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Development and validation of a multiplex HPLC-MS/MS assay for the monitoring of JAK inhibitors in patient plasma

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ABSTRACT

Janus kinase inhibitors (JAKi) are oral small molecules used in the treatment of a broad spectrum of autoimmune and myeloproliferative diseases. JAKi exhibit significant intra- and inter-individual pharmacokinetic variabilities, due to fluctuations in compliance with oral treatments and their metabolism essentially driven by cytochrome P450 enzymes.

Intrinsically, JAKi have dose–response relationship and narrow therapeutic index: therapeutic drug monitoring (TDM) is expected to optimize and adapt their dosage regimen in order to resolve problems of efficacy and tolerance linked to dose and safety.

A sensitive analytical method using multiplex high-performance liquid-chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) was developed and validated for the simultaneous quantification in plasma of the 6 major currently used JAKi, namely abrocitinib, baricitinib, fedratinib, ruxolitinib, tofacitinib, and upadacitinib.

Plasma samples are subjected to protein precipitation with MeOH, using stable isotopically labelled internal standards. The separation of JAKi in supernatants diluted 1:1 with ultrapure H_2O was performed using a C_{18} column Xselect HSS T3 2.5 μm, 2.1x150 mm using a mobile phase composed of formic acid (FA) 0.2% and acetonitrile (+FA 0.1%) in gradient mode. The analytical run time for the multiplex assay was 7 min. JAKi drugs were monitored by electrospray ionization in the positive mode followed by triple-stage quadrupole MS/MS analysis. The method was validated according to SFSTP and ICH guidelines over the clinically relevant concentration ranges (0.5–200 ng/mL for abrocitinib, baricitinib and upadacitinib; 1–400 ng/mL for tofacitinib; 0.5–400 ng/mL for ruxolitinib, and 10–800 ng/mL for fedratinib). This multiplex HPLC-MS/MS assay achieved good performances in term of trueness (91.1-113.5%), repeatability (3.0-9.9%), and intermediate precision (4.5-11.3%).

We developed and validated a highly sensitive method for the multiplex quantification of the JAKi abrocitinib, baricitinib, fedratinib, ruxolitinib, tofacitinib, and upadacitinib in human plasma. The method will be applied for prospective clinical pharmacokinetic studies to determine whether TDM programs for JAKi based on residual drug concentrations can be recommended using disease-specific therapeutic ranges.

1. Introduction

Over the past decade there has been a wealth of innovative treatments targeting inflammatory and autoimmune diseases that provided significant benefit and tolerability, as well as better quality-of-life. Since 1990 s, biological agents (BA), including monoclonal antibodies, provide new treatment paradigms to gain medical insight into the management of patients with autoimmune disorders. They are still recognized as a gold standard for a large number of autoimmune conditions. However, BA have erratic pharmacokinetics and are likely to trigger the production of autoantibodies in the long run. Even though they achieve disease control, their preservation, adverse effects, and intravenous or subcutaneous route of administration remain sources of concern to sustain disease control [1–4].

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In the past ten years, several oral small molecules that act as Janus kinase inhibitors (JAKi) have emerged as a clinically important addition to the therapeutic armamentarium mainly based on BA. JAKi drugs have shown remarkable efficacy in the treatment of several inflammatory diseases, such as inflammatory bowel diseases (IBDs), rheumatoid arthritis (RA), immune-mediated arthropathies, multiple immune-driven dermatological diseases, COVID-19, and several hemato-oncological diseases, including Graft versus Host Disease (GvHD) [5–22].

Although the increasing use and indications of JAKi among several medical specialties were impressive, recent Phase III and IV studies raised concerns regarding their toxicity. Safety issues include major adverse cardiovascular events (MACE), malignancies, infections, venous and arterial thromboembolism [23,24].

JAKi are metabolized mainly by cytochromes P450 (CYP). CYP are characterized by substantial inter-individual variabilities in expression and activity that are modulated by pharmacogenetic factors, inflammatory states, or drug-drug interactions (phenoconversion), as well as by alteration of pathophysiological conditions [25–34]. Further, patient adherence to oral treatments is another factor that likely influence JAKi exposure. There are substantial intra- and inter-individual pharmacokinetic variations for JAKi in real-life population, but the clinical impact remains unknown. TDM programs for patients taking JAKi could target drug concentrations within therapeutic ranges and contain dose-dependent adverse drug reactions (ADRs). Conversely, TDM could address lack of drug response imputable to insufficient exposure, increasing thereby the overall JAKi treatments success rates. Novel TDM approaches are expected to be suited for drugs with dose–response relationship and narrow therapeutic indexes [23,24,35–37].

A validated LC-MS/MS assay that allows the quantification of JAKi concentrations in plasma is instrumental to provide TDM services for patients with a wide range of immune-mediated diseases and, more specifically, for large-scale population pharmacokinetic studies. To our knowledge, there are only a few reports on the development and validation of assays to quantify JAKi in human plasma. Previously published methods for JAKi include a liquid chromatography step on C_{18} support [38–43] or propyl-linked pentafluorophenyl column [44]. Generally, the plasma cleanup process involved either protein precipitation [38–43], solid phase extraction, or liquid–liquid extraction [44]. While few LC-MS/MS analyses have been reported to date, most of them generally focus on only a single, or a limited number of JAKi in human plasma. [38–45].

Our objective was to develop and validate a highly sensitive and rapid multiplex assay by HPLC-MS/MS method using stable isotope-labelled internal standards for the simultaneous quantification of the six major currently-in-use JAKi: abrocitinib, baricitinib, fedratinib, ruxolitinib, tofacitinib, and upadacitinib.

2. Material and methods

2.1. Chemicals, reagents

All chemicals were of analytical grade (purity ≥ 98%) and structures are reported in Fig. 1. Abrocitinib, baricitinib, fedratinib, ruxolitinib, and tofacitinib were purchased from Alsachim (Strasbourg, France) while upadacitinib was purchased from Medchem Express (Monmouth Junction, USA). Their respective stable isotope-labelled internal standard (I.S.) were obtained from Alsachim ([$^2H_7$]-abrocitinib, [$^2H_5$]-baricitinib, [$^2H_9$]-fedratinib, [$^2H_9$]-ruxolitinib, [$^{13}C_3$-^{15}N]-tofacitinib, [^{13}C,$^2H_2$,^{15}N]-upadacitinib). The following HPLC grade solvent (purity ≥ 98%) were purchased from Merck (Darmstadt, Germany): acetonitrile (ACN), methanol (MeOH), formic acid (FA), and dimethylsulfoxide (DMSO). Ultrapure water was produced using the Mili-Q® UF-Plus device from Millipore Corp (Burlington, USA).

Human blank plasma samples used for the preparation of quality controls (QCs) and calibration samples were obtained in accordance with institutional ethical standards from citrated blood of patients with *polycythemia vera* undergoing regular phlebotomy at the Ambulatory Care Unit, Unisanté, University Hospital of Lausanne (Lausanne, Switzerland). Once collected, blood samples were centrifuged at 2000 g for 10 min at +4 °C on a Hettich® Rotanta 4600RF (Bäch, Switzerland).

2.2. Calibration standard, quality control and stable isotope-labelled internal standard

Analyte stock solutions were prepared in DMSO at 2 mg/mL for baricitinib, fedratinib, tofacitinib, and upadacitinib, 1 mg/mL for ruxolitinib, 5 mg/mL for abrocitinib and stored at −20 °C. First, independent working solutions of upadacitinib, abrocitinib, and baricitinib were prepared in MeOH:H_2O 3:1 (v:v) at 100 µg/mL. Working solutions (WS) of abrocitinib (2 µg/mL), baricitinib (2 µg/mL), fedratinib (10 µg/mL), ruxolitinib (4 µg/mL), tofacitinib (4 µg/mL), and upadacitinib (2 µg/mL) were then prepared in MeOH:H_2O 3:1 (v:v) for the preparation of

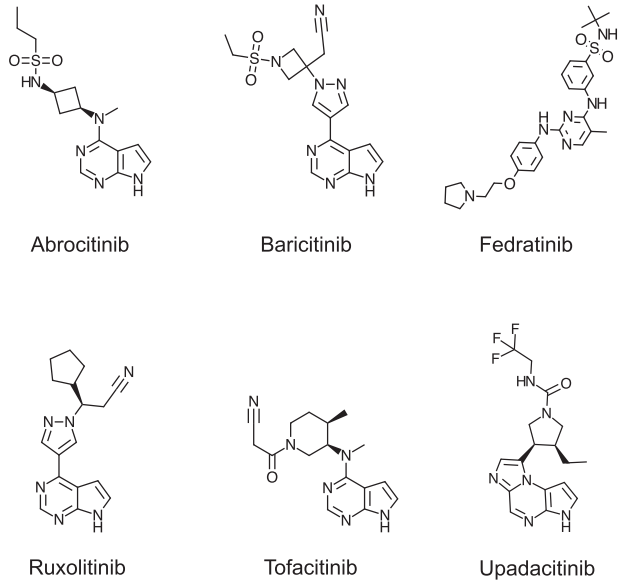

Fig. 1. Chemical structures of the analyzed JAKi.

