y-axis shows the Mitra® volumetric absorptive microsampling concentrations transformed using the blood:plasma ratio derived from the concordance analysis to equivalent plasma concentrations.
QD: Once per day.

Baricitinib was being submitted for global approval at the time these pediatric studies were being planned and there was no opportunity to generate concordance data (to compare PK data in plasma generated from venous blood vs microsampled Mitra VAM blood) within an ongoing adult trial. Therefore, these two pediatric trials were designed to collect concordance data from the older age-group patients (who enrolled first and were able to provide the additional blood sampling given their larger body size).

The initial data showed that the Mitra VAM data to plasma data had a regression, with a slope of approximately 1.29 (blood:plasma ratio for baricitinib) in patients with JIA and 1.32 in patients with AD. It was decided that there was no difference between the two patient populations and to pool all the data for analysis, as shown in Figure 5. These blood:plasma data are in agreement with *in vitro* blood:plasma values reported for baricitinib [31]. The concordance analysis show that Mitra VAM data can be transformed to plasma data equivalents, and vice versa, using the slope of the regression (blood:plasma ratio). The Mitra VAM data, transformed to plasma concentration equivalents, were used to support PK profiling and modelling. Overall, the PK data show good concordance between the two matrices (dried blood microsamples and plasma) and justify the use of either matrix as a viable option for conducting PK analysis.

Training was critical to ensure success. Errors and mistakes can happen and should be expected (Figure 3). Although the percentage of Mitra VAMs samples that were not suitable for analysis were less than 8% (6.8% in the juvenile idiopathic arthritis study and 7.6% in the atopic dermatitis study), this is still a major concern. Additional instructions and training were provided as mishaps became known. Online meetings and newsletters were used to provide reminders and additional training as needed.

These two global studies required resupply of the Mitra VAM collection kits, since the kits have an 18-month expiration. Resupply became a challenging issue as different lots of Mitra VAMs had different blood-wicking volumes. The difference between some lots was over 5% and this was unacceptable, since it would impact the accuracy of the data. Therefore, additional lots for resupply were selected such that the differences in the blood-wicking volume were less than 3% (equivalent to criteria used for verification of pipette calibration). Another

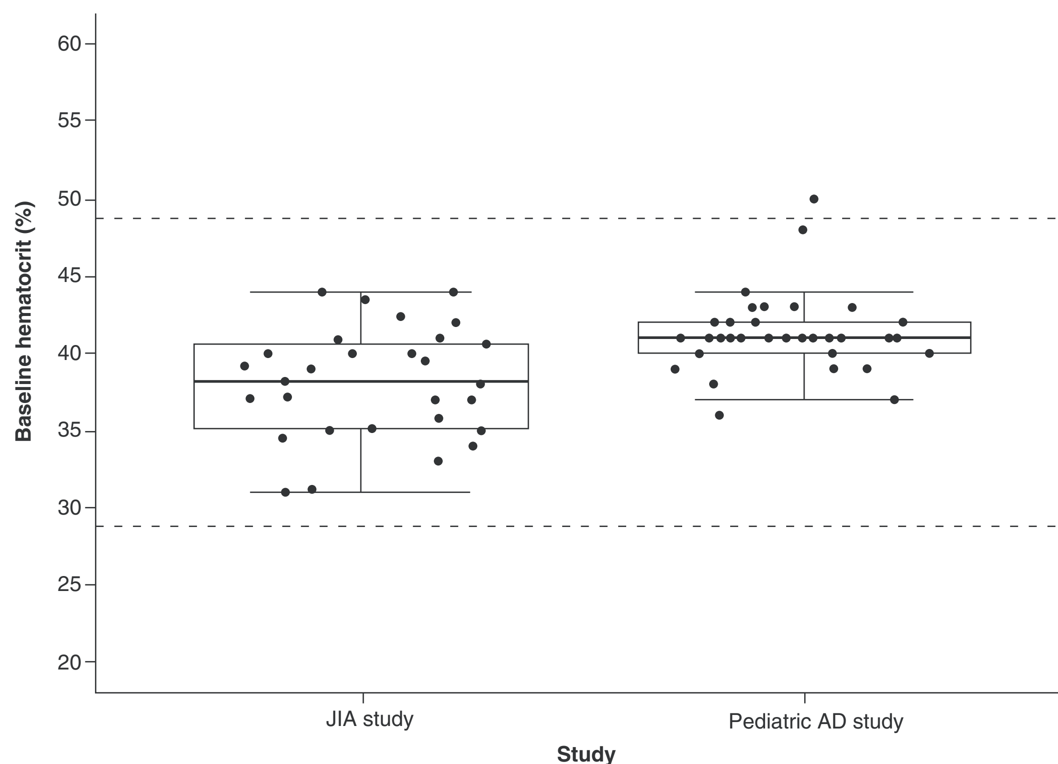

Figure 7. Baseline hematocrit values of patients from whom Mitra® volumetric absorptive microsampling samples were collected.
AD: Atopic dermatitis; JIA: Juvenile idiopathic arthritis.

challenge encountered during these two global trials was the need for translation of patient training materials and instructions into multiple languages, given that these were global studies.

