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

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Abstract

Kinase inhibitors (KIs) and antiandrogen drugs (AAs) are oral anticancer drugs with narrow therapeutic index that exhibit high inter- and intra-individual variability. We describe here a UPLC-MS/MS method for the simultaneous quantification of nine KIs: cobimetinib, dasatinib, ibrutinib, imatinib, nilotinib, palbociclib, ruxolitinib, sorafenib and vemurafenib; two active metabolites of them: N-desmethyl imatinib, N-oxide sorafenib; and two AAs: abiraterone and enzalutamide; with short pre-treatment and run time in order to be easily used in clinical practice for their therapeutic drug monitoring (TDM) and facilitating pharmacokinetics and pharmacokinetics/pharmacodynamics studies. Plasma samples were prepared by a single-step protein precipitation. Analytes were separated on a Waters Acquity UPLC® T3 HSS C18 column by non-linear gradient elution; with subsequent detection by Xevo® TQD triple quadrupole tandem mass spectrometer in a positive ionization mode. Analysis time was 2.8 minutes per run, and all analytes eluted within 1.46-1.97 minutes. The analytical performance of the method in terms of specificity, sensitivity, linearity, precision, accuracy, matrix effect, extraction recovery, limit of quantification, dilution integrity and stability of analytes under different conditions met all criteria for a bioanalytical method for the quantification of drugs. The calibration curves were linear over the range of 1-500 ng/mL for abiraterone, dasatinib and ibrutinib; 5-500 ng/mL for cobimetinib and palbociclib; 10-5,000 ng/mL for imatinib, N-desmethyl imatinib, nilotinib, sorafenib, N-oxide sorafenib and ruxolitinib; 100-50,000 ng/mL for enzalutamide and 100-100,000 ng/mL for vemurafenib with coefficient of correlation above 0.995 for all analytes. This novel method was successfully applied to TDM in clinical practice.

Keywords: liquid chromatography, mass spectrometry, therapeutic drug monitoring, kinase inhibitors, antiandrogens, oral targeted therapies.

1. Introduction

Kinase inhibitors (KIs) such as: cobimetinib, dasatinib, ibrutinib, imatinib, nilotinib, palbociclib, ruxolitinib, sorafenib and vemurafenib; and antiandrogen drugs (AAs) such as: abiraterone acetate and enzalutamide; both belonging to the class of oral targeted therapies characterized by high specificity for single or multiple key biological pathways responsible or implicated in the cancer process.

KIs target molecular aberrations of cancer cells by blocking intracellular signals driving proliferation in malignant cells [1]. They have an important activity on many types of kinases (tyrosine or serine/threonine) involved in tumour growth, angiogenesis, and metastatic progression of cancer [2]. KIs, analysed in this study, are used for treating various haematological malignancies: dasatinib, ibrutinib, imatinib, nilotinib; and solid tumours including gastrointestinal stromal tumours: imatinib; advanced renal cell carcinoma: sorafenib; breast cancer: palpociclib; hepatocellular carcinoma: sorafenib; melanoma and erdheim-chester disease: vemurafenib and cobimetinib [3]. Furthermore, ruxolitinib have been approved for use in the treatment of myelofibrosis and graft versus host disease.

Abiraterone acetate and enzalutamide are both oral antiandrogen drugs approved for treatment of metastatic prostate cancer. Both drugs inhibit tumour growth effects of androgens. Abiraterone inhibits the production of adrenal androgens, whereas enzalutamide functions as an androgen receptor signalling inhibitor [4].

Oral administration of these drugs is associated with a better quality of life but patients prescribed oral therapies struggle with adherence [3]. Moreover, these molecules display large pharmacokinetics (PK) variability. Indeed, they are metabolized mostly by cytochromes P450 3A4 [5], whose activity is known to present a large inter-individual variability and to be influenced by environmental factors such as food or drug-induced interactions [6]. Likewise, inherent factors such as age, gender, medical conditions or genetics contribute to this

variability [3]. A given dose can therefore yield very different exposure levels, favouring the selection of resistant cellular clones in case of sub-therapeutic drug exposure or increasing the risk of adverse reactions at excessive plasma levels. Targets, cancers indication and potential effect of food and drug-drug interaction in their PK are summarized in Table 1 for each drug analysed in this study.

In parallel with this PK variability, some of these drugs display an exposure-response relationship [7]. For example; sorafenib, palbociclib and imatinib show an exposure-toxicity relationship [8–10]. Similarly; abiraterone, enzalutamide, vemurafenib and dasatinib show an exposure-efficacy relationship [11–13]. Furthermore, cardiovascular toxicities associated with most of these latter drugs have an exposure-toxicity relationship yet to be explored [14–17].

The poor adherence, the PK variability and the pharmacokinetics/pharmacodynamics (PK/PD) relationship of these molecules suggest the potential interest of their therapeutic drug monitoring (TDM) [3]. It has been established that the therapeutic use of targeted anticancer drugs could be optimized by an individualization of their dosage, based on plasma concentrations measurement. Target concentrations as well as efficacy and/or toxicity thresholds have been proposed for some molecules although there is currently no consensus [7].

We describe here a rapid, selective, sensitive and simple UPLC-MS/MS method for the simultaneous analysis, in small volume of plasma, of nine KIs: cobimetinib, dasatinib, ibrutinib, imatinib, nilotinib, palbociclib, ruxolitinib, sorafenib and vemurafenib; two of their active metabolites: N-desmethyl imatinib, N-oxide sorafenib; and two AAs: abiraterone and enzalutamide to enable their TDM and support PK studies and research protocols for molecule for which PK/PD relationship needs to be characterized.

2. Materials and methods

2.1. Chemical and reagents

Abiraterone (ABIRA), enzalutamide (ENZA), ibrutinib (IBRU), imatinib (IMA), nilotinib (NILO), N-oxide sorafenib (NO-SORA), palbociclib (PALBO), ruxolitinib (RUXO), sorafenib (SORA), [$^{2}H_4$]-abiraterone (d4-ABIRA), [$^{13}C_6$]-cobimetinib ($^{13}C_6$-COBI), [$^{2}H_5$]-ibrutinib (d5-IBRU), [$^{2}H_8$]-imatinib (d8-IMA), [^{13}C,$^{2}H_3$]-nilotinib (d3-NILO), [$^{2}H_3$]-N-oxide sorafenib (d3-NO-SORA), [$^{2}H_8$]-palbociclib (d8-PALBO), [$^{2}H_9$]-ruxolitinib (d9-RUXO) and [^{13}C,$^{2}H_3$]-sorafenib (d3-SORA) were purchased from Alsachim® (Illkrich, France) while cobimetinib (COBI), dasatinib (DASA), N-desmethyl imatinib (DM-IMA), vemurafenib (VEMU), [$^{2}H_8$]-dasatinib (d8-DASA) and [$^{2}H_6$]-vemurafenib (d6-VEMU) were purchased from LGC® (Augsburg, Germany). The chemical structures of analytes (except metabolites) are shown in Figure 1. Methanol and dimethylsulfoxyde (DMSO) were obtained from Merck® (Darmstadt, Germany). Formic acid and ammonium acetate were obtained from Sigma-Aldrich® (Munich, Germany). Zinc sulphate heptahydrate ($ZnSO_4$*$7H_2O$) was obtained from VWR® (Fontenay-sous-Bois, France). All reagents used were of the highest available analytical grades. Liquid chromatography–MS/MS grade water was purchased from a water distribution hypergrade system Purelab Flex® (ELGA®), and drug-free plasma (blank plasma) from healthy donors was supplied by the French Blood Establishment (Paris, France).