Table 1
Concentration levels of calibration standards and QCs. The calibration and QCs standards selected for the validation ranged from 0.5 to 200 ng/mL for abrocitinib, baricitinib and upadacitinib; from 0.5 to 400 ng/mL for ruxolitinib; 1 to 400 ng/mL for tofacitinib; and from 10 to 800 ng/mL for fedratinib.

Sample type	Levels	Abrocitinib [ng/mL]	Baricitinib [ng/mL]	Fedratinib [ng/mL]	Ruxolitinib [ng/mL]	Tofacitinib [ng/mL]	Upadacitinib [ng/mL]
Calibration standards	8	200.0	200.0	800.0	400.0	400.0	200.0
	7	40.0	40.0	160.0	80.0	80.0	40.0
	6	10.0	10.0	40.0	20.0	20.0	10.0
	5	5.0	5.0	20.0	10.0	10.0	5.0
	4	2.5	2.5	10.0	5.0	5.0	2.5
	3	1.0	1.0	–	2.0	2.0	1.0
	2	0.5	0.5	–	1.0	1.0	0.5
	1	–	–	–	0.5	–	–
QCs	7	200.0	200.0	800.0	400.0	400.0	200.0
	6	80.0	80.0	320.0	160.0	160.0	80.0
	5	20.0	20.0	80.0	40.0	40.0	20.0
	4	5.0	5.0	20.0	10.0	10.0	5.0
	3	2.5	2.5	10.0	5.0	5.0	2.5
	2	0.5	0.5	–	1.0	1.0	0.5
	1	–	–	–	0.5	–	–

calibration and validation standards. The WS were sequentially diluted in MeOH:H_2O 3:1 (v:v) to the desired concentrations prior to being spiked into plasma.

Spiking solutions (50 µL) were diluted 20-fold with blank plasma (950 µL) to obtained spiked plasma that were used for calibration, QC and validation samples. The total added organic solvent volume did not exceed 10% of the biological sample volume according to the recommendations for bioanalytical method validation [46].

A first working solution of labeled internal standards (WS I.S.-1) was prepared in pure MeOH at 100 µg/mL of [$^2H_7$]-abrocitinib, [$^2H_5$]-baricitinib, [^{13}C,$^2H_2$,^{15}N]-upadacitinib. Then, WS I.S.-2 was obtained by diluting WS I.S.-1 in the same solvent at 2 µg/mL and [$^2H_9$]-fedratinib, [$^2H_9$]-ruxolitinib, [$^{13}C_3$-^{15}N]-tofacitinib were diluted at 10 µg/mL, 4 µg/mL, and 4 µg/mL respectively. A protein precipitation solution was prepared from WS I.S-2 and diluted with pure MeOH to yield concentration of 5 ng/mL ([$^2H_7$]-abrocitinib, [$^2H_5$]-baricitinib, and [^{13}C,$^2H_2$,^{15}N]-upadacitinib), 20 ng/mL ([$^2H_9$]-fedratinib); and 10 ng/mL ([$^2H_9$]-ruxolitinib and [$^{13}C_3$-^{15}N]-tofacitinib). All stock solutions were stored at −20 °C.

Height levels of calibration standards were prepared on the first validation day (n = 3) and seven quality controls samples (QCs) (n = 3) were used to cover the relatively large validated JAKi concentration ranges established with respect to clinically relevant concentrations reported in clinical trials or from the few popPK studies [12,14,31,47–58]. Concentration levels of calibration standards and QCs are given in the Table 1.

2.3. Plasma sample treatment procedure

Spiked and blank plasma (50 µL) were subjected to protein precipitation with 150 µL MeOH containing the respective I.S. in a 1.5 mL Eppendorf® plastic vial. Samples were vortexed for approximately 10 s and centrifuged at 20′000 g, 10 min at +4 °C in a Hettich® Mikro 200R benchtop centrifuge (Bäch, Switzerland). A 100 µL-aliquot of supernatant was diluted without delay with 100 µL of ultrapure H_2O into a 1.5 mL glass HPLC vial, securely sealed with a plastic cap and vortexed for approximately 10 s. A volume of 10 µL was injected into HPLC-MS/MS instrument for analysis.

Table 2
Gradient elution program for the separation of the six JAKi.

Time [min]	Flow [mL/min]	% A	% B
0.0	0.3	95.0	5.0
0.2	0.3	95.0	5.0
0.3	0.3	85.0	15.0
3.0	0.3	65.0	35.0
4.8	0.3	10.0	90.0
5.4	0.3	10.0	90.0
5.9	0.6	10.0	90.0
6.0	0.6	95.0	5.0
6.7	0.6	95.0	5.0
6.8	0.3	95.0	5.0
7.0	0.3	95.0	5.0

2.4. HPLC-MS/MS conditions

The liquid chromatography system consisted of a Vanquish split samplers, and a Vanquish Binary F pump coupled to a triple-stage quadrupole TSQ Quantiva™ mass spectrometer (Thermo Fisher Scientific™, USA) operated with the Xcalibur™ package software (v 4.5.). Data acquisition handling and instrument control were performed using QUAL and QUAN browser (Thermofisher Scientific™, USA).

Separation was performed on a Xselect™ HSS T3 2.5 µm, 2.1x150 mm column (Waters™, USA). The aqueous mobile phase was composed of ultrapure H_2O + 0.2% FA (solvent A) and acetonitrile + 0.1% FA (solvent B). Prior to analytical sequences, the HPLC column was conditioned with solvent B (5%) at a flow rate of 0.3 mL/min for 10 min. The autosampler and column chamber temperatures were maintained at +4 °C and +40 °C respectively. The elution gradient is presented in Table 2.

The optimization of MS/MS parameters for all analytes was carried out by direct infusion. Compounds were diluted at 1 µg/mL in MeOH:H_2O (1:1) and infused separately at 10 µL/min into the MS/MS detector. MS/MS conditions were as follows: electrospray ionization in positive mode (ESI^+); spray voltage 3500 V; sheath gas, auxiliary gas, and sweep gas (all nitrogen) pressure 60, 6 and 2 arbitrary units (AU) respectively; vaporizer temperature and ion transfer tube temperature 150 °C and 350 °C respectively; cycle time 0.3 s, fragmentation source 0 V. The calibrated RF Lens was activated. The first Quadrupole (Q1) and third quadrupole (Q3) were both fixed at 1.2 amu mass resolution (Full-Width Half-Maximum, FWHM). The chromatographic filter was activated and the peak width was set to 6 s. At least 20 points per peak were acquired. The collision-induced dissociation (CID) gas pressure in the second quadrupole (Q2) was adjusted to 2 mTorr (argon). The Selective Reaction Monitoring (SRM) detection was used to record Mass spectra (MS) and MS acquisition was done in centroid mode. MS settings, *m/z* transitions, and the collision energy for each analyte and I.S. are reported in Table 3.

2.5. Analytical method validation

The analytical method validation procedure was based on the French

Table 3
MS/MS parameters of the six JAKi of interest (in alphabetical order) and their respective stable isotope-labelled I.S. (*RT: retention time, CE: collision energy*).

Compound	ESI polarity	Transitions [*m/z*] Precursor →product	CE [eV]	RT [min]
Abrocitinib	+	324.10 →149.00	28	3.56
[$^{2}H_7$]-Abrocitinib	+	330.20 →149.00	28	3.54
Baricitinib	+	372.30 →251.11	28	4.05
[H_5]-Baricitinib	+	377.30 →251.11	28	4.04
Fedratinib	+	525.30 →98.00	38	3.93
[$^{2}H_9$]-Fedratinib	+	534.30 →98.00	40	3.91
Ruxolitinib	+	307.40 →186.10	27	5.07
[$^{2}H_9$]-Ruxolitinib	+	316.40 →186.10	27	5.06
Tofacitinib	+	313.40 →149.01	24	3.23
[$^{13}C_3$, ^{15}N]-Tofacitinib	+	317.30 →149.11	28	3.25
Upadacitinib	+	381.40 →256.08	28	4.09
[^{13}C,$^{2}H_2$,^{15}N]-Upadacitinib	+	385.40 →256.08	28	4.09

Society of Pharmaceutical Sciences and Techniques (*Société Française des Sciences et Techniques Pharmaceutiques* - SFSTP) and ICH-M10 guidelines (European Medicine Agency – EMA) [46,59–63].

