RESEARCH ARTICLE

Development and Validation of a Bioanalytical LC-MS/MS Method for Detection of Dasatinib in Rat Plasma

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ABSTRACT

This work presents a novel method for detection of concentration of Dasatinib in rat plasma using Verapamil as internal standard by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method's pharmacokinetic application in rats was also evaluated. Separation was achieved using AB Sciex 4000 instrument, equipped with a Chromasol Garnet C18 polar analytical column (50 × 4.6 mm, 4 µm, 120 Å). The column oven was maintained at 45 ± 2°C, while the autosampler was kept at 10 ± 2°C. The mobile phase consisted of a 60:40 v/v mixture of water and methanol, delivered at flow rate of 0.8 mL/min, with 2 µL an injection volume. The liquid chromatographic procedure was carried out over a four-minute duration. The mass spectrometer operated in positive electrospray ionization molecular structure (+ESI) mode, employing multiple reaction monitoring (MRM) to detect the mass-to-charge ratio transitions for Dasatinib (m/z 488.1 → 401.0) and Verapamil (internal standard, m/z 455.6 → 165.3). were determined using multiple reaction monitoring (MRM). The concentration range for Dasatinib was 500 to 1 ng/mL, with a correlation coefficient of 0.9996. The accuracy and precision for MQC, HQC, LLQC, and LQC were 106.09 %, 107.33%, 99.83%, and 94.15%, respectively. The developed LC-MS/MS method is simple, precise, sensitive and reproduceable. Henceforth it can be used to determine dasatinib.

Keywords: Dasatinib, Verapamil, Rat Plasma, LC MS/MS, Development and validation.

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INTRODUCTION

Dasatinib (BMS-354825), chemically known as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide monohydrate (Fig. 1), is an effective treatment for chronic myeloid leukemia (CML) in cases where imatinib has failed (Y. Sun et al 2017). As an innovative, orally administered, multitargeted kinase inhibitor, dasatinib is effective against break point cluster region–abelson (BCR-ABL) and SRC family kinases (SFKs). Unlike imatinib and its derivative nilotinib (AMN107), dasatinib can bind to multiple conformations (both active and inactive) of the ABL kinase (J. Horinkova et al 2019) (D. Leveque et al 2020) (L. Zhu& L. Chen 2019). It is effective against 21 out of 22 BCR-ABL mutations known to cause resistance to imatinib and also strongly inhibits SFKs involved in imatinib resistance (Y. Wang et al 2020). In spite of that dasatinib is associated with several side effects including headache, nausea, diarrhea, fatigue, peripheral edema and fever. The other popular adverse events reported are diarrhea and gastrointestinal bleeding. Given these potential side effects, it is crucial to develop a reliable analytical method for determining dasatinib levels for therapeutic drug monitoring (M.N. Fornier et al 2011) (P.G. Morris et al 2018) (T.R. Vaidya, S& Ait-Oudhia 2022) (M.A. Fernandez-Peralbo et al 2014).

Bioanalysis involves studying analytes in biological samples, such as biomarkers, medications, and metabolites, through stages like data reporting, sample retrieval, and analysis. The process starts with collecting samples from clinical or preclinical research and sending them to a lab. Crucial to this is sample preparation, which ensures accuracy and reliability by removing impurities from the sample matrix. Techniques like protein precipitation, solid-phase extraction, and liquid-liquid extraction are used to isolate analytes from complex biological matrices. Proper preparation maintains the integrity of analytes and ensures the precision of bioanalytical data (European Pharmacopoeia) (The State Pharmacopeia of Ukraine) (The State Pharmacopeia of Ukraine).

In recent years, various research facilities have reported the utilization of high-throughput bioanalytical procedures for the assessment of antileukemia drugs. Limited number of analytical methods have been reported for dasatinib (S. De Francia et al 2009), which include high-performance thin-layer chromatography [HPTLC], high-performance liquid chromatography [HPLC], radioactive labeling methods, and a liquid chromatography–mass spectrometry

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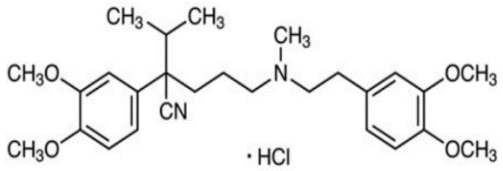

Figure 1: Molecular Structure of Dasatinib

[LC–MS] (M. Clynes& R. O'Connor 2009) (M.Z. Shi et al 2019)method for plasma determination.