2.2. Preparation of stock solutions, standards and quality control samples

Individual stock solutions of each analyte were prepared at 1 mg/mL. Stock solutions of ABIRA, COBI, DASA, ENZA, IBRU, IMA, DM-IMA, NILO, NO-SORA, RUXO, SORA and VEMU were prepared in DMSO, while stock solution of PALBO was prepared in hydrochloric acid 0.1M. Working solutions, obtained by diluting the stock solutions with methanol, were prepared for each analyte. Calibration standard and quality control (QC)

samples were prepared in blank human plasma by spiking with an appropriate volume of each working solutions. The ranges of the different analytes covered in the current method are: 1-500 ng/mL (1-5-10-50-100-250-500) for ABIRA, DASA and IBRU; 5-500 ng/mL (5-10-50-100-250-500) for COBI and PALBO; 10-5,000 ng/mL (10-50-100-500-1,000-2,500-5,000) for IMA, DM-IMA, NILO, NO-SORA, SORA and RUXO; 100-50,000 ng/mL (100-500-2,000-10,000-20,000-50,000) for ENZA and 100-100,000 ng/mL (100-500-2,000-10,000-20,000-50,000-100,000) for VEMU. The QC samples were tested at four different concentrations: high QC (HQC: 80% of upper limit of quantification), medium QC (MQC: 50% of selected range), low QC (LQC: 2-10 times the LLOQ) and QC at LLOQ. Individual stock solutions of each isotopic internal standard (IS) were prepared in adequate solvent at 1 mg/mL. A solution of mix of each IS (ISmix) at 15 ng/mL for d4-ABIRA, d5-IBRU, d6-PALBO, d8-DASA and $^{13}C_6$-COBI; 150 ng/mL for d5-SORA, d3-NILO, d3-NO-SORA, d6-RUXO and d8-IMA; and 1,500 ng/mL for d6-ENZA and d6-VEMU was prepared in methanol. As DM-IMA and IMA display very close chemical structures and a similar chromatographic behaviour, d8-IMA was used as IS for both compounds. All stock solutions, working solutions, calibration standards, ISmix and QC samples were stored at -20°C.

2.3. Instruments and analytical conditions

Chromatography was performed on an Acquity UPLC® system (WATERS®, Milford, Massachusetts, United States) with an autosampler temperature at 10°C. Acquity UPLC® T3 HSS C18 analytical column (2.1 x 100 mm, 1.8 µm particle size) was used for chromatographic separation and column temperature was maintained at 45°C. The mobile phase had a flow rate of 0.4 mL/min with a non-linear gradient elution and the run time analysis was set at 2.8 min. The UPLC system was coupled to a triple quadrupole mass spectrometer: Xevo® TQD (WATERS®, Milford, Massachusetts, United States).

Quantifications were achieved in Multiple Reactions Monitoring (MRM) mode and electrospray ionization was operated in positive mode (ESI+) for each analyte. The source temperature and the desolvation temperature were set at 150°C and 380°C, respectively, with a desolvation gas flow of 800 L/h and a cone gas flow of 30 L/h. The capillary voltage was set at 3.0 kV. Argon was used as collision gas with a flow set at 0.22 mL/min. Chromatographic data acquisition; peak integration and quantification were performed using MassLynx® 4.2 software.

2.4. Samples pre-treatment

Sample preparation was performed by single-step protein precipitation: 100 µL of aqueous $ZnSO_4*7H_2O$ (10%, w/v; pH 5.40) and 200 µL of ISmix were added to 50 µL of human plasma, calibrator or QC samples. The mixture was vortexed for 1 min using a MixMate® Vortex Mixer (Eppendorf®, Sydney, Australia) and centrifuged for 10 min at 18,900 *g* using a Heraeus Biofuge Primo® centrifuge (Thermo Fisher Scientific®, Massachusetts, United States). Finally, the supernatant was transferred to a Waters Acquity® autosampler vial and 10 µL were injected into the LC-MS/MS system using a temperature-controlled autosampler device at 10°C.

2.5. Method validation

The validation was performed according to European Medicines Agency (EMA) guidelines and US Food and Drug Administration (FDA) guidelines for the validation of bioanalytical methods. Parameters included were selectivity, carry-over, linearity, accuracy and precision, lower limit of quantification, matrix effect, extraction recovery, stability in human plasma and dilution integrity.

2.5.1. Selectivity

Six different sources of plasma samples were tested. A selective method should not have

interference of more than 20% of the lower limit of quantification (LLOQ) of the analyte.

2.5.2. Carry-over

As our method is designed to measure very low and very high concentrations simultaneously, a carry-over test was performed. Carry-over was assessed by injecting blank samples after a high concentration calibrator. Carry-over in the blank sample following the high concentration calibrator should not be greater than 20% of the LLOQ of the analyte.

2.5.3. Linearity

Calibration curves were acquired by plotting the peak area ratio of the concentration of each analyte standard to the area of their respective isotopic IS (except for DM-IMA analyzed with d8-IMA) over the range from 1-500 ng/mL for ABIRA, DASA and IBRU; 5-500 ng/mL for COBI and PALBO; 10-5,000 ng/mL for IMA, DM-IMA, NILO, NO-SORA, RUXO and SORA; 100-50,000 ng/mL for ENZA and 100-100,000 ng/mL for VEMU. Each curve was assayed by least square weighted (1/x). Linearity was defined by a coefficient of correlation r $\geq$ 0.995.

2.5.4. Precision and accuracy

The intra-day precision and accuracy were evaluated using 6 different replicates, extracted in the same day, of QC samples at the four concentrations (LLOQ, LQC, MQC and HQC).

The inter-day precision and accuracy were determined by extracting each QC sample (LLOQ, LQC, MQC and HQC) 6 times a day over 3 different days (n = 18 replicates). The concentration of each QC levels was determined using calibration standards prepared on the same day. The precision was calculated as the coefficient of variation (CV, %) within a single run (intra-day assay) and between different runs (inter-day assay), and the accuracy as the percentage ratio of the measured and nominal concentration (mean of measured/nominal x

100). The acceptance limits were CV<15% for precision and within ± 15% of the nominal concentration for accuracy (range from 85-115%).

2.5.5. Lower limit of quantification

The lower limit of quantification (LLOQ) for analytes in human plasma samples was defined as the lowest concentration detectable with a signal-to-noise ratio of at least 10, CV<20% and accuracy of 80-120%. For each analyte, the LLOQ was selected as the lower concentration covered by the selected range.

2.5.6. Matrix effect and extraction recovery

Matrix effect (ME) and extraction recovery (ER) were assessed at three QC levels (LQC, MQC and HQC) in quintuplicate (with five different sources of plasma) for each analytes. The approach involves determination of ratio of peak areas of analyte in three different sets, one consisting of analyte standards in methanol (set A), one prepared in blank matrix extracts and spiked after extraction (set B), and one prepared in blank matrix from the same sources but spiked before extraction (set C). ME and ER were calculated by the following equations: ME (%) = B/A*100 and ER (%) = C/B*100. A value above or below 100% for the ME indicates an ionization enhancement or suppression, respectively. ME was considered negligible for a ratio ranging from 85-115% and CV<15%; ER ranging from 85-115% and CV<15% showing good efficiency of the method.

2.5.7. Stability

The stability of the analytes in plasma was tested by comparing accuracy and precision of three QC levels (LQC, MQC and HQC) kept under different storage conditions using freshly prepared calibrators. The stability of analytes was tested immediately after samples preparation (baseline) and after four conditions: short-term storage at 25°C (72h), short-term

storage at 4°C (1 week), long-term storage at -20°C (8 weeks) and after three cycles of freeze and thaw. In this later condition, samples stored for a minimum of 12h at -20°C, were kept at room temperature for at least 30min followed by freezing in -20°C for a minimum of 12h. The concentrations obtained after these different storage conditions were compared with the baseline concentration of each QC levels. All stability tests were done in quintuplicate per QC level. For each analytes, it was considered to be stable in plasma when measured concentration within ± 15% of the baseline concentration. Stability of extracts kept onboard the autosampler at 10°C during 48h was also tested.

2.5.8. Dilution integrity

The dilution integrity was examined to ascertain that an unknown sample with concentration exceeding the upper limit of compounds calibration range, could be diluted with blank matrix without influencing the accuracy and precision of the measurement. To achieve this, a sample was prepared at higher concentration (1,000 ng/mL for ABIRA, COBI, DASA, PALBO and IBRU; and 10,000 ng/mL for IMA, DM-IMA, NILO, NO-SORA, SORA and RUXO) followed by dilution (1:3) in blank plasma before extraction. Diluted sample was done in quintuplicate. The accuracy and precision of the diluted sample was not to deviate by more than 15%. We did not perform the test for ENZA and VEMU because it is unlikely that concentrations greater than 50,000 and 100,000 ng/mL, respectively, would be found in clinical practice.