2.5.1. Selectivity, specificity and carry-over

Blank human plasma (regular, hemolyzed, and lipemic) and serum from 10 different donors processed with pure MeOH were used to assess the selectivity. Cross-talk interferences (specificity) were then assessed by injecting the highest QC sample (i.e. abrocitinib, baricitinib, upadacitinib: 200 ng/mL; ruxolitinib, tofacitinib: 400 ng/mL, fedratinib: 800 ng/mL) processed with pure MeOH (no I.S.) as well as blank plasma extract sample with I.S. (i.e. [$^{2}H_7$]-abrocitinib, [$^{2}H_5$]-baricitinib, [^{13}C,$^{2}H_2$,^{15}N]-upadacitinib: 5 ng/mL; [$^{2}H_9$]-fedratinib at 20 ng/mL; [$^{2}H_9$]-ruxolitinib, [$^{13}C_3$-^{15}N]-tofacitinib: 10 ng/mL). To determine carry-over, blank plasma was injected immediately after the highest calibration sample (i.e. abrocitinib, baricitinib, upadacitinib: 200 ng/mL; ruxolitinib, tofacitinib: 400 ng/mL; fedratinib: 800 ng/mL) processed with I.S.

2.5.2. Quantitative evaluation of matrix effect

The matrix effect was quantitatively determined by spiking blank plasma (regular, hemolyzed, and lipemic) and serum from 10 different donors. The assessment was performed for three levels of concentration (low, medium, high concentration), covering the calibration range. Each level for each matrix was extracted according to the analytical procedure and analyzed once.

2.5.3. Extraction recovery

Extraction recovery (ER) was quantitatively evaluated based on Matuszewski's approach [75]. Three sets of samples at low (abrocitinib,

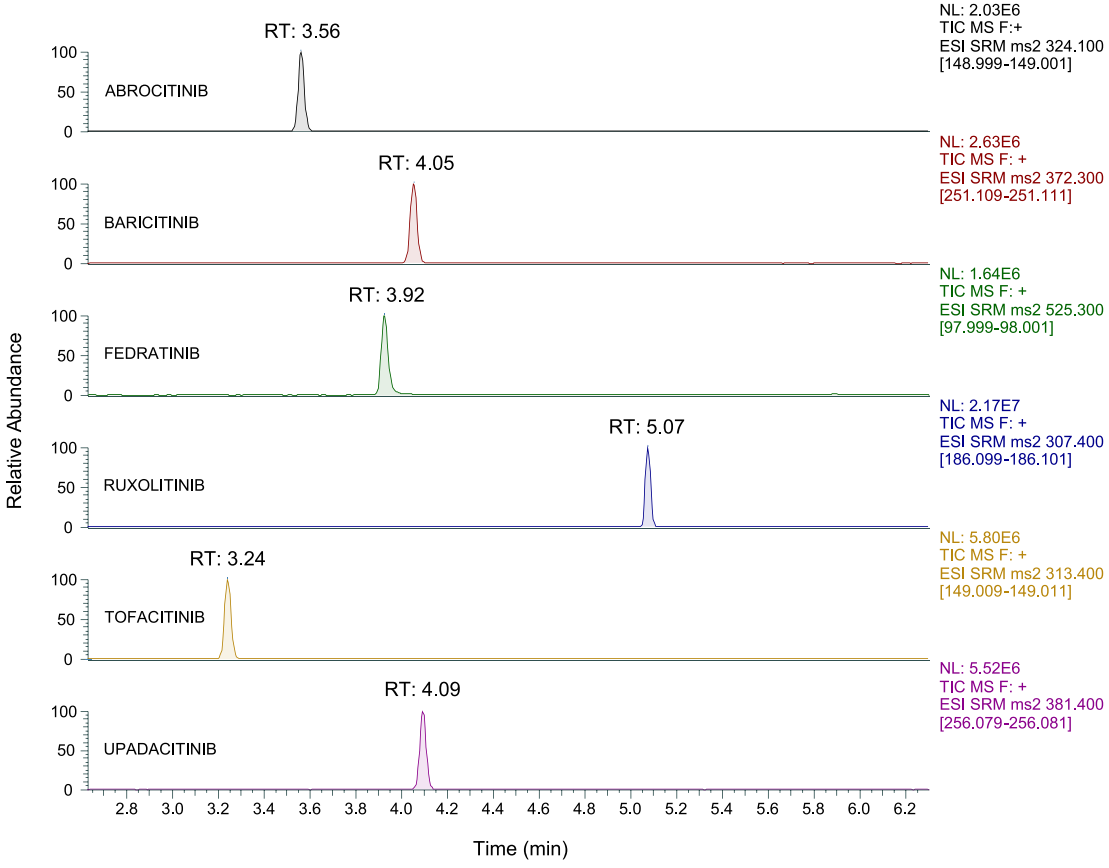

Fig. 2. Chromatographic profile of six JAKi: abrocitinib 10 ng/mL, baricitinib 10 ng/mL, fedratinib 40 ng/mL, ruxolitinib 20 ng/mL, tofacitinib 20 ng/mL, upadacitinib 10 ng/mL. JAKi profiles are given in alphabetical order. The corresponding internal standards are not shown.

Table 4
Internal standard trueness-normalized matrix effect for JAKi, RSD: relative standard deviation.

Compound	Concentration [ng/mL]	Trueness normalized by the I.S. Range [%]	Mean [%]	RSD [%]
Abrocitinib	5	88 –110	98	7
	10	88 – 112	101	8
	160	86 – 110	97	8
Baricitinib	5	92 – 121	107	8
	10	98 – 119	111	6
	160	92 – 115	103	7
Fedratinib	20	72 – 111	97	13
	40	88 – 120	106	10
	640	88 – 111	97	7
Ruxolitinib	10	93 – 114	109	5
	20	96 – 127	116	9
	320	89 – 114	102	6
Tofacitinib	10	86 – 106	98	6
	20	87 – 111	103	8
	320	86 – 107	96	7
Upadacitinib	5	94 – 110	101	7
	10	93 – 113	105	7
	160	88 – 108	97	7

baricitinib, upadacitinib: 10 ng/mL; fedratinib: 40 ng/mL; tofacitinib, ruxolitinib: 20 ng/mL), medium (abrocitinib, baricitinib, upadacitinib: 50 ng/mL; fedratinib: 200 ng/mL; tofacitinib, ruxolitinib: 100 ng/mL), and high (abrocitinib, baricitinib, upadacitinib: 100 ng/mL; fedratinib: 400 ng/mL; tofacitinib, ruxolitinib: 200 ng/mL) concentration were considered and prepared as follows: seven blank plasma spiked after extraction and seven blank plasma spiked before extraction. Average analyte to I.S. peak area ratio was considered for each set of samples to calculate the internal standard-normalized extraction recovery (n-ER).

2.5.4. Trueness, precision, accuracy profile, limits of quantification, and linearity

In the validation phase, trueness and precision were evaluated at 8 concentration levels through replicate analyses (n = 3) over three nonconsecutive days.

Trueness (systematic error) was assessed using the bias, which is the percentage difference between the measured and nominal value, while repeatability (intra-day variances) and intermediate precision (intra-day and inter-day variances) were used to assess precision (random error) [60,64,65]. At each concentration level, the precision parameters were calculated and reported as relative standard deviation (RSD) [66]. Initially, calibrators were prepared in duplicate and QC samples in triplicate covering the expected range of concentrations. The β-expectation tolerance represents the concentration range in which β% of future results are expected to lie. Accuracy profiles represent the combination of both random and systematic error (total analytical error) and were obtained by using a β-value of 95% [67–70]. The lower limit of quantification (LLOQ) is defined as the lowest concentration at which the β-expectation tolerance interval exceeds the acceptance limits (±30%) and was determined from the absolute accuracy profiles [46,71].

Several calibration curve models were tested to adequately describe the response concentration profile. The best calibration model was selected based on estimates of trueness and precision, the narrowest β-expectation tolerance interval, and the LLOQ [66]. By setting the β value to 0.95, it is then possible to estimate the measurement uncertainty (MU) value from the data collected during the validation phase [72,73].

The method's ability to provide proportional quantitative results was assessed each day of validation by using ordinary least squares regression of back-calculated concentrations against nominal concentrations for validation standards.

2.5.5. Limits of detection

The limit of detection (LOD) was assessed by analyzing processed plasma samples spiked with different concentrations of JAKi: the lowest QC standard was subjected to 2-, 5-, and 10-fold dilution in plasma. The LOD values were determined not only by visual inspection of the chromatograms but also by calculating the signal intensity as compared to the blank plasma (i.e. at least 3-times higher than the background "blank" signal).

2.5.6. Integrity to dilution

To ensure the reliability of QC samples outside the analytical range, a dilution integrity experiment was conducted using concentrations greater than 2 times the highest calibrator, which were then diluted 10-fold in blank plasma from 5 different donors or water, to fall within the validated plasma concentration ranges.

