Therapeutic Drug Monitoring of 9 new anticancer agents by High-Performance Liquid Chromatography-Tandem Mass Spectrometry

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Introduction

The treatment of some cancers has shifted from conventional chemotherapy drugs to chronic treatment with molecular targeted therapies. Targeted therapies include drugs such as Tyrosine kinase inhibitors (eg: Imatinib, Dasatinib, Nilotinib, Sunitinib, Sorafenib, Vandetanib, Lapatinib, Vatalanib and Erlotinib) that present better efficiency and lower side effects than conventional anti cancer drugs.

Goal

The goal was to develop and validate a fast, specific and sensitive method for the quantitation of Tyrosine kinase inhibitors (eg: Imatinib, Dasatinib, Nilotinib, Sunitinib, Sorafenib, Vandetanib, Lapatinib, Vatalanib and Erlotinib) in plasma samples using liquid chromatography coupled to mass spectrometry.

Method

Equipment

The liquid chromatography consisted of a Thermo Scientific (Courtaboeuf, France) Accela® autosampler and a quaternary pump. Separation was performed on an Hypersil Gold® PFP (2.1x100 mm; pore size 1.9 µm) analytical column placed in a thermostated column heater at 50°C. The chromatographic system was coupled to a triple quadrupole (TSQ) Quantum Ultra mass spectrometer (MS) from Thermo Fisher Scientific, Inc. equipped with an Ion Max electrospray ionization (ESI) interface and operated with XCalibur 2.07 software (Thermo Fisher Scientific, Courtaboeuf France).

LC conditions

The mobile phase used for chromatography was 10 mM ammonium formate buffer containing 0.1% (v/v) formic acid (solution A), and acetonitrile with 0.1 % (v/v) formic acid (solution B). The mobile phase was delivered using the following stepwise gradient elution program: initial conditions of 95:5 (A:B) maintained for 0.5 minutes, run from 95:5 (A:B) at 0.5 minutes to obtain 5:95 (A:B) at 2 minutes, conditions 5:95 (A:B) maintained from 2 to 4 minutes, wash using 100% of phase C from 4 to 7 minutes, run from 5:95 (A:B) at 7.01 minutes to 95:5 (A:B) at 7.5 minutes, conditions 95:5 (A:B) maintained to 10 minutes for equilibration. The flow was 300 µl/min. The thermostated column heater was set at 50°C and the autosampler was maintained at 4°C.

MS conditions

The MS conditions were as follows: ESI in positive mode, capillary temperature: 325 °C: 10V, tube lens voltages range: reported in Table 1; spray voltage: 3500 V; sheath and auxiliary gas (nitrogen) flow-rate: 45 and 25 (arbitrary units), respectively. The Q2 collision gas (argon) pressure was 1.5 mTorr. Data are acquired in selected reaction monitoring (SRM) mode.
The SRM transitions, the collision energy and ions ratio for each analyte are reported in Table 1.

Sample preparation

Calibrators and QCs preparation

For each drug, two primary stock solutions were prepared at 1 mg/ml by dissolving 10-mg base equivalent aliquots of each drug in 10 mL of methanol. Stock solutions were mixed together in order to get 2 methanolic working solutions containing all drugs at 100 µg/mL, 10 µg/mL and 1 µg/mL.
The first set was used for the preparation of the calibration standards ranging from 2 to 250 ng/mL for BORT, DASA and SUNI and from 50 to 3 500 ng/mL for the others drugs. The second set was used for the preparation of the 5 quality controls (QCs): 7, 75, 150, 750 and 1 500 ng/mL for each drug.

Only QCs at 7, 75 and 150 ng/mL were used for BORT, DASA and SUNI while QCs at 75, 150, 750 and 1 500 ng/mL were used for the other TKIs. A 0.5 mg/mL d8-imatinib, internal standard (IS) stock solution was prepared by dissolving 1 mg of the chemical in 2 ml of methanol. Plasma calibration samples and three plasma quality control (QC) samples were prepared by adding the appropriate volume of each working solution to blank plasma.

Analyte	Retention time	Precursor Ion	Product ion	TL/CE	Product ion	CE	Ion Ratio
Bortezomib	3.11	367.1	226.0	192/-18	208.0	-28	60
Dasatinib	3.01	488.2	401.0	184/-29	231.9	-38	40
Erlotinib	3.12	394 .2	277.9	136/-21	336.0	-22	40
Imatinib	2.96	494.3	394.1	170/-25	222.0	-27	20
D8-Imatinib	2.96	502.3	394.1	170/-25			
Lapatinib	3.28	581.1	349.9	185/-36	364.9	-38	75
Nilotinib	3.26	530.1	288.9	199/-29	261.0	-42	45
Sorafenib	3.59	465.1	251.9	176/-31	270.0	-21	75
Sunitinib	3.06	399.2	282.9	134/-28	326.0	-20	60
Vandetanib	2.99	475.1	83.1	142/-32	111.9	-64	15