2.6. Clinical application

This UPLC-MS/MS quantification method was applied to measurement of oral targeted therapies in plasma of patients. Peripheral venous blood samples were taken as part of the routine clinical care from adult cancer patients treated with oral targeted therapies to perform

TDM. Blood samples were collected into lithium heparin tubes before taking the drugs (C_{trough}) at steady state. Based on their reported Tmax, blood samples were also collected at 1h or 3 h after taking the drug (C_{max}) for RUXO and DASA, respectively, because the peak concentration are associated with clinical efficacy for both drugs. For IBRU, three successive samples were collected: at C_{trough} and 2h (C_{max}) + 4h after taking the drug (concentration in the elimination phase) to determine area under the curve (AUC). AUC was derived from plasma concentration–time data by noncompartmental method using Phoenix WinNonLin® 4.1 software (Certara, St. Louis, Missouri). $AUC_{0\text{-}24h}$ was estimated considering that, at steady state, the concentration found 24h after taking the drug was equal to the C_{trough}. Plasma samples were prepared by centrifuging collected blood samples for 5 min at 4,500 *g*. All plasma samples were frozen at -20°C until analysis, and were processed and analysed as described above. French regulations on non-interventional observational studies do not require patient's consent when analyzing data obtained from routine care. Approval for data collection was obtained from the Commission Nationale de l'Informatique et des Libertés (n°1491960v0).

3. **Results**

3.1. Optimization of LC-MS/MS conditions

Electrospray positive mode yielded a better spectrometer response than the negative mode. To achieve symmetrical peak shapes, good resolution and a short chromatographic run time, a mobile phase consisting of (A) water-formic acid (100:0.1, v/v)-ammonium acetate 2 mM (pH* 2.82) and (B) methanol-formic acid (100:0.1, v/v)-ammonium acetate 2 mM (pH* 4.30) was used in the experiments using non-linear gradient elution. Mass spectrometry parameters for the LC-MS/MS determination of each analyte and their respective IS are shown in Table 2.

3.2. Sample pre-treatment

Tandem mass spectrometry is sufficiently selective and sensitive to allow a simple and fast pre-treatment procedure as described. The efficiency of the pre-treatment was evident from high extraction recovery values and minimized matrix effects.

3.3. Method validation

3.3.1. Selectivity and carry-over

Six different sources of plasma samples without analytes but containing the following drugs: voriconazole, posaconazole, topiramate, diazepam, levetiracetam, lacosamide, clonazepam, lansoprazole, paracetamol, tramadol, furosemide, ceftriaxone, levofloxacin, rifampicin and amoxicillin were tested. These drugs were tested due to their relatively common use and for their possible concomitant administration in our cohort of patients. No interference with endogenous compounds or tested drugs was observed above 20% of the LLOQ of the analytes and with the same transitions and retention times of the studied analytes or their respective isotopic IS. All analytes and ISs were eluted within 1.45-1.97 min. The retention time of each analyte are shown in Table 2. The carry-over observed with the different analytes was less than 20% of the LLOQ [ABIRA (1.2%), COBI (0.8%), DASA (2%), ENZA (1.8%), IBRU (0.3%), IMA (0.5%), DM-IMA (0.2%), NILO (0.7%), NO-SORA (1.3%), PALBO (3.2%), RUXO (2.1%), SORA (0.7%) and VEMU (1.3%)]. Furthermore, no carry over was observed for any of the IS used.

3.3.2. Linearity

Calibration curves were linear with coefficient of correlation greater than r = 0.9972 for all analytes (ranged from 0.9972 to 0.9999). All calibrators, analyzed on seven different days, were measured with an accuracy ranged from 88-112% and coefficient of variation less than 11.6%. The linear regression equations of each analyte are shown in Table 2.

3.3.3. Accuracy and precision

Intra- and inter-day precision and accuracy outcomes of QC samples are shown in Table 3 and Table 4, respectively. The intra- and inter-day coefficients of variation ranged from 0.8% to 9.4% and from 1.4% to 12.3% respectively, for all analytes at all tested concentrations (LQC, MQC and HQC). Likewise, the inter-day accuracy ranged from 89-110%, for all the analytes at all tested concentrations (LQC, MQC and HQC).

3.3.4. Lower limit of quantification

The LLOQ was established at 1 ng/mL for ABIRA, DASA and IBRU; 5 ng/mL for COBI and PALBO; 10 ng/mL for IMA, DM-IMA, NILO, NO-SORA, SORA and RUXO; and at 100 ng/mL for ENZA and VEMU (Table 3 and 4). The chromatogram of the different analytes at their LLOQ is shown in Figure 2.

3.3.5. Matrix effect and extraction recovery

Matrix effect and extraction recovery for all the analytes ranged from 87-122% and 76-113%, respectively, and were stable over the concentration range for each of them, as shown in Table 5. The result of matrix effect indicated that there was no significant ionization suppression or maximization resulting from sample matrices. Moreover, the method resulted in high recovery value at all QCs showing good efficiency except for abiraterone (Table 5). Abiraterone showed relative low recovery in this experiment. However, this relative low recovery did not interfere significantly with the quantitative determination of abiraterone concentration in plasma, as judged by linearity, accuracy and precision.

3.3.6. Stability

Table 5 shows the stability of each analyte in plasma. All analytes were stable in plasma at 25°C up to 72h, except for IBRU for which degradation was observed after 24h. For IBRU,

we dosed each QC level, stored at 25°C, every hour for 8 consecutive hours to precisely establish the length of stability at room temperature. No degradation was observed during 8h. Therefore, IBRU degrades between 8-24h at room temperature. All analytes, except for ibrutinib, were stable in plasma at 4°C up to 1 week. Plasma stability of ibrutinib was demonstrated at 4°C up to 48h. Likewise, all analytes were stable in plasma at -20°C up to 8 weeks. Regarding freeze and thaw stability, all analytes were stable after three freeze and thaw cycles. Furthermore, after extraction, the extracts were stable for at least 48h when kept onboard the autosampler at 10°C.

3.3.7. Dilution integrity

The accuracy (% true) and precision (% CV) of the diluted samples were: ABIRA (98; 2.9), COBI (96; 3.1), DASA (100; 4.3), IBRU (95; 5.2), IMA (98; 1.6), DM-IMA (103; 3.6), NILO (100; 1.3), NO-SORA (105; 1.9), PALBO (98; 2.3), RUXO (101; 1.2) and SORA (110; 2.1).

3.4. Clinical application

This validated UPLC-MS/MS method was successfully applied to the TDM of eighty adult patients with various haematological malignancies, prostate cancer, graft versus host disease, renal carcinoma or Erdheim-Chester disease and treated with oral targeted therapies, especially ibrutinib, dasatinib, imatinib, nilotinib, sorafenib, abiraterone, enzalutamide, ruxolitinib, vemurafenib and cobimetinib which are the most frequently required as part of TDM in our hospital. The analytes were easily detected and measured in patients' plasma. The results are summarized in Table 6. Moreover, as shown in Figure 3, no interferences were observed between the studied targeted therapies and endogenous compounds or others drugs given to participating cancer patients.

4. Discussion

We describe here a method for the simultaneous quantification of nine KIs (cobimetinib, dasatinib, ibrutinib, imatinib, nilotinib, palbociclib, ruxolitinib, sorafenib and vemurafenib), and two AAs (abiraterone and enzalutamide). We specifically chose these molecules to meet the request of clinicians and because most of them have an exposure-response relationship well studied [8–13] for which an established or accepted target concentration exists [7]. Furthermore, cardiovascular toxicities associated with ibrutinib, abiraterone and enzalutamide have been demonstrated by our team [14–17] and this method will allow us to explore the possible exposure-cardiovascular toxicities relationship with these drugs.

Each KI generates an important number of metabolites that are often inactive. However, sorafenib and imatinib have active metabolites: N-oxide sorafenib and N-desmethyl imatinib, respectively, which seems relevant to quantify.

Based on the high recovery, relative low intra- and inter-day CVs, and good linearity, the present method is suitable for detection and quantification of each analyte in human plasma.