2.5.7. Stability studies

Short-term stability at high and low concentrations were evaluated in plasma and in whole blood at room temperature (RT) and +4 °C, for up to 96 h. In addition, stability after three freeze–thaw cycles was also assessed. Samples were analyzed immediately after the preparation, and then after a freezing period of at least 12 h. Medium-term stability was assessed with plasma samples frozen at −20 °C and −80 °C for 2 months. Finally, short-term stability of the extract in vials was assessed by injecting QCs stored in the autosampler maintained at +4 °C for 24 h, 48 h, and 72 h. At t_0, QCs were injected immediately after their preparation. Each sample was prepared and analyzed in triplicate. The average concentrations measured for each time point were compared with the average concentrations of the samples prepared at t_0.

2.6. Clinical application

Initially, patients' blood samples were analyzed as laboratory quality control analyses for the formal demonstration of assay applicability. After extensive analytical method validation, JAKi levels were determined in patients' samples collected within the framework of a Swiss-wide observational population pharmacokinetic-pharmacodynamics study of JAKi. For this purpose, 78 blood samples from patients treated with JAKi for various autoimmune or myeloproliferative diseases were collected in EDTA-K monovettes at the occasion of their usual medical follow-up. The preanalytical sample preparation was performed by centrifuging blood samples for 5 min, 2000 g, at +4 °C. Plasma were then transferred into propylene tubes and stored at −80 °C until batch analyses. Samples were processed and analyzed with the method described above. The patient provided written informed consent for the use of patient related data for scientific purposes. The study protocol has been approved by the Institutional Ethics Committee in 2023.

3. Results

3.1. Analytical method development

The MS/MS parameters, precursor and product ions were optimized and the optimal parameters are summarized in Table 3 the ESI^+ mode was found to be the most sensitive for all compounds. A suitable separation of abrocitinib, baricitinib, fedratinib, ruxolitinib, tofacitinib, and upadacitinib was obtained by optimizing the chromatographic conditions. The best peak shapes and retention times were obtained with H_2O + 0.2% FA and ACN + 0.1% FA (mobile phase) and a Xselect™ HSS T3 2.5 μm, 2.1x150 mm LC column. The gradient program for the mobile phase was optimized to improve separation and reduce analytical time to 7 min. The multiplex chromatographic profile of the six JAKi is shown in the Fig. 2. MeOH proved to be the best solvent for plasma protein precipitation whilst providing the best signal sensitivity of JAKi

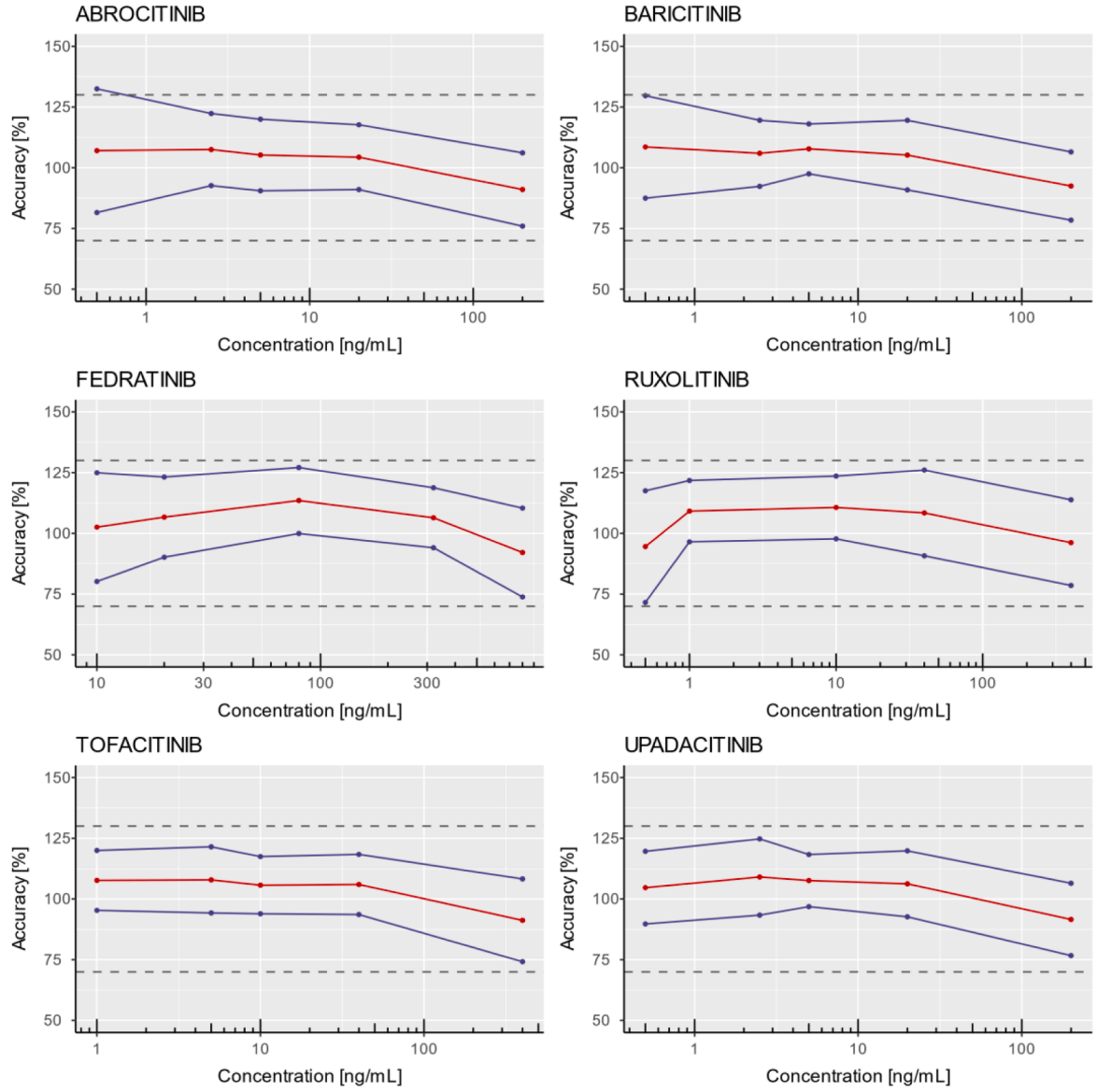

Fig. 3. Accuracy profiles over the validated domain in human plasma for abrocitinib, baricitinib, fedratinib, ruxolitinib, tofacitinib, and upadacitinib. Trueness (red solid line), upper and lower β-expectation tolerance intervals (β = 95) (blue solid lines) and acceptance limits (α = ±30, grey dotted lines).

compared to ACN. Peak shapes were improved by diluting 1:1 the supernatants with milliQ H_2O prior to injection into HPLC column. The injection volume was set at 10 μL to give the best compromise between sensitivity and peak shape. ESI source parameters such as background noise and signal sensitivity were adjusted to obtain the best signal-to-noise ratio. By adjusting the I.S. concentration, the response functions were improved while limiting variability at low concentrations and interferences.

3.2. Validation of the method

3.2.1. Selectivity, cross-talk, and carry-over

Serum and plasma (regular, hemolyzed, and lipemic) from 10 different donors were compared, and no significant matrix interferences were observed in the *m/z* transition of abrocitinib, baricitinib, fedratinib, ruxolitinib, tofacitinib, and upadacitinib at their respective retention times, demonstrating good selectivity (see Figure S1 of the Supplementary Material).

The injection of a blank plasma processed with the precipitation solution containing I.S. and the highest calibration sample processed with pure MeOH did not reveal significant reciprocal cross-talk interferences between the six JAKi and their corresponding I.S. (see Figure S2 of the Supplementary Material).

A significant carry-over effect was observed for fedratinib, ruxolitinib, and tofacitinib, when a blank plasma was analyzed immediately after the highest calibration sample, providing signal peaks greater than 20% of the LLOQ. Such effect could be circumvented by programming the injection of a blank MeOH and a blank plasma extract sample (25 μL volume) immediately after the highest calibration level and after each patient's sample. With these conditions, the carry-over of fedratinib in the following blank sample corresponds to 4% of the response measured for the lowest calibrator, while ruxolitinib and tofacitinib were not detected at all.

3.2.2. Quantitative evaluation of matrix effect

Quantitative results of the assessment of the matrix effect are summarized in Table 4. Values for at least two concentration levels for each matrix (serum, regular, hemolyzed, and lipemic plasma) were within 85 − 115% and the RSD observed between the different matrices did not exceed 15% for each level.

Table 5
Trueness and accuracy of JAKi over their validated range and their respective limit of quantification. (*LLOQ: lower limit of quantification, ULOQ: upper limit of quantification)*.