Most approaches used to quantify pharmaceuticals have concentrated on individual agent isolation and have been

regularly applied to plasma determination or pharmaceutical analysis of the agent. The high-performance liquid chromatography–ultraviolet [HPLC–UV] (E. Kralj et al 2012) method has restricted sensitivity and specificity and required a relatively long analysis duration to achieve sufficient chromatographic separation.

In contrast, the selectivity and selectivity of tandem mass spectrometry [MS/MS] (N.A. Lankheet et al 2013) have led to a growing trend of developing fast analytical methods in recent years. These advancements in MS/MS technology are particularly beneficial for creating more efficient and accurate bioanalytical procedures for antileukemia drugs like dasatinib (I. Andriamanana et al 2013) (A. Haouala et al 2009).Our objective was to develop and optimize a straightforward, user-friendly, sensitive, and specific LC–MS method for detecting dasatinib in rat plasma. This method can be effectively used in pharmacokinetic studies of dasatinib in rats following intragastric administration.

MATERIALS AND METHOD

Chemicals and reagents

Dasatinib and Verapamil reference samples were generously supplied by MSN Labs Hyderabad. Water were obtained from Thomas baker, All the chemicals, methanol, Propan-2-ol, acetonitrile suitable for LCMS analysis, were obtained from the chemical division of Rankem. The Institutional Animal Ethics Committee (IAEC) approved the experimental procedure based on IAEC No. 02/AP/2022.

Condition of liquid chromatography and mass spectroscopy:

The liquid chromatography and mass spectrometry analysis was performed using an AB Sciex 4000 instrument, equipped with a Chromasol Garnet C18 polar analytical column (50 × 4.6 mm, 4 µm, 120 Å). The column oven was maintained at 45 ± 2℃, while the autosampler was kept at 10 ± 2°C. The mobile phase consisted of a 60:40 v/v mixture of water and methanol, delivered at a flow rate of 0.8 mL/min, with an injection volume of 2 µL. The liquid chromatographic procedure was carried out over a 4 minute duration. The mass spectrometer operated in positive electrospray ionization molecular structure (+ESI) mode, employing multiple reaction monitoring (MRM) to detect the mass-to-charge ratio transitions for Dasatinib (m/z 488.1 → 401.0) and Verapamil (internal standard, m/z 455.6 → 165.3). This

Table 1: Dasatinib linearity results

Final conc. in ng/mL	Dasatinib peak area
Blank	00
1	0.366
2.5	4.720
5	20.130
10	39.133
25	53.155
50	74.240
100	89.069
250	92.250
500	99.325
Y Intercept	0.00126
Slope	0.0140
Regression coefficient (r^2)	0.9958

setup ensured precise and efficient separation and detection of the analytes, as illustrated in Figures 2 and 3.

Calibration Standards and Quality Control Samples

Individual stock solutions of dasatinib (2 mg/mL) and Verapamil (IS) (2 mg/mL) were prepared in acetonitrile. Working solutions for calibration and controls were drawn up from these stock solutions by dilution with acetonitrile. A working standard solution of IS at 1.0 mg/mL was prepared by diluting the IS stock solution with acetonitrile. Quality control (QC) samples were similarly prepared to the calibration standards, with concentrations of 4000, 2000, and 30 ng/mL. All solutions were stocked at 2-8°C and brought to room temperature before use.

Dasatinib calibration standards were produced by spiking blank rat plasma with appropriate amounts of the working solutions. Calibration plots were constructed in the range of 5000–1 ng/mL for dasatinib in rat plasma, with specific concentrations of 1, 2.5, 5, 10, 25, 50, 100, 250, and 500 ng/mL. QC samples were formulated in the similar manner as the calibration standards, with plasma concentrations of 3, 200, and 400 ng/mL. Both the analytical standards and QC samples were stocked at -20°C.

Bio-analytical Method Validation:

Method validation was performed according to ICH M10 guidelines.

Selectivity

We utilized blank samples from minimum of six independent sources/ lots to evaluate selectivity. The blank sample/s are processed without including an internal standard (IS) or analyte.

Linearity

Preparing a calibration curve using the same biological matrix as the samples is essential. Separate calibration curves are required for each analyte to be measured. The method's range refers to the concentration interval within which accuracy, precision, and linearity have been demonstrated.