Several LC-MS/MS analytical methods have been published to quantify one or more KI but few methods quantify up to 9 KIs. Van Dyk et al. [18] described a simultaneous quantification of 18 KIs in human plasma including dasatinib, ibrutinib, imatinib, nilotinib, ruxolitinib, sorafenib and vemurafenib. Likewise, Andriamanana et al. [19] described the simultaneous analysis of 9 KIs including dasatinib, imatinib, nilotinib and sorafenib; whereas Huynh et al. [20] described a method for quantification of 14 KIs including cobimetinib, dasatinib, ibrutinib, imatinib, nilotinib, sorafenib and vemurafenib. In these three methods, the authors did not quantify the active metabolites of imatinib and sorafenib, while some studies suggest their importance for TDM. The AUC (N-oxide SORA) and the ratio [AUC (N-oxide SORA)/AUC (SORA)] seems to be reliable predictors of adverse effects [21]. Likewise, a

correlation between imatinib + N-desmethyl imatinib exposure and hematologic toxicity were showed [10]. Janssen et al. [22] have also developed a method for the quantification of 9 KIs in human plasma including only cobimetinib and palbociclib. The authors thus made a choice different from ours on the selection of the molecules to be measured. Finally, Merienne et al. [23] and Bouchet et al. [24] reported a technique quantifying 17 and 9 KIs, respectively, including dasatinib, imatinib and its metabolite, nilotinib, ruxolitinib and sorafenib but using a solid-phase extraction procedure more complex than the protein precipitation extraction used in our method. The method we developed was designed to perform therapeutic monitoring of these drugs in a routine setting. This requires that our method should be simple, fast and practical. The stringent workup for preparation of calibration and QCs plasma samples containing thirteen different analytes is counterbalanced by a simplified extraction step.

Likewise, several LC-MS/MS analytical methods have been published to quantify one or more AAs. Van Nuland et al. [25] described a method for simultaneous quantification of abiraterone, enzalutamide and their major metabolites in human plasma and Kim et al. [26] reported a method for simultaneous quantification of abiraterone, enzalutamide, N-desmethyl enzalutamide and bicalutamide. Both methods allow quantification of active metabolites of abiraterone and enzalutamide unlike our method. However both methods focus only on antiandrogen drugs. Furthermore, some studies suggest that these metabolites (N-desmethyl enzalutamide and Δ(4)-abiraterone) are unlikely to have meaningful contribution to the pharmacodynamics activity of abiraterone and enzalutamide. No exposure-response relationship was found in the PK/PD studies, which does not support the need for the monitoring of their plasma concentration in clinical practice [27,28].

Finally, there are many methods in the literature for the measurement of KIs or AAs but to our knowledge, neither combined both. Our method is the first that allows simultaneous quantification of 9 KIs, 2 metabolites of them and 2 AAs in human plasma. The measurement

of all these drugs in a single run is advantageous in light of the possible combined use of multiple KIs in future clinical practice. Indeed, several trials using combinations of KIs are listed on the United States clinical trials registration site: for example nilotinib/imatinib in gastrointestinal stromal tumours, chronic myeloid leukemia and Ph+/Bcr-Abl+ acute lymphoblastic leukemia (NCT01089595, NCT01819389), ruxolitinib/dasatinib or nilotinib in chronic myeloid leukemia (NCT03654768), cobimetinib/vemurafenib in melanoma (NCT02537600, NCT03224208). Similar to melanoma, combination approaches using cobimetinib/vemurefenib have been used successfully in Erdheim-Chester disease [29]. In addition, there is some potential for the KI/AA combination in future clinical practice: Hongxi Wu et al. [30] demonstrated in their study that sorafenib therapy improved the efficacy of enzalutamide in the castration-resistant prostate cancer (CRPC) model, indicating a promising therapeutic strategy for clinical CRPC patients, and a phase I/II study of enzalutamide with and without sorafenib in advanced hepatocellular carcinoma patients is in progress (NCT02642913). The abiraterone/dasatinib combination in men with mCRPC are also been tested [31]. Moreover, our method is one of the few to quantify palbociclib in human plasma using mass spectrometry detection. Only five recent papers describe methods for the quantification of palbociclib in human plasma using LC-MS/MS [22,32–35] while the existence of a exposure-toxicity relationship is possible [9].

Concerning the selected ranges of concentrations tested, the LLOQ of analytes was lower in some methods compared with those we used. This can be explained by the fact that their plasma volume, and their injection volume into the LC-MS/MS system were higher as compared to those we used (50 µL and 10 µL respectively). Furthermore, this parameter depends on the sensitivity of the mass spectrometer used. We estimated that it was not necessary to improve this parameter since our LLOQs are already under the measured concentration in most patients. Likewise, none of the patients' samples were measured above

the ULOQs. In addition, in case of concentrations exceeding our ULOQs, the dilution integrity test shows that the sample can be diluted in blank plasma without affecting analyte response and assay precision or accuracy. This new method has large concentration range which makes it suitable for the measurement of the maximum and minimum concentrations reported for all studied drugs and is therefore applicable to TDM.

In most published methods, the volume of plasma samples and run time analysis varies from 50-300 µL, and 5-15 min, respectively [18–20,22–26,32]. Our method is rapid with fast sample preparation and run times (2.8 min) and requires only a small volume of plasma (50 µL), which could reduce the time required for quantification of large number of samples and the blood volume collected from the patients. In the method of Jolibois et al. [33] the run time was 2.5 min but the volume of plasma samples was 150 µL. In contrast, the volume of plasma samples was 10 µL but the run time was 6.5 min in the method of Posocco et al. [34]. Furthermore, with respect to other methods, our method is the one of the few in which each analyte (except for N-desmethyl Imatinib) are analyzed with respect to their respective isotopic internal standard.

Concerning stability, we found that ibrutinib should be dispatched to the laboratory without delay due to instability at room temperature. Huynh et al. [20] showed in their study that ibrutinib was stable in plasma for at least 4h at room temperature. We showed that this was the case for at least 8h. This may be important in order to manage shipping of samples coming from other hospitals. Likewise, all analytes were stable for at least 2 months at -20°C in plasma. This is particularly important for clinical research protocols, where samples may need to be stored for a long time before they can be assayed.

Finally, the first clinical experience with the method confirms its suitability for clinical application (Table 6). We are currently working to incorporate 18 additional KIs (afatinib, alectinib, axitinib, bosutinib, brigatinib, cabozantinib, capmatinib, crizotinib, dabrafenib,

erlotinib, gefitinib, lenvatinib, osimertinib, pazopanib, ponatinib, ribociclib, trametinib, vandetanib) in order to be able to quantify most kinase inhibitors used in clinical practice to provide a TDM platform for oral targeted therapies.

5. Conclusion

We have developed and validated a rapid, sensitive, selective, accurate, precise and reliable UPLC-MS/MS method for the simultaneous quantification of nine kinase inhibitors, two of their active metabolites and two antiandrogen drugs in human plasma. This method is currently used in clinical practice for TDM of oral targeted therapies for which an established or accepted target concentration exists. In the future, this method could be adapted to incorporate additional KIs and AAs for which PK/PD relationship needs to be studied.

Credit authorship contribution statement

Benoit Llopis: Conceptualization, Validation, Visualization, Writing - original draft, Writing – review & editing
Pascal Robidou: Technical realization, review & editing
Nadine Tissot: Investigation, review & editing
Bruno Pinna: Investigation, review & editing
Paul Gougis: Investigation, review & editing
Luca Campedel: Investigation, review & editing
Fleur Cohen Aubart: Investigation, review & editing
Baptiste Abbar: Investigation, review & editing
Damien Roos Weil: Investigation, review & editing
Madalina Uzunov: Investigation, review & editing
Joseph Gligorov: Investigation, review & editing
Joe-Elie Salem: Investigation, review & editing
Christian Funck-Brentano: Investigation, Supervision, Writing – review & editing
Noël Zahr: Conceptualization, Validation, Supervision, Writing – review & editing

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.

