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Isotope dilution direct injection mass spectrometry method for determination of four tyrosine kinase inhibitors in human plasma

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ABSTRACT

Background: Therapeutic drug monitoring is recommended for the optimal management of patients with several malignant diseases. The aim of this study was to develop and validate an isotope dilution direct injection mass spectrometry method for the high throughput determination of tyrosine kinase inhibitors in plasma from leukemic and cancer patients.
Methods: The plasma for analysis was deproteinated by methanol and the centrifuged supernatant was directly injected to mass spectrometer without separation step. Multiple reaction monitoring modes on a hybrid triple quadrupole – linear ion trap mass spectrometer (5500 QTRAP) were used for the detection and quantification of imatinib, nilotinib, lapatinib, and dasatinib.
Results: We developed a fast method with analysis time of 55 s and 19 s in multiple injection setting. The method was successfully validated and applied to the patient plasma samples. In order to overcome insufficient sensitivity of dasatinib, multiple reaction monitoring cube mode in linear ion trap (MRM^3) was successfully applied. The limits of quantification were in the range 1.0–5.5 ng/ml. Imprecisions were lower than 6.9% and the accuracy of the quality control samples ranged between 99.0 and 107.9%.
Conclusions: Isotope dilution direct injection mass spectrometry method allows high-throughput therapeutic drug monitoring of tyrosine kinase inhibitors in plasma. The method offers low-cost analyses as a result of its speed and the exclusion of separation step and can be advantageously used in routine clinical practice. The method can be applied on various drugs and biochemical markers with the use of triple quadrupole instruments.

1. Introduction

Cancer is one of the most extensive causes of death in the world. Thanks to advanced understanding of cancer cell biology e.g. signaling pathways that are deregulated in tumor cells as well as finding of mutations in oncogenes and grow suppressor genes, novel multiple targets and approaches for the treatment of malignancies have been identified.

Particularly small molecule inhibitors of mutant tyrosine kinases became significant promise for upcoming era of targeted therapy. Tyrosine kinases (TKs) play central role in regulation of essential processes in cell cycle especially in differentiation, proliferation, growth and apoptosis. Any disturbance of TK activity leads to several human disorders including leukemia, and solid tumors such as gliomas, tumors of bladder, lung, head, neck and breast [1,2].

X-ray crystallographic studies of the TKs' tertiary structure enabled and accelerated selective drug development [3]. In recent years, a large number of tyrosine kinase inhibitors (TKIs) have been developed and many preclinical and clinical studies have been reported. Imatinib mesylate (formerly STI571) has been developed as the first and currently has been the most extensively investigated TKI. This small molecule inhibits c-Abl, PDGFR, and c-Kit TKs, hence it is used for the treatment of gastrointestinal stromal tumors, mastocytosis, hypereosinophilic syndrome and myeloproliferative disorders, especially chronic myeloid leukemia [1,4]. Resistance or intolerance of imatinib (IMA) remains the most serious problem associated with the failure of treatment in patients with suboptimal response. Second-generation tyrosine kinase inhibitors (TKIs) such as dasatinib (DAS) and nilotinib (NIL) are a possible solution of this problem. Both of these have shown significant clinical activity in patients with CML who became resistant or intolerant to IMA or other therapies [5,6]. Other TKIs such as lapatinib (LAP), erlotinib, gefitinib, trastuzumab and others are used for the treatment of many disorders e.g. breast, colon, lung and other solid tumors [2,7].

Therapeutic drug monitoring (TDM) is widely useful important tool for improving the treatment benefits by evaluation of patient

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compliance with therapy, individualizing daily dosage because of inter-patient variability, diagnosing of undertreatment, avoiding toxicity, monitoring and detecting of drug–drug or food–drug interactions and guiding withdrawal of therapy. TDM has also been shown to be cost-advantageous for many drugs [8]. Several studies proved correlation between IMA through plasma levels and clinical response [9–14]. In general, patients with optimal treatment response have higher IMA plasmatic concentration than patients with suboptimal response.

Methods for the detection and quantification of IMA and other TKIs in human plasma have been published [15–18]. The most commonly used technique is liquid chromatography coupled with single or triple quadrupole mass spectrometry. Sample preparation is usually based on simple protein precipitation by organic solvents. Separations are performed on short C18 RP columns with a typical particle size of 5 μm. Analyses for simultaneous quantification of TKIs take several minutes, including the column wash and re-equilibration steps [15,16]. We developed an ultra-high-performance liquid chromatographic method for TKI determination with a separation time of 2.2 min. High effectivity was made possible by 1.7 μm C18 RP particles [17]. Another approach covers separation on HILIC column with short analysis time of 1 min [18]. Compounds are usually ionized by electrospray in positive mode. In tandem mass spectrometry, products of molecular ion fragmentation are detected in multiple reaction monitoring (MRM) mode. Quantification is mostly based on the addition of deuterated internal standards [16,17].

Isotope dilution direct injection method coupled with tandem mass spectrometry became one of the essential techniques for quantitative determination of small molecules in clinical practice. It is extensively used in newborn screening programs [19–21] and plays significant role in diagnosing of various metabolic disorders. Number of metabolites in human body fluids serves as biomarkers for many types of diseases including prostate and colorectal cancer [22,23], Crohn's disease [24], cardiologic defects [25] or selected metabolic disorders [26–29].