Accuracy and Precision

In the time of method development, it is crucial to verify the technique's suitability for validation by analyzing replicate QCs at varying in concentrations within the assay range. This involves analyzing replicate QCs at varying

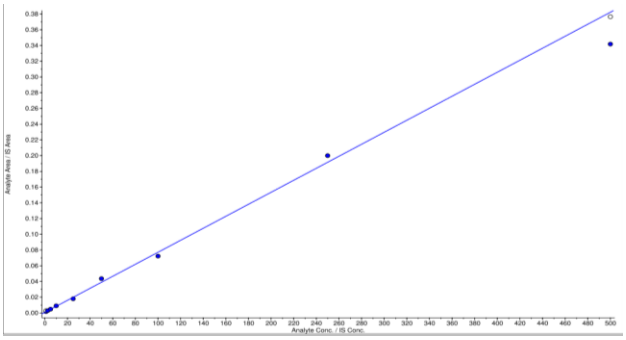

Figure 2: Representative calibration curve

Table 2: Calculated concentrations obtained for precision and accuracy batches

Injections	Observed Concentration (ng/mL)			
	HQC	MQC	LQC	LLOQ QC
Standard Deviation	1.45980	0.63620	0.01700	0.01050
CV (Precision %)	2.37	1.79	2.86	5.44
Accuracy (%)	106.09	107.33	99.83	94.15

concentrations. Method validation studies must include minimum of six independent runs, each with a calibration curve and several sample concentrations measured in repetition, to calibrate accuracy and precision.

Recovery

Six sets of QC samples either thawed or freshly prepared from the deep freezer. The IS was added to these samples before the injection. A 100% extraction of the analyte was accomplished by processing blank matrix samples from one single lot. These samples then injected alongside six sets of quality control dilutions at low, middle, and high concentrations. An IS was included in the process. At each QC level and for the internal standard, the %CV of recovery should be below 15.00%. The overall mean recovery %CV must be below 20.00% for each QC levels.

Matrix Effect

The matrix effect refers to a disparity in analyte reactivity caused due to interfering and sometimes undetectable components within sample matrix. To evaluate this, eight different batches of screened plasma were used to prepare two replicates of blank plasma samples. The LQC concentration was spiked with the IS using one set of eight independent blank matrices, while the HQC concentration was spiked with IS using a different set. The analysis was performed with spiked analyte(s) and IS added to the reconstitution solution to create a set of aqueous sample(s) which matched the final LQC and HQC concentrations.

Stability

Stability tests a crucial part to ensure that analyte concentration remain consistent allover sample preparation, analysis, processing and storage. To evaluate the analyte's stability within the matrix, quality controls at both low and high concentrations are utilized. When storage conditions are established at time zero, aliquots of the quality controls are analyzed at specified intervals. It is essential to perform minimum of triple stability tests for each concentration level, time point and storage condition. According to FDA, these stability measures are recommended for biological studies. Any alteration in the analyte can affect chromatographic behavior, adding complexity to the method development process.

Application of Bio-Analytical Method to Pharmacokinetics Study:

A cohort of six Wistar rats male, weighing between 220 to 250 g, was used for the pharmacokinetic experiments. The rats were housed in ventilated cages with adequate food and water for seven days prior to the study. They were fasted overnight before the administration of the dose. A pharmacokinetic investigation of molnupiravir was conducted using this cohort. The animal study protocol was approved by the Institute of Animal Ethics Committee (registered number 02/AP/2022). A single dose of 10mg/kg Dasatinib tablet powder was administered orally to the rats. Blood samples were collected at 0 , 0.5, 1, 2, 3, 6, 12 and 24 hrs hours post-dose. At each time interval, 5 mL of blood was collected into K2 EDTA vacutainer containers. Additionally, a predose sample was obtained to evaluated the potential plasma interference. The gathered samples were subjected to centrifugation to