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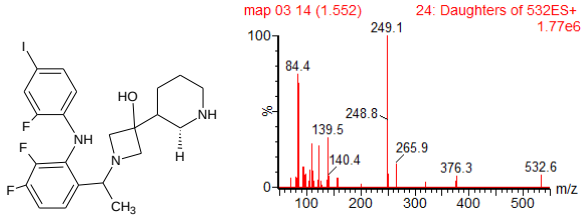

Cobimetinib

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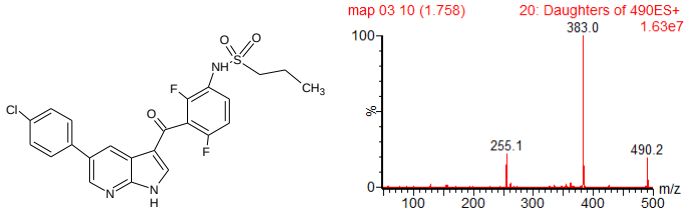
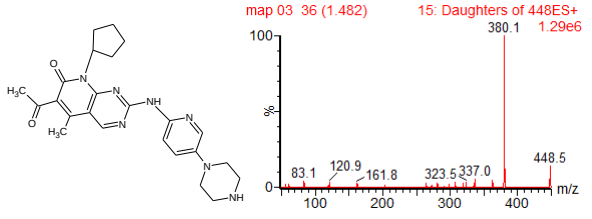
Formula Weight: 531.3530796
[M+H]+: 532.106708 Da



Vemurafenib

Molecular Formula: $C_{23}H_{18}ClF_2N_3O_3S$
Formula Weight: 489.9221264
[M+H]+: 490.079822 Da



Palbociclib

Molecular Formula: $C_{24}H_{29}N_7O_2$
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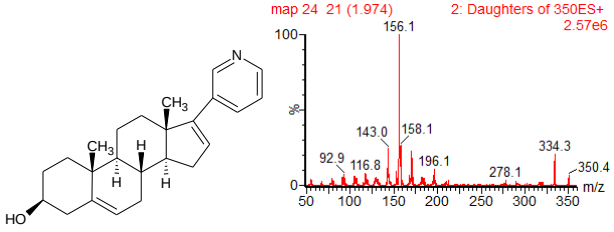
[M+H]+: 448.24555 Da



Abiraterone

Molecular Formula: $C_{24}H_{31}NO$
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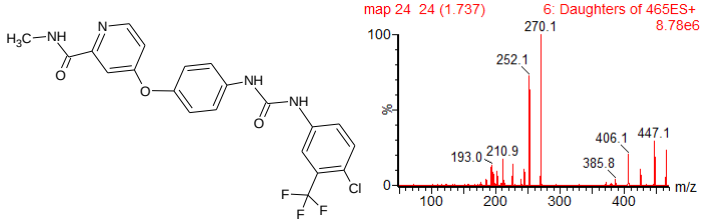
[M+H]+: 350.247841 Da



Sorafenib

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Formula Weight: 464.8249496
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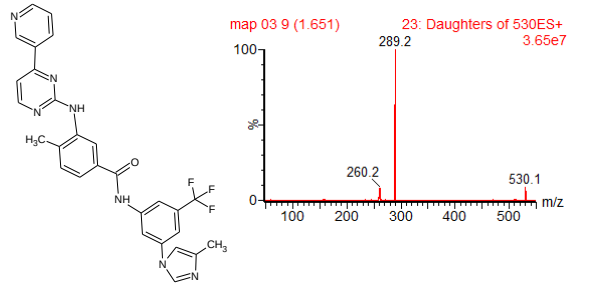

Nilotinib

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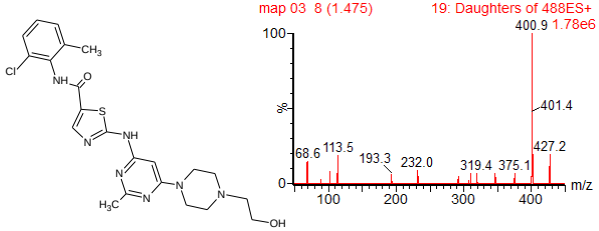

Dasatinib

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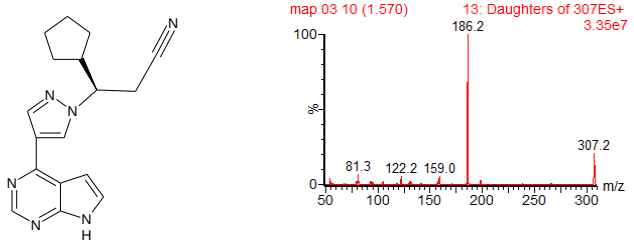

Ruxolitinib

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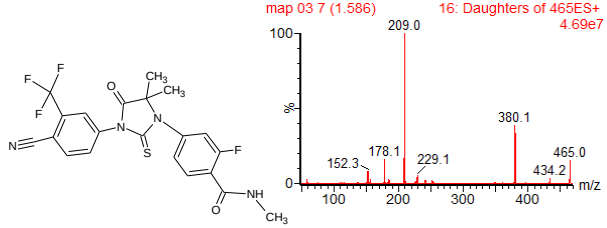
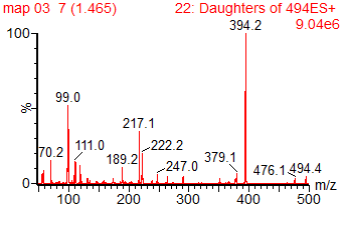


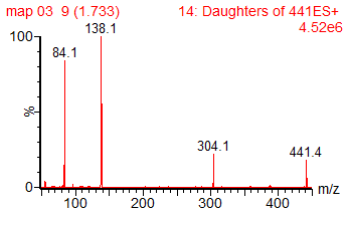
Enzalutamide

Molecular Formula: $C_{21}H_{16}F_4N_4O_2S$
Formula Weight: 464.4359528
[M+H]+: 465.100285 Da



Imatinib

Molecular Formula: $C_{29}H_{31}N_7O$
Formula Weight: 493.60274
[M+H]+: 494.266285 Da



Ibrutinib

Molecular Formula: $C_{25}H_{24}N_6O_2$
Formula Weight: 440.49706
[M+H]+: 441.20335 Da

Figure 1. Chemical structures of analytes and theoretical characteristics relevant to their detection by mass spectrometry.

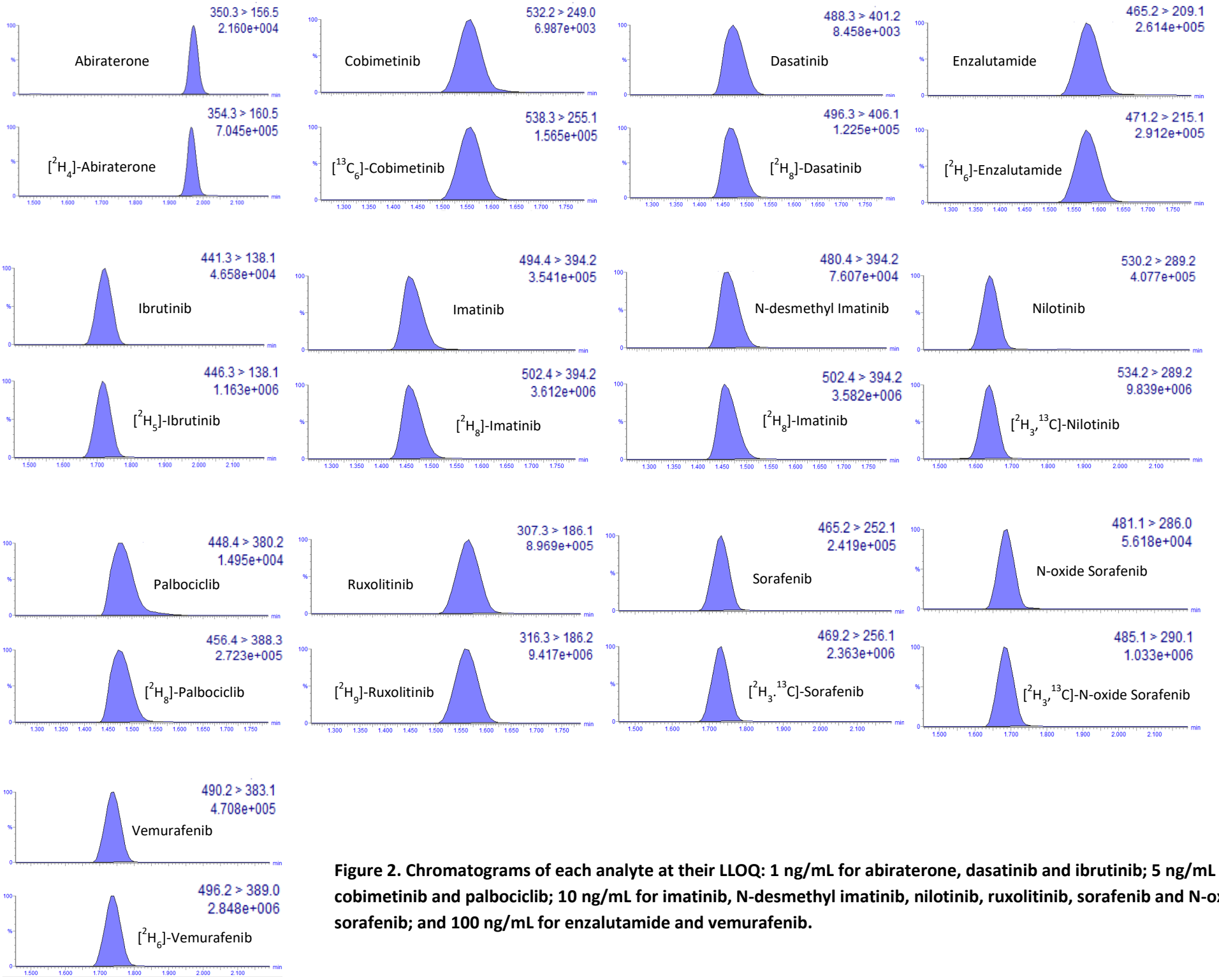
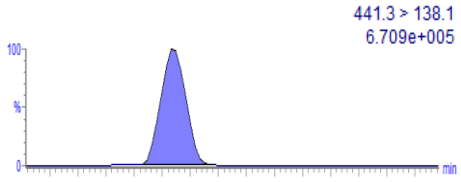
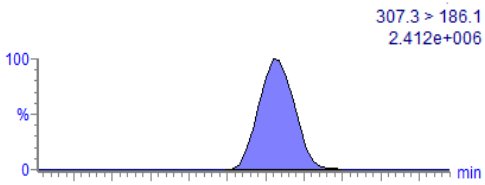