			Precision				
Compound	Concentration [ng/mL]	Trueness [%]	Repeatability [%]	Intermediate precision [%]	Relative uncertainty [%]	LLOQ [ng/mL]	ULOQ [ng/mL]
Abrocitinib	0.5	107.0	9.6	11.3	30.2	0.5	200
	2.5	107.5	6.6	6.6	16.1		
	5	105.3	6.5	6.5	15.9		
	20	104.4	4.2	5.9	17.8		
	200	91.1	3.0	6.7	27.0		
Baricitinib	0.5	108.6	9.3	9.3	22.8	0.5	200
	2.5	105.9	6.0	6.0	14.7		
	5	107.8	4.1	4.5	11.7		
	20	105.2	3.5	6.3	22.4		
	200	92.5	3.5	6.2	21.7		
Fedratinib	10	102.6	9.9	9.9	24.2	10	800
	20	106.7	7.3	7.3	17.9		
	80	113.5	3.1	6.0	22.4		
	320	106.4	5.4	5.5	13.4		
	800	92.1	4.2	8.1	29.6		
Ruxolitinib	0.5	94.5	6.3	10.2	33.9	0.5	400
	1	109.1	4.4	5.6	15.6		
	10	110.7	5.7	5.7	14.0		
	40	108.4	4.8	7.8	26.3		
	400	96.2	3.6	7.8	31.4		
Tofacitinib	1	107.6	3.6	5.5	17.3	1.0	400
	5	107.9	6.0	6.0	14.7		
	10	105.7	5.2	5.2	12.8		
	40	106.0	3.8	5.5	16.8		
	400	91.2	5.2	7.5	23.1		
Upadacitinib	0.5	104.7	6.6	6.6	16.2	0.5	200
	2.5	109.0	6.9	6.9	17.0		
	5	107.6	4.7	4.7	11.6		
	20	106.2	4.1	6.0	18.6		
	200	91.6	3.7	6.6	23.3		

Table 6
Stability assay. Evolution over time of JAKi concentrations in plasma at +4 °C and room temperature (RT) with respect to t_0 values. Each time point represents the mean % difference from the initial nominal concentrations (analysed at t_0) of the 2 levels of concentrations (1.5 and 150.0 ng/mL for abrocitinib, baricitinib, upadacitinib; 1.5 and 300.0 ng/mL for ruxolitinib, 3.0 and 300.0 ng/mL for tofacitinib; and 30.0 and 600.0 ng/mL for fedratinib).

Compounds	Time [h]	Plasma				Whole blood			
		Low concentration		High concentration		Low concentration		High concentration	
		RT	+4 °C	RT	+4 °C	RT	+4 °C	RT	+4 °C
Abrocitinib	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	6.0	−5.5	−1.6	−2.2	−1.5	−3.2	2.8	0.4	−1.5
	24.0	−3.0	0.1	−0.4	−2.4	−1.3	−0.4	0.5	1.2
	96.0	−4.8	−1.3	−2.0	−1.3	−1.8	5.4	−1.3	0.2
Baricitinib	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	6.0	−2.0	3.3	−2.3	−0.9	−2.4	−5.2	0.7	−3.0
	24.0	0.6	2.9	−1.3	−1.1	−3.7	−6.6	2.0	0.7
	96.0	−3.4	−0.2	−1.1	−0.9	−2.6	−19.3	1.6	−0.7
Fedratinib	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	6.0	−5.7	−8.0	0.5	−1.3	−4.5	−8.1	−9.9	6.9
	24.0	−3.1	−7.2	−1.7	−3.5	4.5	−11.4	−8.0	1.5
	96.0	−3.2	−10.1	1.4	−2.3	−2.5	−5.8	1.9	6.7
Ruxolitinib	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	6.0	−3.1	0.5	−1.0	−1.2	7.4	6.6	8.7	−0.9
	24.0	−0.9	0.4	−0.2	−2.1	13.6	7.4	18.7	4.6
	96.0	−2.5	0.8	−1.2	−0.6	23.2	11.9	27.3	6.8
Tofacitinib	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	6.0	−3.8	0.9	−1.8	−0.8	−2.3	0.9	−1.1	−0.2
	24.0	−2.5	1.8	−1.0	−1.4	−2.8	0.7	−1.6	−0.3
	96.0	−3.1	0.4	−2.6	−0.9	−11.2	−2.1	−5.1	−1.4
Upadacitinib	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	6.0	−3.2	0.9	−2.1	−0.6	−1.2	6.8	0.0	−0.8
	24.0	−2.4	2.4	−0.7	−1.6	−2.3	4.3	0.4	1.1
	96.0	−4.6	3.1	−1.3	−1.2	−2.1	12.0	1.0	5.2

3.2.3. Extraction recovery

Table S1 (Supplementary Material) shows the quantitative results of the evaluation of the n-ER. The mean n-ER of the analytes ranged between 96.78% and 115.21% and the analytical RSD values were between 1.11% and 13.68%.

3.2.4. Trueness, precision, accuracy profile, limits of quantification, and linearity

Quadratic log–log regression models best described the response-concentration profile in terms of coefficient of determination and back-calculated calibration samples within ± 15% (±20% at LLOQ) of

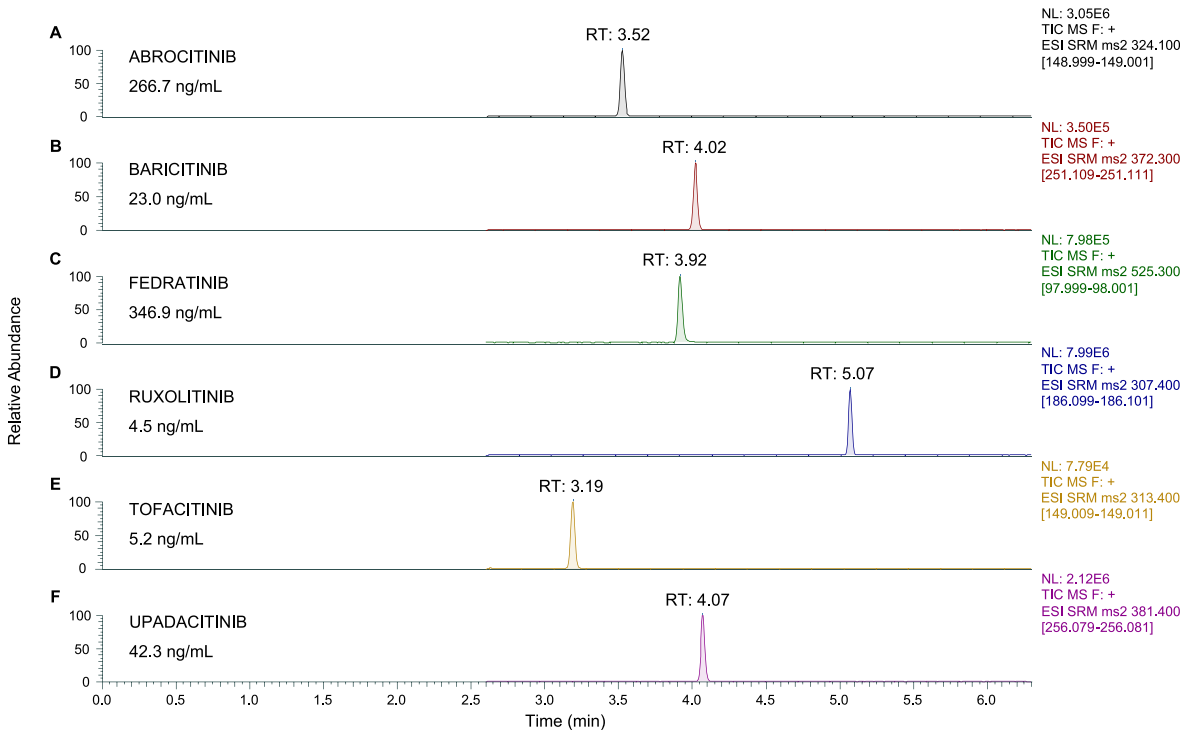

Fig. 4. Chromatographic profiles of plasma samples collected from **(A)** a 46-year-old patient suffering from severe atopic dermatitis treated with 50 mg abrocitinib once daily (QD). The sample was collected 4 h after the last intake. TDM measured a concentration of 266.7 ng/mL, **(B)** a 79-year-old patient suffering from severe eczema was treated with 4 mg baricitinib QD. The blood sample was collected 6 h after the last intake and the concentration measured was 23.0 ng/mL, **(C)** a polymedicated 76-year-old patient with unstable myelofibrosis (hepatic, renal, and cardiac dysfunction) treated with fedratinib 100 mg QD. The blood sample was collected 1 h after the last intake. TDM showed a fedratinib plasma concentration of 346.9 ng/mL, **(D)** a 55-year-old patient with stable graft versus host disease treated with ruxolitinib 10 mg twice daily (BID). The measured concentration was 4.5 ng/mL, 11 h after the last intake **(E)** a 33-year-old patient with sarcoidosis treated with tofacitinib 5 mg BID. The concentration measured 10 h after the last dose intake was 5.2 ng/mL, **(F)** a 46-year-old patient diagnosed with stable atopic dermatitis and vitiligo treated with upadacitinib 15 mg QD is shown. Blood sample was collected 4 h after the last dose intake and the concentration measured was 42.3 ng/mL. The respective internal standards are not shown in the figure for clarity.

the nominal concentration. Intercepts were comprised between −0.20 and −0.03 and the correlation coefficient (R^2) was higher than 0.99 for all calibration curves for the six JAKi and for the 3 days of validation.