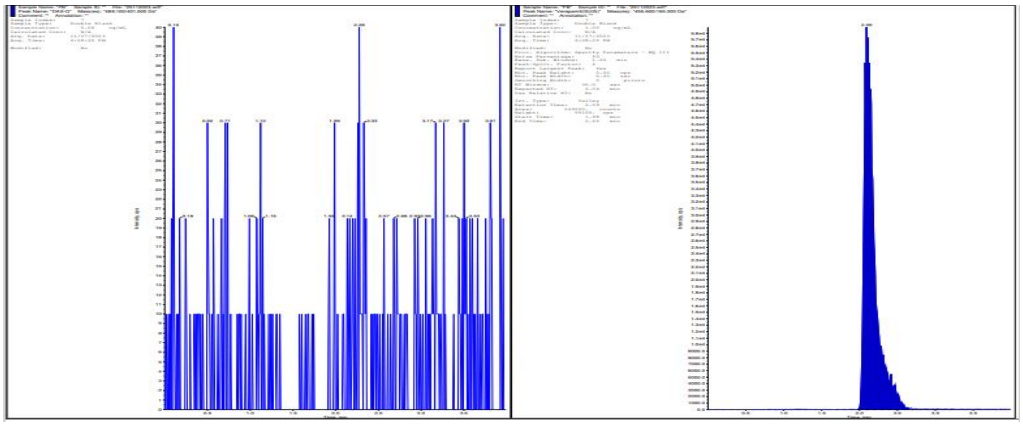

Figure 3: Representative ion chromatogram for the blank plasma (a) and LLOQ (b) with a 2 µL injection volume.

Table 3: Recovery of Dasatinib

S.No	Observed response								
	Extra HQC	Aqs HQC	% Recovery	Extra MQC	Aqs MQC	% recovery	Extra LQC	Aqs LQC	% recovery
MEAN .	1099562.7	1291075.3	85.17	1056168.0	1324165.5	79.77	1080442.8	1326906.0	81.43
S D	31372.14	5708.78	2.480	32510.02	4376.02	2.602	32866.70	8889.17	2.432
% CV	2.85	0.44	2.91	3.08	0.33	3.26	3.04	0.67	2.99
Mean recovery					82.12				
SD					2.766				

Table 4: Dasatinib QC sample stability results using LC-MS/MS.

Stability	Nominal concentration ng/ml	Standard deviation	CV (Precision %)	% Accuracy
Bench top stability	50	1.59400	2.52	102.64
Auto sampler stability		1.40400	2.37	102.23
Short term		2.68200	4.38	105.54
Long-term (64 days)		1.59400	2.69	102.54
Freeze and thaw stability		1.38300	2.32	102.70

obtain plasma, and was stored at -80°C. Four concentrations of spiked plasma and quality control (QC) samples were analyzed. The pharmacokinetics of Dasatinib were calculated using WinNonlin (Version 5.2).

RESULTS AND DISCUSSION

Selectivity

No peaks from endogenous compounds were observed at the drug's retention time in any of the six evaluated blank plasma extracts. The responses in blank plasma were consistently below 14.9% of the signal at the LLOQ of 1 ng/mL for Dasatinib (DAS) and below 0.62% for the internal standard (IS). Figure 3 presents a representative ion chromatogram for the blank plasma (a) and LLOQ (b) with a 2 µL injection volume.

Linearity:

The seven-point calibration curve demonstrated linearity over the concentration range of 1-500 ng/mL. The calibration model, chosen based on the data, employed quadratic regression with a $1/x^2$ weighting factor. Within this concentration range, the regression coefficient (r^2) of the calibration curves consistently exceeded 0.9958. accuracy of 88-110 across the tested concentration. Quality control samples passed the accuracy of ± 15% of theoretical value.

Accuracy and Precision

To evaluate the intra-assay precision and accuracy, six duplicates containing molnupiravir were analyzed at three different (QC) levels. The inter-assay precision was determined by analyzing these three levels of QC samples in independent runs. The proposed method's mean accuracy ranged from 94.15% to 107.33%, while the precision (%CV) for the low (LQC), middle (MQC), and high (HQC) quality controls ranged from 1.79% to 5.44%. Table 2 summarizes these findings.

Recovery

Coefficient of variation (CV) or relative standard deviations (RSD) of the responses of Dasatinib peaks of replicate injections of aqueous samples are varying between 0.33 % to 0.67 %. Coefficient of variation (CV) or relative standard deviations (RSD) of the response of

Table 5: Pharmacokinetic parameters from the noncompartmental model analysis

PK Parameter	Unit	Value
$t_{1/2}$	h	3.97
T_{max}	h	4.00
C_{max}	ng/ml	8.50
AUC	ng/ml*h	66.06

Dasatinib peaks of extracted samples are varying between 2.85 % to 3.08%. The average recoveries of the Dasatinib at all three concentration levels ranged from 79.77 % to 85.17 %. The mean recovery of Dasatinib is 82.12 %.