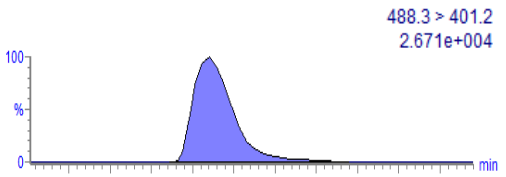
Figure 2. Chromatograms of each analyte at their LLOQ: 1 ng/mL for abiraterone, dasatinib and ibrutinib; 5 ng/mL for cobimetinib and palbociclib; 10 ng/mL for imatinib, N-desmethyl imatinib, nilotinib, ruxolitinib, sorafenib and N-oxide sorafenib; and 100 ng/mL for enzalutamide and vemurafenib.



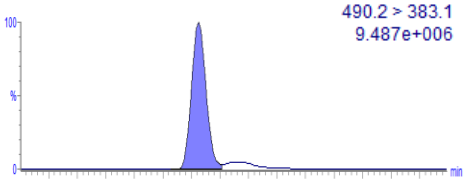
Ibrutinib (280 mg QD)
C_{trough} = 27.6 ng/mL



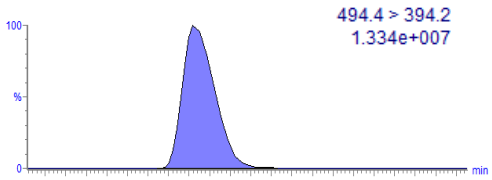
Ruxolitinib (20 mg BID)
C_{trough} = 82.7 ng/mL



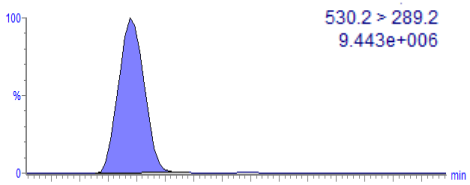
Dasatinib (100 mg QD)
C_{max} = 15.7 ng/mL



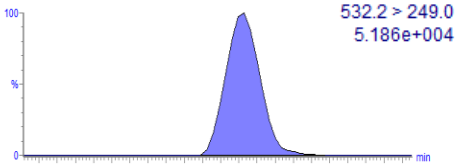
Vemurafenib (240 mg QD)
C_{trough} = 10,947 ng/mL



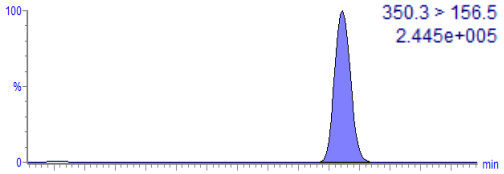
Imatinib (400 mg QD)
C_{trough} = 2,024 ng/mL



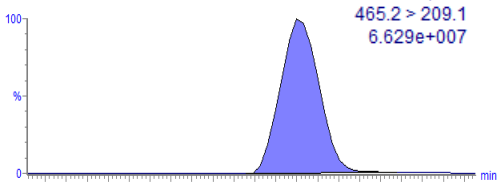
Nilotinib (300 mg BID)
C_{trough} = 960.2 ng/mL



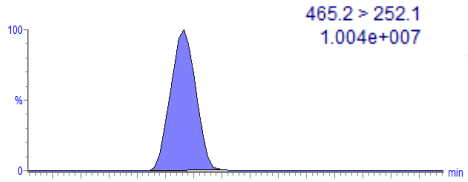
Cobimetinib (40 mg QD)
C_{trough} = 12.5 ng/mL



Abiraterone (1,000 mg QD)
C_{trough} = 15.6 ng/mL



Enzalutamide (160 mg QD)
C_{trough} = 16,770 ng/mL



Sorafenib (400 mg BID)
C_{trough} = 4,699 ng/mL

Figure 3. Typical chromatograms of the targeted therapies obtained in plasma of patients.

Table 1. Pharmacological characteristics of oral targeted therapies analysed in this study (data from summary of products characteristics)

Drug (INN)	Targets	Cancer indication	Metabolic pathway	Food effect AUC	Inhibitors effect CYP 3A4	Inductors effect CYP 3A4
Abiraterone acetate	CYP 17A1	Prostate	CYP 3A4	x 10	/	-55% AUC
Cobimetinib	MEK	Melanoma	CYP 3A4/5 UGT 2B7	/	Increase AUC	Decrease AUC
Dasatinib	Bcr-Abl, Src, c-Kit,PDGFR, EphR	CML, ALL Phi+	CYP 3A4	+14%	4 x Cmax 5 x AUC	-81% Cmax -82% AUC
Enzalutamide	Androgen receptors	Prostate	CYP 2C8 CYP 3A4/5	/	+41 to +326% AUC	-37% AUC
Ibrutinib	BTK	Mantel cell lymphoma, CLL	CYP 3A4	+160%	29 x Cmax 24 x AUC	-90% Cmax and AUC
Imatinib	Bcr-Abl, PDGFR, c-Kit	CML, ALL Phi+, GIST	CYP 3A4	-11%	+26% Cmax +40% AUC	-54% Cmax -74% AUC
Nilotinib	Bcr-Abl, PDGFR, c-Kit	CML	CYP 3A4	+29 to 82%	1,8 x Cmax 3 x AUC	-64% Cmax -80% AUC
Palbociclib	CDK4/6	Breast (HR+, HER2-) with concomittant hormone therapy	CYP 3A4 SULT 2A1	+12 to +21%	+34% Cmax +87% AUC	-70% Cmax -85% AUC
Ruxolitinib	JAK1/JAK2	myelofibrosis, GVH	CYP 3A4 CYP 2C9	/	+33 to +47% Cmax +91 to +232% AUC	-70% AUC
Sorafenib	VEGFR, PDGFR, B-Raf, C-Raf, c-Kit, Fit-3, MEK	Hepatocellular carcinoma, tyroid, renal	CYP 3A4 UGT	-29 to +14%	/	-37% AUC
Vemurafenib	B-Raf (V600E)	Melanoma, Erdheim-chester disease	CYP 3A4	+200%	Increase Cmax and AUC	Decrease Cmax and AUC

ALL: acute lymphoblastic leukemia, AUC: area under the curve, Bcr-Abl: breakpoint cluster region-Abelson complex, B-Raf: serine/threonine-protein kinase B-Raf, BTK: Burton tyrosine kinase, CDK4/6: cyclin-dependent kinase 4/6, c-Kit: tyrosine-protein kinase Kit, CLL: chronic lymphocytic leukemia, CML: chronic myeloid leukemia, C-Raf: serine/threonine-protein kinase C-Raf, CYP 17A1: 17α-hydroxylase/C17,20-lyase, CYP 3A4: cytochome P450 3A4, EphR: erythropoietin-producing human hepatocellular receptor, GIST: Gastrointestinal stromal tumours, GVH: graft versus host disease, HER2: human epidermal growth factor receptor, HR: hormonal receptor, JAK1/JAK2: janus kinase 1 and 2, MEK: mitogen-activated protein kinase, PDGFR: platelet-derived growth factor receptor, Src: tyrosine-protein kinase Src, SULT: Sulfotransferase, UGT: UDP-glycosyltransferase, VEGFR: vascular endothelial growth factor receptor.