The validated calibration ranges that have been selected vary between 0.5 and 200 ng/mL for abrocitinib, baricitinib, upadacitinib; 0.5 to 400 ng/mL for ruxolitinib; 1 to 400 ng/mL for tofacitinib; and 10 to 800 ng/mL for fedratinib. Trueness, repeatability, and intermediate precision were comprised between 91.1 and 113.5%, 3.0–9.9%, and 4.5–11.3% respectively, and were therefore suitable for the quantification of clinically relevant plasma levels of the six considered JAKi.

The accuracy profiles determined for abrocitinib, baricitinib, fedratinib, ruxolitinib, tofacitinib, and upadacitinib are shown in the Fig. 3 and the β-expectation tolerance interval lies within the acceptance limits of ± 30% for biological samples [74]. The LLOQ and the upper limit of quantification (ULOQ) were then defined as the highest and lowest concentrations of the calibration samples. As the lowest concentration of abrocitinib and baricitinib calibration samples lied close to the acceptance limit of +30%, the concentration of 0.5 ng/mL was considered as the LLOQ (Table 5).

3.2.5. Limits of detection

The LOD determined in human plasma was 0.25 ng/mL for abrocitinib and baricitinib, 0.5 ng/mL for fedratinib, 0.05 ng/mL for ruxolitinib and upadacitinib, and 0.1 ng/mL for tofacitinib.

3.2.6. Integrity to dilution

A dilution integrity experiment was performed to ensure the reliability of sample concentrations exceeding the ULOQ values. A 10-fold dilution by blank plasma and water gave appropriate results and were considered as suitable. The average bias was 2.1% for abrocitinib, 5.4% for baricitinib, 8.7% for fedratinib, 8.1% for ruxolitinib, 4.0% for tofacitinib, and 5.4% for upadacitinib.

3.2.7. Stability studies

Short term stability results showed that abrocitinib, fedratinib, tofacitinib, and upadacitinib were stable for 96 h in whole blood or in plasma samples at +4 °C or at room temperature. Similar results were obtained for baricitinib except at low concentrations where it was only stable for 48 h at +4 °C in whole blood samples. Ruxolitinib was stable in plasma, (+4 °C and room temperature) up to 96 h. In whole blood, later compound was stable at +4 °C but was unstable at room temperature after 6 h (Table 6). Finally, after three freeze–thaw cycles, no significant degradation was observed for any JAKi (remaining within −15% of T_0 values). After subjecting abrocitinib, baricitinib, fedratinib, ruxolitinib, tofacitinib, and upadacitinib to medium-term stability studies, it was observed that their concentration remained unchanged following one month storage at both −20 °C and −80 °C (data not shown). Finally, all analytes were stable in HPLC vials kept in the autosampler for 72 h at +4 °C after the extraction procedure.

3.3. Clinical application

The LC-MS/MS method has been successfully applied to the analyses of 78 patients' samples receiving one of the six JAKi. The measured concentrations were in line with those reported in the literature and were within the calibration range. Two ruxolitinib concentrations from the same patient were inferior to the LLOQ. However, these low concentrations were mostly attributed to the fact that the patient was on a

very low dosage (5 mg twice a week). Two peak concentrations of abrocitinib measured in samples from the same patient were also above the range of calibration (240.5 ng/mL and 266.7 ng/mL).

Typical chromatographic profiles of patients treated with JAKi are shown in the Fig. 4. Of note, no additional peaks were observed in these selected *m/z* transition, even during prolonged series of patients' samples analyses, highlighting the very high selectivity of the proposed LC-MS/MS method.

4. Conclusion

A sensitive and selective HPLC-MS/MS method was developed to quantify the six currently used JAKi in human plasma in an analytical run lasting 7 min. This assay was validated according to the international recommendations for bioanalytical assay over a large concentration range that covers the plasma concentration reported so far for abrocitinib, baricitinib, fedratinib, ruxolitinib, tofacitinib, and upadacitinib. After extensive validation, this assay is intended to be integrated in our routine TDM service. Indeed, information on circulating concentrations of JAKi will help practitioners for a better follow-up or their patients with regard to not only efficacy but also tolerability, possibly explaining some instances of less than expected clinical responses, or so far-unexplained toxicities, as well as long-term safety concerns, and their modulation by potential drug-drug interactions and pharmacogenetic traits, and finally by the fluctuating compliance to oral treatments.

CRediT authorship contribution statement

Jérémie Tachet: Conceptualization, Validation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **François Versace:** Conceptualization, Supervision, Validation, Writing – review & editing. **Thomas Mercier:** Conceptualization, Supervision, Validation, Writing – review & editing. **Thierry Buclin:** Conceptualization, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Writing – original draft. **Laurent A. Decosterd:** Conceptualization, Resources, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Eva Choong:** Writing – original draft, Resources, Writing – review & editing. **François R. Girardin:** Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2023.123917.