Matrix effect

The back-calculated concentrations for the high-quality control (HQC) and low-quality control (LQC) levels showed mean accuracies of 99.86% and 98.76%, respectively. As these results met the acceptance criteria of 85.00% to 115.00%, the current method demonstrated no significant ionization effects.

Stability

To evaluate stability of Dasatinib in plasma, tests were conducted using six replicates of quality control samples at both low and high concentrations. Appropriate volumes of Dasatinib standard solutions were added to create drug-free plasma samples. The results, shown in Table 4, fell within the acceptable range, indicating that Dasatinib demonstrates favorable stability.

Pharmacokinetic Application:

The method was attempted on a pharmacokinetic study in rats. The main pharmacokinetic parameters which followed noncompartmental model analysis been summarized in Table 4. The mean plasma concentration–time curve following the oral administration of a single 10 mg/kg dose of dasatinib is presented in Figure 4.

CONCLUSION

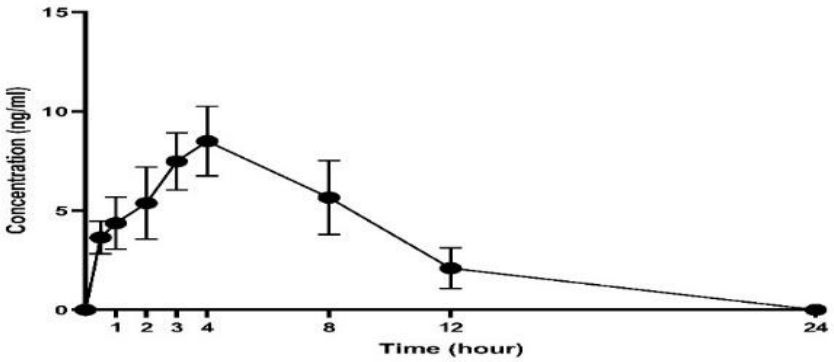

Figure 4: The mean plasma concentration–time curve following the oral administration of dasatinib.

A specific, straightforward, and sensitive LC-MS method with gradient elution were developed and validated for determining dasatinib in rat plasma in the concentration range of 1-500 ng/ml. This method was validated to meet the requirements for the pharmacokinetic determination of dasatinib in rat plasma.