Table 1: Retention time, precursor molecular ion/product ion for quantification, precursor molecular ion/product ion for confirmation and detection parameters (tube lens voltage (TL)/collision energy(CE)) for each analyte

Plasma sample extraction procedure

Aliquots of 50 µl of the plasma unknowns, blank, calibration standards and QCs were placed in appropriate labeled 1.5 mL microcentrifuge tubes and mixed with 200 µl of acetonitrile containing 20 ng/mL IS. After automatic vortexing for 10 minutes, each sample was centrifuged at 6 000g at 4°C for 15 minutes. Hundred microliters of supernatant were diluted two-fold using the mobile phases A and B in a 50/50 (v/v) ratio. After capping and vortexing, the vials were transferred into the autosampler tray that was maintained at +4°C. Twenty-five microliters aliquots of the extract were injected into the HPLC system.

Results

Chromatograms

The proposed method enables the simultaneous quantification of commonly used TKIs in 50µL-plasma aliquots by liquid chromatography coupled with tandem MS. Typical chromatographic profiles of the highest calibrator sample containing all are shown in Fig. 2.

Internal standard, calibration curve and lower limit of quantification

Imatinib-D8 was used as IS with a satisfactory chromatographic profile and a negligible memory effect. Calibration curves over the entire ranges of concentrations were best described by 1/x weighted linear regression of the peak-area ratio of each TKI to IS *versus* the concentrations of the respective TKI in each standard sample.

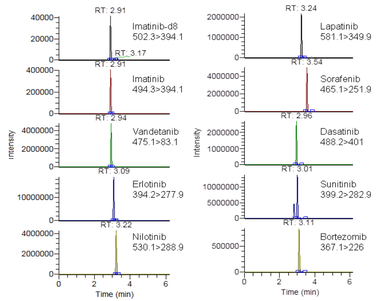

Fig. 1: Chromatogram of the highest calibrator sample containing each TKI.

This model was optimal for the 9 TKIs. Standard curves, prepared from different biological plasmas (EDTA), were performed in plasma on twenty consecutive days. The assay proved to be linear and acceptable, as the regression coefficients were >0.99 for each of the twenty standard curves excepted for sorafenib (mean r^2 0.9894) (Table 2).

A linearity test has been performed to compare theorical values, mean and standard deviations of the back-calculated values to each nominal concentration used in the low and the high standard curves. Then the accuracies were calculated for each analyte. In all cases, slopes of these linear curves were ranging between 0.9987 to 1.019 and statistics showed slopes significantly different from 1 ($p<0.0001$). The LLOQ was established at 2 ng/mL for BORT, DASA and SUNI and 50 ng/mL for the others drugs in human plasma.

Analyte		Slope	Intercept	R^2
BORT	Mean	0.000179	0.0000483	0.9935
	CV	14.8	142.9	0.48
DASA	Mean	0.000989	-0.0004033	0.9967
	CV	9.3	181.7	0.26
ERLO	Mean	0.00820	0.2222	0.9913
	CV	7.1	40.8	0.46
IMAT	Mean	0.0198	-0.009083	0.9980
	CV	5.4	137.8	0.10
LAPA	Mean	0.000286	-0.0004005	0.9964
	CV	11.5	164	0.22
NILO	Mean	0.002519	-0.02377	0.9911
	CV	3.88	91.8	1.44
SORA	Mean	0.000657	-0.020596	0.9894
	CV	10.8	24.0	0.69
SUNI	Mean	0.00514	0.00121	0.9919
	CV	6.9	183.9	0.46
VAND	Mean	0.0000199	-0.002118	0.9943
	CV	12.2	163.0	0.32

Table 2: Data detailing the slopes, intercepts, coefficient correlations (r^2) for 9TKIs (n=20).

Accuracy and precision

Precision and accuracy determined with 3 and 4 controls samples are given in Table 3. The levels of control samples were selected to reflect low, medium and high range of the two sets of calibration curves. They were chosen to encompass the clinically range of concentrations found in patients plasma. The mean intra-assay precision was similar over the entire concentration range and lower than 8.2 %. Overall, the mean inter-day precision was good with CVs within 5.3 and 13.8%. The intra-assay and **inter-assay** bias from the nominal concentrations of QCs for each considered TKI were contained between and 86.8 and 113.5 %. Ratios of ion transitions were reproducible for all TKIs and standard deviation for all of them below 25%.