References

[1] S. Danese, L. Vuitton, L. Peyrin-Biroulet, Biologic agents for IBD: practical insights, Nat. Rev. Gastroenterol. Hepatol. 12 (9) (2015) 537–545.
[2] V. Strand, A. Balsa, J. Al-Saleh, L. Barile-Fabris, T. Horiuchi, T. Takeuchi, et al., Immunogenicity of Biologics in Chronic Inflammatory Diseases: A Systematic Review, BioDrugs 31 (4) (2017) 299–316.
[3] S. Alexandre, K. Andreas, B. Sytske Anne, S.S. Josef, H. Désirée van der, C. Roberto, et al., Safety of synthetic and biological DMARDs: a systematic literature review informing the 2022 update of the EULAR recommendations for the management of rheumatoid arthritis, Ann. Rheum. Dis. 82 (1) (2023) 107.
[4] P. Prasad, S. Verma, G.NK. Surbhi, V. Chaturvedi, S.A. Mittal, Rheumatoid arthritis: advances in treatment strategies, Mol. Cell. Biochem. 478 (1) (2023) 69–88.
[5] R. Zeiser, N. von Bubnoff, J. Butler, M. Mohty, D. Niederwieser, R. Or, et al., Ruxolitinib for Glucocorticoid-Refractory Acute Graft-versus-Host Disease, N. Engl. J. Med. 382 (19) (2020) 1800–1810.
[6] R. Zeiser, N. Polverelli, R. Ram, S.K. Hashmi, R. Chakraverty, J.M. Middeke, et al., Ruxolitinib for Glucocorticoid-Refractory Chronic Graft-versus-Host Disease, N. Engl. J. Med. 385 (3) (2021) 228–238.
[7] T. Bieber, E.L. Simpson, J.I. Silverberg, D. Thaçi, C. Paul, A.E. Pink, et al., Abrocitinib versus Placebo or Dupilumab for Atopic Dermatitis, N. Engl. J. Med. 384 (12) (2021) 1101–1112.
[8] P.C. Taylor, E.C. Keystone, D. van der Heijde, M.E. Weinblatt, M.L. Del Carmen, J. Reyes Gonzaga, et al., Baricitinib versus Placebo or Adalimumab in Rheumatoid Arthritis, N. Engl. J. Med. 376 (7) (2017) 652–662.
[9] C. Traboulsi, F. Ayoub, A. Silfen, T.G. Rodriguez, D.T. Rubin, Upadacitinib Is Safe and Effective for Crohn's Disease: Real-World Data from a Tertiary Center, Dig. Dis. Sci. 68 (2) (2023) 385–388.
[10] E.V. Loftus, J. Panés, A.P. Lacerda, L. Peyrin-Biroulet, G. D'Haens, R. Panaccione, et al., Upadacitinib Induction and Maintenance Therapy for Crohn's Disease, N. Engl. J. Med. 388 (21) (2023) 1966–1980.
[11] B. Barberio, D.J. Gracie, C.J. Black, A.C. Ford, Efficacy of biological therapies and small molecules in induction and maintenance of remission in luminal Crohn's disease: systematic review and network meta-analysis, Gut 72 (2) (2023) 264.
[12] Ponce-Bobadilla AV, Stodtmann S, Eckert D, Zhou W, Liu W, Mohamed MA-O. Upadacitinib Population Pharmacokinetics and Exposure-Response Relationships in Ulcerative Colitis Patients. 2022(1179-1926 (Electronic)).
[13] E. Guttman-Yassky, D. Thaci, A.L. Pangan, H.C. Hong, K.A. Papp, K. Reich, et al., Upadacitinib in adults with moderate to severe atopic dermatitis: 16-week results from a randomized, placebo-controlled trial, J. Allergy Clin. Immunol. 145 (3) (2020) 877–884.
[14] E. Muensterman, B. Engelhardt, S. Gopalakrishnan, J.K. Anderson, M.F. Mohamed, Upadacitinib pharmacokinetics and exposure-response analyses of efficacy and safety in psoriatic arthritis patients - Analyses of phase III clinical trials, Clin. Transl. Sci. 15 (1) (2022) 267–278.
[15] O. Kwon, M.M. Senna, R. Sinclair, T. Ito, Y. Dutronc, C.-Y. Lin, et al., Efficacy and Safety of Baricitinib in Patients with Severe Alopecia Areata over 52 Weeks of Continuous Therapy in Two Phase III Trials (BRAVE-AA1 and BRAVE-AA2), Am. J. Clin. Dermatol. 24 (3) (2023) 443–451.
[16] Pfizer. Summary of product characteristics - Cibinqo. European Medicines Agency (EMA); 2021. Contract No.: EMEA/H/C/005452.
[17] EEIG BMSP. Summary of product characteristics - Inrebic. European Medicines Agency (EMA); 2021. Report No.: EMEA/H/C/005026 - IAIN/0009.
[18] B.V. ELN. Summary of product characteristics - Olumiant. European Medicines Agency (EMA); 2022. Report No.: EMEA/H/C/004085 - II/0029/G.
[19] Europharm N. Summary of product characteristics - Jakavi. European Medicines Agency (EMA); 2022. Report No.: EMEA/H/C/002464 - II/0053.
[20] Europe P. Summary of product characteristics - Xeljanz. European Medicines Agency (EMA); 2022. Report No.: EMEA/H/C/004214 - II/0044.
[21] AbbVie. Summary of product characteristics - Rinvoq. European Medicines Agency (EMA); 2022. Report No.: EMEA/H/C/004760 - II/0016.
[22] J. Tachet, A. Dumusc, C. Conrad, F. Grandoni, Y. Chalandon, C. Ribi, et al., Janus kinase inhibitors : new perspectives for precision medicine ? Rev. Med. Suisse 8 (800) (2022) 1979–1983.
[23] S.R. Ytterberg, D.L. Bhatt, T.R. Mikuls, G.G. Koch, R. Fleischmann, J.L. Rivas, et al., Cardiovascular and Cancer Risk with Tofacitinib in Rheumatoid Arthritis, N. Engl. J. Med. 386 (4) (2022) 316–326.
[24] C.A. Salinas, A. Louder, J. Polinski, T.C. Zhang, H. Bower, S. Phillips, et al., Evaluation of VTE, MACE, and Serious Infections Among Patients with RA Treated with Baricitinib Compared to TNFi: A Multi-Database Study of Patients in Routine Care Using Disease Registries and Claims Databases, Rheumatol Ther. 10 (1) (2023) 201–223.
[25] T. Buclin, Y. Thoma, N. Widmer, P. André, M. Guidi, C. Csajka, et al., The Steps to Therapeutic Drug Monitoring: A Structured Approach Illustrated With Imatinib, Front. Pharmacol. 11 (2020) 177.
[26] K. Moran, K. Null, Z. Huang, T. Lissoos, S. Kane, Retrospective Claims Analysis Indirectly Comparing Medication Adherence and Persistence Between Intravenous Biologics and Oral Small-Molecule Therapies in Inflammatory Bowel Diseases, Adv. Ther. 36 (9) (2019) 2260–2272.

[27] C. Lenoir, V. Rollason, J.A. Desmeules, C.F. Samer, Influence of Inflammation on Cytochromes P450 Activity in Adults: A Systematic Review of the Literature, Front. Pharmacol. 12 (2021), 733935.

[28] F. Girardin, Y. Daali, M. Gex-Fabry, M. Rebsamen, P. Roux-Lombard, A. Cerny, et al., Liver kidney microsomal type 1 antibodies reduce the CYP2D6 activity in patients with chronic hepatitis C virus infection: Metabolic impact of autoantibodies in HCV, J. Viral Hepat. 19 (8) (2012) 568–573.

[29] V. Aslanis, K. Umehara, F. Huth, T. Ouatas, S. Bharathy, A.A. Butler, et al., Multiple administrations of fluconazole increase plasma exposure to ruxolitinib in healthy adult subjects, Cancer Chemother. Pharmacol. 84 (4) (2019) 749–757.

[30] V. Veeravalli, R.P. Dash, J.A. Thomas, R.J. Babu, L.M.V. Madgula, N.R. Srinivas, Critical Assessment of Pharmacokinetic Drug-Drug Interaction Potential of Tofacitinib, Baricitinib and Upadacitinib, the Three Approved Janus Kinase Inhibitors for Rheumatoid Arthritis Treatment, Drug Saf. 43 (8) (2020) 711–725.

[31] M.-E.-F. Mohamed, B. Klünder, A.A. Othman, Clinical Pharmacokinetics of Upadacitinib: Review of Data Relevant to the Rheumatoid Arthritis Indication, Clin. Pharmacokinet. 59 (5) (2020) 531–544.

[32] X. Wang, M.E. Dowty, A. Wouters, S. Tatulych, C.A. Connell, V.H. Le, et al., Assessment of the Effects of Inhibition or Induction of CYP2C19 and CYP2C9 Enzymes, or Inhibition of OAT3, on the Pharmacokinetics of Abrocitinib and Its Metabolites in Healthy Individuals, Eur. J. Drug Metab. Pharmacokinet. 47 (3) (2022) 419–429.

[33] E.Q. Wang, V. Le, J.A. Winton, S. Tripathy, S. Raje, L. Wang, et al., Effects of Renal Impairment on the Pharmacokinetics of Abrocitinib and Its Metabolites, J. Clin. Pharmacol. 62 (4) (2022) 505–519.

[34] A. Walton, J. Paik, A. Quebe, C.L. Kannowski, C. Choong, S. Anderson, et al., Frequency of Prescription Claims for Drugs that May Interact with Janus Kinase Inhibitors Among Patients with Rheumatoid Arthritis in the US, Rheumatol. Therapy. 8 (1) (2021) 599–607.

[35] N.H.G. Holford, L.B. Sheiner, Kinetics of pharmacologic response, Pharmacol. Ther. 16 (2) (1982) 143–166.

[36] L.B. Sheiner, T.M. Ludden, Population pharmacokinetics/dynamics, Annu. Rev. Pharmacol. Toxicol. 32 (1992) 185–209.

[37] C.N. Harrison, N. Schaap, A.M. Vannucchi, J.-J. Kiladjian, R.V. Tiu, P. Zachee, et al., Janus kinase-2 inhibitor fedratinib in patients with myelofibrosis previously treated with ruxolitinib (JAKARTA-2): a single-arm, open-label, non-randomised, phase 2, multicentre study, The Lancet Haematol. 4 (7) (2017) e317–e324.

[38] D. Koller, V. Vaitsekhovich, C. Mba, J.L. Steegmann, P. Zubiaur, F. Abad-Santos, et al., Effective quantification of 11 tyrosine kinase inhibitors and caffeine in human plasma by validated LC-MS/MS method with potent phospholipids clean-up procedure Application to therapeutic drug monitoring, Talanta. 208 (2020), 120450.

[39] C. Pressiat, H.-H. Huynh, A. Plé, H. Sauvageon, I. Madelaine, C. Chougnet, et al., Development and Validation of a Simultaneous Quantification Method of Ruxolitinib, Vismodegib, Olaparib, and Pazopanib in Human Plasma Using Liquid Chromatography Coupled With Tandem Mass Spectrometry, Ther. Drug Monit. 40 (3) (2018).

[40] B. Llopis, P. Robidou, N. Tissot, B. Pinna, P. Gougis, F.C. Aubart, et al., Development and clinical validation of a simple and fast UPLC-ESI-MS/MS method for simultaneous quantification of nine kinase inhibitors and two antiandrogen drugs in human plasma: Interest for their therapeutic drug monitoring, J. Pharm. Biomed. Anal. 197 (2021), 113968.

[41] Y. Liu, Z. He, H. Liang, M. Han, J. Wang, Q. Liu, et al., A high-throughput UHPLC-MS/MS method for the determination of eight anti-tumor drugs in plasma, Anal. Biochem. 676 (2023), 115230.

[42] K.D. Bharwad, P.A. Shah, P.S. Shrivastav, P. Singhal, Development and validation of a rapid and sensitive UPLC–MS/MS assay for the quantification of tofacitinib in human plasma, Biomed. Chromatogr. 33 (4) (2019) e4458.