Acknowledgments

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REFERENCES

1. Y. Sun., X. Lin., J.C. Aske., P. Ye., C. Williams., M. Abramovitz., B.R. Leyland-Jones. Dasatinib attenuates overexpression of Src signaling induced by the combination treatment of veliparib plus carboplatin in triple-negative breast cancer. Cancer Chemother Pharmacol, 2019;84(6):1241-56.
2. J. Horinkova., M. Sima.., O. Slanar.. (2019).Pharmacokinetics of Dasatinib. Prague Med. Rep. 2019;120(2-3):52-63.
3. D. Leveque., G. Becker., K. Bilger., S. Natarajan-Ame. Clinical Pharmacokinetics and Pharmacodynamics of Dasatinib. Clin. Pharmacokinet. 2020;59(7):849-856.
4. L. Zhu., L. Chen. Progress in research on paclitaxel and tumor immunotherapy. Cell Mol. Biol. Lett. 2019;24:40.
5. Y. Wang, Y. Ding, Y. Xu, C. Wang, Y. Ding, M. Gao, C. Ma, X. Ma, L. Li. Mixed micelles of TPGS and Soluplus® for co-delivery of paclitaxel and fenretinide: in vitro and in vivo anticancer study. Pharm. Dev. Technol. 2020;25(7):865-73.
6. M.N. Fornier, P.G. Morris, A. Abbruzzi, G. D'Andrea, T. Gilewski, J. Bromberg, C. Dang, M. Dickler, S. Modi, A.D. Seidman, N. Sklarin, J. Chang, L. Norton, C.A. Hudis. A phase I study of dasatinib and weekly paclitaxel for metastatic breast cancer. Ann. Oncol.2011;22(12):2575-81.
7. P.G. Morris, S. Rota, K. Cadoo, S. Zamora, S. Patil, G. D'Andrea, T. Gilewski, J. Bromberg, C. Dang, M. Dickler, S. Modi, A.D. Seidman, N. Sklarin, L. Norton, C.A. Hudis, M.N. Fornier. Phase II Study of Paclitaxel and Dasatinib in Metastatic Breast Cancer. Clin. Breast Cancer. 2018;18(5):387-94.
8. T.R. Vaidya, S. Ait-Oudhia. Experimental and computational assessment of the synergistic pharmacodynamic drug-drug interactions of a triple combination therapy in refractory HER2- positive breast cancer cells. J. Pharmacokinet Pharmacodyn. 2022;49(2)227-41.
9. M.A. Fernandez-Peralbo, F. Priego-Capote, M.D. Luque de Castro, A. Casado-Adam, A. Arjona-Sanchez, F.C. Munoz-Casares. LC-MS/MS quantitative analysis of paclitaxel and its major metabolites in serum, plasma and tissue from women with ovarian cancer after intraperitoneal chemotherapy. J. Pharm. Biomed. Anal. 2014;91:131-7.
10. European Pharmacopoeia 10th Ed. European Directorate for the Quality of Medicines & Healthcare of the Council of Europe. Strasbourg Cedex. 2020. https://www.edqm.eu/en/european_pharmacopoeia_10th_ edition
11. The State Pharmacopeia of Ukraine 3rd vol. 2014. State Enterprise "Ukrainian Scientific Pharmacopoeial Centre. State Service of Ukraine on medicines and Drug Control, Ministry of Health. St. Kharkiv.
12. The State Pharmacopeia of Ukraine 2nd vol. 2004. State Enterprise "Ukrainian Scientific Pharmacopoeial Centre. State Service of Ukraine on medicines and Drug Control, Ministry of Health. St. Kharkiv.
13. S. De Francia, A. D'Avolio, F. De Martino, E. Pirro, L. Baietto, M. Siccardi, M. Simiele, S. Racca, G. Saglio, F. Di Carlo, G. Di Perri. New HPLC – MS Method for the simultaneous quantification of the antileukemia drugs imatinib, dasatinib and nilotinib in human plasma. J Chromatogr. B: Anal. Technol. Biomed. Life Sci. 2009;877(18-19):1721–1726.
14. M. Clynes, R. O'Connor. Development of a high-performance liquid. chromatographic-mass spectrometric method for the determination of cellular levels of the tyrosine kinase inhibitors lapatinib and dasatinib. J. Chromatogr. B. 2009;877(31):3982-90.
15. M.Z. Shi, T.Y. Xing, J.J. Chen, B. Jiang, X. Xiao, J. Yang, J. Zhu, C. Guo, J.D. Hu, Y.L. Han. Effect of

Xiao-Ai-Ping injection on paclitaxel pharmacokinetics in rats by LC-MS/MS method. J. Pharm. Biomed. Anal.2019;174:728-733.

16. . E. Kralj, J. Trontelj, T. Pajic, A. Kristl. Simultaneous measurement of imatinib, nilotinib and dasatinib in dried blood spot by ultrahigh performance liquid chromatography tandem mass spectrometry. J. Chromatogr. B. 2012;903:150-6.
17. N.A. Lankheet, M.J. Hillebrand, H. Rosing, J.H. Schellens, J.H. Beijnen, A.D. Huitema. Method development and validation for the quantification of dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, sorafenib and sunitinib in human plasma by liquid chromatography coupled with tandem mass spectrometry. Biomed. Chromatogr. 2013;27(4):466-76.
18. I. Andriamanana, I. Gana, B. Duretz, A. Hulin. Simultaneous analysis of anticancer agents bortezomib, imatinib, nilotinib, dasatinib, erlotinib, lapatinib, sorafenib, sunitinib and vandetanib in human plasma using LC/MS/MS, J. Chromatogr. B. 2013;926:83-91.
19. A. Haouala, B. Zanolari, B. Rochat, M. Montemurro, K. Zaman, M.A. Duchosal, H.B. Ris, S. Leyvraz, N. Widmer, and L.A. Decosterd. Therapeutic Drug Monitoring off the new targeted anticancer agents imatinib, nilotinib, dasatinib, sunitinib, sorafenib and lapatinib by LC tandem mass Spectroscopy. J. Chromatogr. B: Anal. 2009;877:1982–96.