Table 2. Calibration range, linear regression equation, correlation coefficient, retention time, MRM transition, collision energie, cone potential and dwell time for each tested analyte.

Analyte	Calibration Range (ng/mL)	Calibration curve Linear regression Equation	Coefficient of Correlation (r)	Retention Time (min)	MRM-Transition (m/z)	Collision Energie (V)	Cone Potential (V)	Dwell Time (s)
Abiraterone	1-500	y = 0.0138 x – 0.0072	0.9995	1.97	350.3 > 156.5	50	60	0.005
Cobimetinib	5-500	y = 0.0216 x – 0.0012	0.9999	1.56	532.2 > 249.0	35	50	0.005
Dasatinib	1-500	y = 0.0305 x + 0.0130	0.9990	1.47	488.3 > 401.0	30	60	0.005
Enzalutamide	100-50,000	y = 0.0002 x + 0.0079	0.9988	1.57	465.2 > 209.1	25	50	0.005
Ibrutinib	1-500	y = 0.0145 x + 0.0234	0.9988	1.71	441.3 > 138.1	25	60	0.005
Imatinib	10-5,000	y = 0.0018 x – 0.0028	0.9999	1.46	494.4 > 394.2	25	60	0.005
N-desmethyl Imatinib	10-5,000	y = 0.0005 x + 0.0040	0.9972	1.46	480.4 > 394.2	30	40	0.005
Nilotinib	10-5,000	y = 0.0016 x – 0.0012	0.9999	1.64	530.2 > 289.2	30	60	0.005
N-oxide Sorafenib	10-5,000	y = 0.0020 x – 0.0056	0.9997	1.68	481.1 > 286.0	25	50	0.005
Palbociclib	5-500	y = 0.0204 x – 0.0240	0.9997	1.48	448.4 > 380.2	28	50	0.005
Ruxolitinib	10-5,000	y = 0.0035 x + 0.0046	0.9993	1.57	307.3 > 186.1	25	50	0.005
Sorafenib	10-5,000	y = 0.0039 x – 0.0068	0.9999	1.73	465.2 > 252.1	30	50	0.005
Vemurafenib	100-100,000	y = 0.0007 x – 0.0123	0.9998	1.74	490.2 > 383.1	26	45	0.005
[$^{2}H_{4}$]-Abiraterone	/	/	/	1.96	354.3 > 160.5	50	60	0.005
[$^{13}C_{6}$]-Cobimetinib	/	/	/	1.55	538.3 > 255.1	35	45	0.005
[$^{2}H_{8}$]-Dasatinib	/	/	/	1.46	496.3 > 406.1	30	60	0.005
[$^{2}H_{6}$]-Enzalutamide	/	/	/	1.57	471.2 > 215.1	25	50	0.005
[$^{2}H_{5}$]-Ibrutinib	/	/	/	1.72	446.3 > 138.1	25	60	0.005
[$^{2}H_{8}$]-Imatinib	/	/	/	1.45	502.4 > 394.2	25	55	0.005
[$^{2}H_{3}$,^{13}C]-Nilotinib	/	/	/	1.63	534.2 > 289.2	30	60	0.005
[$^{2}H_{3}$,^{13}C]-N-oxide Sorafenib	/	/	/	1.68	485.1 > 290.1	25	60	0.005
[$^{2}H_{8}$]-Palbociclib	/	/	/	1.47	456.4 > 388.3	28	50	0.005
[$^{2}H_{9}$]-Ruxolitinib	/	/	/	1.56	316.3 > 186.2	25	50	0.005
[$^{2}H_{3}$.^{13}C]-Sorafenib	/	/	/	1.73	469.2 > 256.1	30	50	0.005
[$^{2}H_{6}$]-Vemurafenib	/	/	/	1.73	496.2 > 389.1	26	45	0.005

Table 3. Assay precision. Data detailing intra-day precision (n = 6) and inter-day precision (n = 6/day; 3 days : n = 18) of each analyte in human plasma.

Analyte	Precision (% CV)							
	LLOQ		LQC		MQC		HQC	
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
Abiraterone	9.9	11.8	2.6	5.5	1.9	2.1	2.5	2.3
Cobimetinib	9.2	14.7	7.2	8.9	2.9	3.3	3.4	4.3
Dasatinib	11.3	19.2	6.8	8.2	4.0	5.2	4.1	4.9
Enzalutamide	8.4	10	8.3	9.3	7.2	7.6	5.7	6.5
Ibrutinib	11.4	14.8	2.4	2.5	1.1	7.0	9.4	11.3
Imatinib	3.1	2.9	2.0	2.8	0.8	1.7	1.5	3.2
N-desmethyl Imatinib	7.8	13.8	4.5	5.6	2.6	5.2	2.8	4.4
Nilotinib	5.2	6.4	1.6	1.7	0.9	1.5	0.9	1.4
N-oxide Sorafenib	6.5	7.2	3.5	3.8	1.6	3.2	1.8	3.1
Palbociclib	5.9	6.3	4.3	7.3	3.2	5.1	2.3	3.8
Ruxolitinib	2.9	7.8	1.6	3.1	0.9	1.5	1.1	1.6
Sorafenib	4.2	11.2	2.5	2.4	5.4	5.9	8.5	9.7
Vemurafenib	3.6	6.4	2.1	3.7	2.7	12.3	4.5	9.8

CV: coefficient of variation

Table 4. Assay accuracy. Data detailing inter-day accuracy (n = 6/day; 3 days: n = 18) of each analyte in human plasma.

Analyte	Concentration (ng/mL)											
	LLOQ			LQC			MQC			HQC		
	Nominal	Mean Measured ± SD	Accuracy (% true)	Nominal	Mean Measured ± SD	Accuracy (% true)	Nominal	Mean Measured ± SD	Accuracy (% true)	Nominal	Mean Measured ± SD	Accuracy (% true)
Abiraterone	1	1.15 ± 0.14	115	10	10.4 ± 0.6	104	100	96 ± 2	96	400	408 ± 9	102
Cobimetinib	5	4.9 ± 0.7	98	10	9.4 ± 0.8	94	100	92 ± 3	92	400	381 ± 16	95
Dasatinib	1	1.02 ± 0.20	102	10	9.9 ± 0.8	99	100	92 ± 5	92	400	383 ± 19	96
Enzalutamide	100	114 ± 11	114	1,000	1,010 ± 94	101	10,000	9,260 ± 704	93	40,000	39,200 ± 2,548	98
Ibrutinib	1	0.95 ± 0.14	95	10	9.9 ± 0.2	100	100	96 ± 7	96	400	395 ± 45	99
Imatinib	10	11.4 ± 0.3	114	100	98 ± 3	98	1,000	975 ± 17	98	4,000	3,945 ± 126	99
N-desmethyl Imatinib	10	9.5 ± 1.3	95	100	110 ± 6	110	1,000	1,090 ± 57	109	4,000	4,033 ± 177	101
Nilotinib	10	11.2 ± 0.7	112	100	108 ± 2	108	1,000	1,084 ± 16	108	4,000	4,390 ± 61	110
N-oxide Sorafenib	10	11.0 ± 0.8	110	100	102 ± 4	103	1,000	1,030 ± 33	103	4,000	4,157 ± 129	104
Palbociclib	5	5.5 ± 0.3	109	10	9.4 ± 0.7	94	100	89 ± 5	89	400	388 ± 15	97
Ruxolitinib	10	9.9 ± 0.8	100	100	105 ± 3	105	1,000	1,062 ± 16	106	4,000	4,220 ± 68	106
Sorafenib	10	9.3 ± 1.0	93	100	94 ± 2	94	1,000	973 ± 57	97	4,000	3,712 ± 360	93
Vemurafenib	100	107 ± 7	107	1,000	960 ± 36	96	10,000	8,900 ± 1,095	89	80,000	74,400 ± 7,291	93

SD: standard deviation

Table 5. Matrix effect (ME), extraction recovery (ER) and stability of each analyte at LQC, MQC and HQC in human plasma (n = 5)