Concentration	BORT		DASA		SUNI	
	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision
2	98.4	19.8	106.9	16.2	119.8	20.0
5	93.2	19.5	101.5	8.2	99.7	6.3
10	93.9	8.9	98.3	11.9	97.6	7.0
20	108.2	13.1	97.8	6.0	93.9	11.0
50	104.0	10.1	97.2	7.7	90.7	5.1
100	98.2	5.8	98.4	5.6	91.3	4.0
250	99.5	3.9	102.2	3.0	105.8	2.1

Concentration	ERLO		IMAT		LAPA	
	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision
50	91.8	13.8	93.4	12.8	105.5	7.7
100	94.3	10.5	98.1	9.9	96.8	6.6
200	113.9	7.1	107.6	7.8	109.2	6.1
500	109.0	5.7	98.3	5.9	90.5	7.8
1000	103.5	5.1	100.2	5.1	96.8	6.1
2000	101.4	4.4	99.2	3.8	99.6	4.8
3500	94.9	3.6	100.8	2.3	101.9	2.7

Concentration	NILO		SORA		VAND	
	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision
50	111.5	7.8	113.1	5.4	86.5	16.2
100	99.7	4.6	98.9	3.3	91.7	11.0
200	111.1	4.5	108.2	5.8	111.2	17.4
500	98.5	5.3	91.8	5.7	103.8	10.7
1000	93.0	7.0	91.2	8.4	108.5	5.0
2000	97.5	3.3	98.3	2.7	101.3	2.4
3500	103.2	3.0	105.3	3.3	96.0	3.3

Table 3: Assay performance data of the low calibration samples for BORT, DASA, SUNI and of the high calibration samples for ERLO, IMAT, LAPA, NILO, SORA, VAND in human plasma (n=20)

Selectivity and specificity

No peaks from endogeneous compounds were observed at the drugs retention time in any of the 10 blank plasma extracts evaluated. The endogeneous responses in blank plasma were always below 6.5 % of the signal at the LLOQ of 2 ng/mL for BORT, DASA, SUNI and at 50 ng/mL for the others. The endogeneous responses in plasma provided from polymedicated patients were always less than 7.1% of the signal at each LLOQ. There were no effects of others concomitant treatments (40 mg/l of amikacin, 20 mg/l of gentamycin, 25 mg/l of vancomycin, ceftazidime, imipenem and cisplatin, 0.5 mg/l of morphine, 3 mg/l of docetaxel, 5 mg/l of voriconazole, posaconazole, itraconazole and fluconazole).

Extraction recovery and matrix effect

The assessment of matrix effects and extraction recoveries is reported in Table 5. A value above or below 100% for the matrix effects indicates an ionization enhancement or suppression, respectively. Matrix effects and extraction yields were ranged from 84.6 to 109 % and 84.0 to 101.2% respectively. Overall recoveries were ranged from 77.8 to 93.3 % for lower concentrations, 78.6 to 98.4% for medium concentrations and from 79.8 to 105.6 % for higher concentrations. The extraction recovery of D8-imatinib was 93.7%. There was no effect of hyperbilirubinemia, hyperlipemia and haemolysis on matrix effect as evaluated in medium CQs.

Stability

The stability of TKIs in human plasma samples was studied with low and high QC samples left at room temperature up to 48h. The variations are contained within ± 15% of starting concentrations indicating that TKIs can be considered stable at RT excepted for lapatinib which decreases of -36% at RT after 24h and of -76% after 48h. It has been demonstrated that lapatinib was stable at RT for 6 hours. Sunitinib is sensitive to light and decreases by -15% after 48h even light protection. By contrast, all TKIs in plasma samples left during the same period of time at +4°C were found stable.
QC samples prepared in human plasma undergoing three freeze-thaw cycles showed no significant degradation (variation < 8.2 %) for all analytes.
Long-term stability studies indicated that all analytes were stable in human plasma when stored at -70°C for 150 days (ratios between 96.0 to 100.5%, degradation < 7.9%).
The stability of stock solutions held at -70°C and left in the dark for 10 months showed decrease less than 6% for each analyte.
In neutral extracts, all analytes were stable up to 7h when left in the autosampler without any degradation allowing more than 40 samples to be analyzed simultaneously within a single chromatographic batch.

External quality controls

The external quality controls (low and high concentrations) for imatinib (18 laboratories), nilotinib and dasatinib (9 laboratories) showed a good accuracy (97.2 to 101.4%) in comparison to data obtained from others laboratories.

Application to biological samples

We applied the assay to the analysis of samples obtained from patients receiving imatinib, nilotinib, dasatinib, sunitinib or sorafenib.
DASA, IMAT and NILO were frequently detected in patients with chronic myeloid leukemia (n=75). In 71 patients treated with 400 (84%) or 600 mg imatinib daily, detected though concentrations were around 871 ng/mL (median: 789 ng/mL). Among these 71 patients, 45 % of them presented a major molecular response associated with a trough concentration higher than 1,000 ng/mL such as recommended [50].
We applied the assay to samples provided from an obese patient treated with 50 mg sunitinib for a renal carcinoma. The profile of SUNI concentrations measured in this obese woman showed no difference with AUC (1592 ± 41 ngh/ml) observed in patients without obesity.

Conclusion

In overall, the method that has been developed is precise, accurate and sensitive. It concerns nine inhibitors of tyrosine kinase acquired in a single run Confirmation is performed using confirmation/quantification ion ratios criteria. The method is very simple and therefore used in a routine environment for clinical studies; it is also possible to add new TKIs that could potentially have an interest in clinical practices and performed a partial analytical validation. The dynamic range of the concentrations allow to carry out some pharmacokinetics studies.