[43] J. Martens-Lobenhoffer, S. Tomaras, E. Feist, S.M. Bode-Böger, Quantification of the janus kinase 1 inhibitor upadacitinib in human plasma by LC-MS/MS, J. Chromatogr. B 1188 (2022), 123076.

[44] A.S. Abdelhameed, M.W. Attwa, A.A. Kadi, An LC–MS/MS method for rapid and sensitive high-throughput simultaneous determination of various protein kinase inhibitors in human plasma, Biomed. Chromatogr. 31 (2) (2017) e3793.

[45] S. Tripathy, D. Wentzel, X. Wan, O. Kavetska, Validation of enantioseparation and quantitation of an active metabolite of abrocitinib in human plasma, Bioanalysis 13 (19) (2021) 1477–1486.

[46] ICH guideline M10 on bioanalytical method validation and study sample analysis, (2022).

[47] X. Zhao, X.Y. Sheng, C.D. Payne, X. Zhang, F. Wang, Y.M. Cui, Pharmacokinetics, Safety, and Tolerability of Single- and Multiple-Dose Once-Daily Baricitinib in Healthy Chinese Subjects: A Randomized Placebo-Controlled Study, Clin. Pharmacol. Drug Dev. 9 (8) (2020) 952–960.

[48] C. Vong, S.W. Martin, C. Deng, R. Xie, K. Ito, C. Su, et al., Population Pharmacokinetics of Tofacitinib in Patients With Moderate to Severe Ulcerative Colitis, Clin. Pharmacol. Drug Dev. 10 (3) (2021) 229–240.

[49] Corporation I. Clinical Pharmacology and Biopharmaceutics review(s) - Jakafi. Food and Drug Administration (FDA); 2011. Report No.: 202192Orig1s000.

[50] Lilly E. Clinical Pharmacology and Biopharmaceutics review(s) - Olumiant. Food and Drug Administration (FDA); 2017. Report No.: 207924Orig1s000.

[51] J. Wojciechowski, B.K. Malhotra, X. Wang, L. Fostvedt, H. Valdez, T. Nicholas, Population Pharmacokinetics of Abrocitinib in Healthy Individuals and Patients with Psoriasis or Atopic Dermatitis, Clin. Pharmacokinet. 61 (5) (2022) 709–723.

[52] X. Zhang, L. Chua, C. Ernest Ii, W. Macias, T. Rooney, L.S. Tham, Dose/Exposure-Response Modeling to Support Dosing Recommendation for Phase III Development of Baricitinib in Patients with Rheumatoid Arthritis. CPT: Pharmacometrics & Systems, Pharmacology 6 (12) (2017) 804–813.

[53] K. Ogasawara, S. Zhou, G. Krishna, M. Palmisano, Y. Li, Population pharmacokinetics of fedratinib in patients with myelofibrosis, polycythemia vera, and essential thrombocythemia, Cancer Chemother. Pharmacol. 84 (4) (2019) 891–898.

[54] X. Chen, W.V. Williams, V. Sandor, S. Yeleswaram, Population Pharmacokinetic Analysis of Orally-Administered Ruxolitinib (INCB018424 Phosphate) in Patients With Primary Myelofibrosis (PMF), Post-Polycythemia Vera Myelofibrosis (PPV-MF) or Post-Essential Thrombocythemia Myelofibrosis (PET MF), J. Clin. Pharmacol. 53 (7) (2013) 721–730.

[55] B. Klünder, M.-E.-F. Mohamed, A.A. Othman, Population Pharmacokinetics of Upadacitinib in Healthy Subjects and Subjects with Rheumatoid Arthritis: Analyses of Phase I and II Clinical Trials, Clin. Pharmacokinet. 57 (8) (2018) 977–988.

[56] B. Klünder, R.K. Mittapalli, M.-E.-F. Mohamed, A. Friedel, P. Noertersheuser, A. A. Othman, Population Pharmacokinetics of Upadacitinib Using the Immediate-Release and Extended-Release Formulations in Healthy Subjects and Subjects with Rheumatoid Arthritis: Analyses of Phase I-III Clinical Trials, Clin. Pharmacokinet. 58 (8) (2019) 1045–1058.

[57] A. Nader, S. Stodtmann, A. Friedel, M.-E.-F. Mohamed, A.A. Othman, Pharmacokinetics of Upadacitinib in Healthy Subjects and Subjects With Rheumatoid Arthritis, Crohn's Disease, Ulcerative Colitis, or Atopic Dermatitis: Population Analyses of Phase 1 and 2 Clinical Trials, J. Clin. Pharmacol. 60 (4) (2020) 528–539.

[58] Pfizer. Clinical Pharmacology and Biopharmaceutics review(s) - Xeljanz. Food and Drug Administration (FDA); 2011. Report No.: 203214Orig1s000.

[59] P. Hubert, P. Chiap, J. Crommen, B. Boulanger, E. Chapuzet, N. Mercier, et al., The SFSTP guide on the validation of chromatographic methods for drug bioanalysis: from the Washington Conference to the laboratory, Anal. Chim. Acta 391 (2) (1999) 135–148.

[60] E. Chapuzet, N. Mercier, S. Bervoas-Martin, B. Boulanger, P. Chevalier, P. Chiap, et al., Méthodes chromatographiques de dosage dans les milieux biologiques : stratégie de validation - Rapport d'une commission SFSTP, STP Pharma Pratiques. 7 (3) (1997) 169.

[61] R.-a. Xu, Q. Lin, X. Qiu, J. Chen, Y. Shao, G. Hu, et al., UPLC-MS/MS method for the simultaneous determination of imatinib, voriconazole and their metabolites concentrations in rat plasma, J. Pharm. Biomed. Anal. 166 (2019) 6–12.

[62] C. Tang, X. Niu, L. Shi, H. Zhu, G. Lin, Xu R-a. In vivo Pharmacokinetic Drug-Drug Interaction Studies Between Fedratinib and Antifungal Agents Based on a Newly Developed and Validated UPLC/MS-MS Method, Front. Pharmacol. 11 (2021).

[63] Y. Zhang, Y.-n. Liu, S. Xie, X. Xu, R.-a. Xu, Evaluation of the inhibitory effect of quercetin on the pharmacokinetics of tucatinib in rats by a novel UPLC–MS/MS assay, Pharm. Biol. 60 (1) (2022) 621–626.

[64] P. Hubert, J.J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, et al., Harmonization of strategies for the validation of quantitative analytical procedures: A SFSTP proposal—part I, J. Pharm. Biomed. Anal. 36 (3) (2004) 579–586.

[65] B. Boulanger, P. Chiap, W. Dewe, J. Crommen, P. Hubert, An analysis of the SFSTP guide on validation of chromatographic bioanalytical methods: progresses and limitations, J. Pharm. Biomed. Anal. 32 (4) (2003) 753–765.

[66] E. Rozet, A. Ceccato, C. Hubert, E. Ziemons, R. Oprean, S. Rudaz, et al., Analysis of recent pharmaceutical regulatory documents on analytical method validation, J. Chromatogr. A 1158 (1) (2007) 111–125.

[67] E. Rozet, C. Hubert, A. Ceccato, W. Dewé, E. Ziemons, F. Moonen, et al., Using tolerance intervals in pre-study validation of analytical methods to predict in-study results: The fit-for-future-purpose concept, J. Chromatogr. A 1158 (1) (2007) 126–137.

[68] D. Hoffman, R. Kringle, A Total Error Approach for the Validation of Quantitative Analytical Methods, Pharm. Res. 24 (6) (2007) 1157–1164.

[69] M. Feinberg, Validation of analytical methods based on accuracy profiles, J. Chromatogr. A 1158 (1) (2007) 174–183.

[70] S. Rudaz, M. Feinberg, From method validation to result assessment: Established facts and pending questions, TrAC Trends Anal. Chem. 105 (2018) 68–74.

[71] C.T. Viswanathan, S. Bansal, B. Booth, A.J. DeStefano, M.J. Rose, J. Sailstad, et al., Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays, Pharm. Res. 24 (10) (2007) 1962–1973.

[72] M. Feinberg, B. Boulanger, W. Dewé, P. Hubert, New advances in method validation and measurement uncertainty aimed at improving the quality of chemical data, Anal. Bioanal. Chem. 380 (3) (2004) 502–514.

[73] A.-L. Gassner, J. Schappler, M. Feinberg, S. Rudaz, Derivation of uncertainty functions from validation studies in biological fluids: Application to the analysis of caffeine and its major metabolites in human plasma samples, J. Chromatogr. A 1353 (2014) 121–130.

[74] Bioanalytical Method Validation - Guidance for Industry, (2018).

[75] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the Assessment of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC−MS/MS, Analytical Chemistry 75 (13) (2003) 3019–3030.