Analyte	QC level	ME (%) + CV (%)	ER (%) + CV (%)	Stability: % true + CV (%)			
				25°C for 72h	4°C for 1 week	-20°C for 8 weeks	3 freeze/thaw cycles
Abiraterone	LQC	88 + 6.4	77 + 5.9	108 + 6.6	108 + 5.3	104 + 4.2	111 + 7.6
	MQC	90 + 10.8	76 + 9.7	97 + 1.3	100 + 3.5	100 + 2.1	103+ 10.4
	HQC	87 + 9.9	82 + 9.9	97 + 2.2	107 + 4.4	104 + 1.3	97 + 4.4
Cobimetinib	LQC	113 + 7.2	111 + 2.1	92 + 4.9	102 + 12.7	103 + 2.8	99 + 6.7
	MQC	115 + 2.0	103 + 3.8	97 + 3.3	104 + 4.2	98 + 3.6	103 + 3.2
	HQC	112 + 4.3	105 + 1.3	100 + 2.8	105 + 3.6	101 + 1.9	96 + 0.9
Dasatinib	LQC	92 + 12.2	103 + 11.6	94 + 5.8	109 + 3.8	94 + 6.3	91 + 4.7
	MQC	95 + 4.2	99 + 7.9	95 + 5.7	102 + 8.1	98 + 2.3	96 + 2.6
	HQC	96 + 3.0	111 + 0.9	99 5.8	99 + 7.5	99 + 1.2	95 + 5.7
Enzalutamide	LQC	100 + 12.3	98 + 12.4	96 + 9.7	103 + 11.3	92 + 10.8	104 + 11.3
	MQC	98 + 5.9	102 + 2.4	94 + 11.1	104 + 1.5	92 + 2.9	108 + 3.8
	HQC	101 + 5.4	103 + 3.3	104 + 9.6	103 + 6.0	94 + 1.2	107 + 3.9
Ibrutinib	LQC	92 + 13.1	107 + 11.5	65 + 7.5	72 + 8.5	90 + 5.0	107 + 4.9
	MQC	109 + 11.7	104 + 9.8	68 + 3.3	76 + 2.9	108 + 2.2	99 + 6.6
	HQC	105 + 2.9	101 + 0.8	74 + 10.0	80 + 10.4	107 + 2.4	95 + 10.5
Imatinib	LQC	101 + 2.7	99 + 4.1	96 + 3.0	99 + 3.6	99 + 0.8	88 + 1.8
	MQC	92 + 1.2	100 + 1.5	96 + 1.5	99 + 1.5	98 + 1.6	90 + 0.7
	HQC	98 + 4.0	111 + 1.7	97 + 0.5	99 + 1.9	100 + 1.2	89 + 2.7
N-desmethyl Imatinib	LQC	117 + 3.1	95 + 3.7	94 + 2.2	93 + 3.1	91 + 1.5	94 + 2.1
	MQC	104 + 1.3	102 + 2.3	101 + 3.5	97 + 1.7	99 + 3.3	93 + 3.2
	HQC	114 + 4.4	109 + 1.4	104 + 1.5	98 + 5.2	101 + 2.0	96 + 4.3
Nilotinib	LQC	92 + 6.3	105 + 5.8	97 + 2.4	99 + 5.0	102 + 1.4	107 + 1.4
	MQC	98 + 5.8	94 + 7.3	94 + 0.6	96 + 1.6	100 + 0.7	101 + 2.6
	HQC	103 + 3.3	97 + 4.2	97 + 1.1	95 + 2.3	100 + 0.4	100 + 0.6
N-oxide Sorafenib	LQC	97 + 11.7	107 + 6.8	101 + 1.8	97 + 6.1	96 + 4.8	112 + 5.1
	MQC	94 + 4.4	102 + 0.8	94 + 1.3	96 + 3.7	97 + 2.4	102 + 4.8
	HQC	102 + 2.9	110 + 6.1	98 + 1.0	98 + 2.8	97 + 1.2	101 + 2.1
Palbociclib	LQC	122 + 11.5	113 + 2.0	94 + 6.1	108 + 2.3	95 + 4.6	103 + 9.7
	MQC	92 + 2.9	108 + 2.1	95 + 2.2	105 + 3.2	94 + 2.7	96 + 2.6
	HQC	95 + 3.6	111 + 6.1	96 + 1.6	100 + 5.9	98 + 0.9	97 + 5.6
Ruxolitinib	LQC	98 + 8.8	108 + 2.7	97 + 0.5	98 + 3.3	96 + 1.5	98 + 2.2
	MQC	95 + 6.9	101 + 1.0	97 + 0.9	99 + 2.3	102 + 1.3	99 + 2.4
	HQC	101 + 3.3	101 + 3.2	99 + 1.1	101 + 3.1	101 + 1.4	101 + 2.7
Sorafenib	LQC	98 + 12.8	92 + 9.7	100 + 2.0	101 + 6.1	95 + 1.1	112 + 3.6
	MQC	90 + 10.9	94 + 4.3	96 + 0.8	105 + 2.0	99 + 1.6	98 + 9.6
	HQC	92 + 9.8	104 + 6.2	91 + 0.9	106 + 3.1	98 + 1.8	98 + 4.9
Vemurafenib	LQC	107 + 4.7	100 + 3.4	101 + 4.5	98 + 3.4	103 + 1.9	102 + 2.0
	MQC	99 + 6.4	92 + 1.3	92 + 1.1	98 + 1.6	98 + 1.5	108 + 2.7
	HQC	103 + 3.9	98 + 1.5	95 + 0.7	99 + 2.9	94 + 2.2	106 + 1.8

CV: coefficient of variation. n: number of replicates

Table 6. Results of the samples of patients treated with oral targeted therapies.

Drug (INN)	Number of patients	Dose (mg)	Steady-state C_{trough}¢ (ng/mL)	C_{max} ¤ (ng/mL)	$AUC_{0\text{-}24h}$ ¥ ($ng.h^{-1}.mL^{-1}$)	Established or accepted target concentration Ref [7]
Abiraterone	7	1,000 mg QD	12.0 [3.1-16.3]*	/	/	C_{trough} > 8.4 ng/mL
Cobimetinib	19	20 [20-40]* mg QD	54.0 [6.5-227.8]*	/	/	C_{trough} : 75-290 ng/mL**
Dasatinib	2	100 mg QD	2.1 ; 4.7	15.7 ; 28.3	/	C_{trough} : 1.4-3.4 ng/mL; C_{max} > 50 ng/mL
Enzalutamide	4	160 mg QD	13,322 [9,240-16,770]*	/	/	C_{trough} > 10,000 ng/mL
Ibrutinib	15	420 [140-560]* mg QD	5.5 [1.2-80.1]*	113.1 [6.4-355.5]*	918 [69-3,210]*	C_{max} < 170 ng/mL, AUC: 680 ± 517 $ng.h^{-1}.mL^{-1}$
Imatinib	4	400 mg QD	805.1 [141.8-2,024]*	/	/	C_{trough} > 1,000 ng/mL
N-desmethyl Imatinib	/	/	213.1 [97.2-432.7]*	/	/	/
Nilotinib	2	300 mg BID	960.2 ; 1,601	/	/	C_{trough} : 480-1,580 ng/mL
Ruxolitinib	5	15 [15-20]* mg BID	37.3 [11.7-82.7]*	162.4 [139.8-204.2]*	/	C_{trough} : 5.4-17.4 ng/mL; C_{max} : 140-277 ng/mL
Sorafenib	7	400 [200-400]* mg BID	4,242 [3,475-5,684]*	/	/	C_{trough} : 3,750-4,300 ng/mL
N-oxide Sorafenib	/	/	432.3 [142.7-941.4]*	/	/	/
Vemurafenib	15	240 [240-960]* mg BID	16,654 [7,583-67,774]*	/	/	C_{trough} > 40,000 ng/mL**

* Median [min-max], QD: once a day, BID: two times a day

** Therapeutic range in melanoma. No data in erdheim-chester disease

¢ For all patients, the samples were taken at least 5 days after the start of treatment or change in dose

¤ Cmax was measured at T1h, T2h and T3h after taking the drug for ruxolitinib, ibrutinib and dasatinib, respectively

¥ AUC0-24h was estimated using 3 successive samples at T0, T2h and T4h by noncompartmental method using WinNonLin® software