The adoption and execution of Mitra VAM to collect blood samples for PK was highly successful in both studies and generated data that met the trial objectives and trial completion [27,28]. While Mitra VAM PK samples were collected by healthcare practitioners during clinic visits, the study protocols allowed for flexibility of collection at home by the patients or caregivers; there was an occasion, during the COVID-19 pandemic, where a patient was unable to return to the clinic due to the COVID-19 restrictions and the patient's parents successfully collected three blood samples at home. The samples were stored as instructed with desiccant, sealed inside the foil bag and returned to the clinic once the COVID-19 restrictions were lifted. The samples were successfully analyzed and generated valid data.

Although a formal questionnaire was not used to solicit feedback on the use of VAM, positive feedback was received from clinical sites, such as "greatly helped with enrollment" and "some patients wouldn't have participated without it". This reaffirms the known difficulties in enrolling pediatric clinical trials and the importance of blood sampling (with its related discomfort and inconvenience) as a key factor influencing the choice to participate. The experience and learnings from these two studies have enabled the adoption of the Mitra VAM blood sampling in subsequent studies [32].

Conclusion

We have demonstrated the successful adoption and execution of microsampling using the Mitra VAM blood collection device to collect PK samples from two phase III global pediatric trials. The collection of very small volumes of blood via a finger stick (minimally invasive compared to venipuncture) was critical to enable the collection and evaluation of a complete PK profile, especially in the youngest age group (2–6 years) and comply with TBVs that can be collected safely, per protocol. The flexibility offered to the patients to collect the complete PK profile over multiple visits enabled patients to participate in the trial without having to spend time away from school and spending extended periods of time at the clinic (needed to enable the collection of complete PK profiles).

Another key factor that contributed to the success of these two trials was the inclusion of additional experiments to establish stability to cover short-term temperature excursions and related mishaps that could happen during shipping, especially when involving multiple global sites.

The concordance analysis between the plasma concentration and Mitra VAM concentration data supported the continued implementation of the Mitra VAM blood collection, and the ISR data generated from both studies proved that the methodology was robust. One of the challenges was the loss of several samples due to improper sampling (e.g., tips not being completely filled), and this will continue to be an issue that needs to be carefully monitored and managed (provide additional training, reminders, etc.) until these techniques become routine.

In addition to the advantages afforded by microsampling, such as being able to collect samples without visiting and spending time at the clinic as well as being able to easily implement its use across sites and regions that may not have been accessed previously (due to lack of infrastructure such as freezers, centrifuges and dry ice for shipping), it also affords patient convenience, which is probably the most important factor impacting enrollment and completion of trial procedures.

Summary points

- This study trialled the successful development of a sensitive liquid chromatography with tandem mass spectrometry assay to quantify baricitinib concentrations from a 10-μl Mitra® volumetric absorptive microsampling (VAM) sample.
- It established concordance between traditional plasma concentrations and dried blood concentrations, which enables the transformation of blood data to plasma equivalents and vice versa.
- The Mitra VAM samples were stable for 710 days when stored at room temperature and also stable when exposed to temperature extremes (47.5°C and -20°C) for 96 h.
- Incurred sample reanalysis was conducted in both studies using the second Mitra VAM tip and it was greater than 83% in both studies, exceeding the acceptance criteria and demonstrating the robustness of the analytical procedure.
- Training materials and detailed instructions were provided to all clinical sites. A training video was also made available.
- Overall, approximately 7% of microsamples collected were unsuitable for analysis due to improper sampling, predominantly due to incomplete sampling.

Author contributions

All authors were involved in the overall design and execution of the study. In addition, ER Wickremsinhe was involved in the development, validation, and execution of the microsampling technique, L Lee was involved in the validation and data review, R Decker was involved in the analysis of data, E Lelle and L Carlton were involved in clinical operations, and S Keller and A Prakash were involved in overall study management.

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Financial & competing interests disclosure

All the authors are/were employees of Eli Lilly and company at the time the work was conducted. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The clinical protocols were approved by Institutional Review Boards before patient recruitment, and each patient provided written informed consent before enrollment. The studies were conducted in accordance with the protocol and with the consensus ethics principles derived from international ethics guidelines, including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines, applicable ICH GCP guidelines and applicable laws and regulations.

Data sharing statement

The use of data was in accordance with the study protocols and as recorded in clinical trials.gov. NCT03773978 “A Study of Baricitinib in Participants From 2 Years to Less Than 18 Years Old with Juvenile Idiopathic Arthritis (JIA)” and NCT03952559 “A Study of Baricitinib (LY3009104) in Children and Adolescents With Atopic Dermatitis (AD)”.

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