Commonly MRM mode is used for accurate measurement. However some small analytes offer poor analytical parameters due to interferences in biological extracts and MRM^3 mode can be applied with advance. This approach provides easy, highly selective and extremely sensitive analyses [30–32].

In this work we developed and validated a fast and inexpensive isotope dilution direct injection method coupled with tandem mass spectrometry for the simultaneous determination of TKIs in human plasma without the need of chromatographic separation using common sample preparation.

2. Materials and methods

2.1. Chemicals and reagents

Imatinib mesylate, nilotinib, and dasatinib were purchased from LC Laboratories (Woburn, MA, USA) and deuterated standards (D8-imatinib, D6-nilotinib, D8-dasatinib, D4-lapatinib-ditosylate) and lapatinib from TLC PharmaChem (Vaughan, Ontario, Canada). Formic acid, ammonium hydroxide, water, and methanol were all LC/MS grade and were purchased from Sigma (St. Louis, MO, USA); dimethylsulfoxide (DMSO) was obtained from Lachema (Brno, Czech Republic). Plasma samples of healthy volunteers and patients in tri-potassium salts of ethylenediaminetetraacetic acid (K_3EDTA) were obtained from Hemato-Oncology Clinic, University Hospital Olomouc (Czech Republic). The internal quality control (IQC) samples for imatinib, nilotinib, and dasatinib were obtained from Chromsystems (Munich, Germany), and the external quality control (EQC) samples for imatinib were obtained from Service de pharmacologie clinique (Center Hospitalier Universitaire de Bordeaux, France).

2.2. Standard solutions, quality control samples

Stock solutions of IMA mesylate and D8-IMA were prepared by dissolving them in methanol to a yield concentration of 1 mg/mL expressed as free substances. IMA was further diluted in methanol to obtain solutions with concentration levels in the range 0.1–100 μg/mL. The internal standard was finally diluted in methanol to a working concentration of 22.5 ng/mL. NIL, DAS, LAP, and their deuterated internal standards (D6-NIL, D8-DAS, D4-LAP-ditosylate) were dissolved in DMSO to a yield concentration of 1 mg/mL expressed as free substances. All the drugs were subsequently diluted in methanol to obtain a concentration of 0.01–10 μg/mL for DAS, 0.1–100 μg/mL for NIL, 0.1–200 μg/mL for LAP, 50 ng/mL for D8-DAS, 100 ng/mL for D6-NIL, and 75 ng/mL for D4-LAP. The calibration standards and controls were prepared from drug-free plasma from healthy volunteers enriched with particular analytes to the required concentration. All the solutions were stored at −20 °C. Lyophilized IQC samples were dissolved in 2 mL of pure water and the definite aliquot was processed by a standard procedure as described below. The EQC plasma sample aliquots were processed in the same way.

2.3. Sample preparation

This study was conducted in accordance with the Helsinki Declaration, and the protocol was reviewed by the hospital ethics committee. All the patients who were examined gave their informed consent to participation in the study before blood sampling. The blood from healthy control volunteers and patients treated with TKI was put into a test tube with the addition of an anticoagulant (EDTA), and subsequently centrifuged (1200 × *g*; 5 min). A small volume of the plasma (20 μL) was precipitated by methanol enriched by an adequate internal standard (180 μL) in 1.5 mL Eppendorff tubes. Subsequently, the sample was put into a sonicator for 1 min, shaken for 5 min, cooled for 30 min at −20 °C, and centrifuged for 5 min at 14,300 × *g*. The supernatant was placed into 350 μL glass vial (12 mm × 32 mm, fused insert) and directly injected into a mass spectrometer or stored in a freezer at −20 °C before analysis.

2.4. Isotope dilution direct injection analysis

TKI plasma levels were measured using UltiMate 3000 RS (Dionex, Sunnyvale, CA). The samples were maintained in a thermostated autosampler rack with the temperature set to +5 °C during analysis.

In order to prevent carryover of plasma samples and enhance ionization organic solvents with addition of formic acid are commonly used for direct injection analyses [33]. Based on the physical and chemical properties of TKIs and deproteination solvent the mobile phase consisting of methanol and 0.1% formic acid was chosen. The flow rate was set at 0.30 mL/min for 0.00–0.12 min and 0.40–0.60 min; in the measuring period between 0.12 and 0.40 min the flow rate was reduced to 0.03 mL/min. The samples (0.5 μL) were injected directly into a mass spectrometer without using chromatographic separation. In order to obtain maximum sample throughput with optimized multiple injection in one analysis the flow rate was increased to 0.5 mL/min and injections of all samples were performed in sequence every 19 s.

Table 1
Optimized mass spectrometry parameters for analyzed compounds.

	MRM transition	DP (V)	CE (V)	CXP (V)
IMA	494.0 → 394.1	16	39	34
IMA	494.0 → 217.2	16	35	18
D8-IMA	502.0 → 394.1	16	39	34
D8-IMA	502.0 → 225.2	16	35	18
NIL	529.9 → 289.1	41	43	24
NIL	529.9 → 259.1	41	77	22
D6-NIL	536.3 → 295.1	41	39	8
D6-NIL	536.3 → 266.1	41	69	18
DAS	488.9 → 401.0	31	41	34
DAS	488.0 → 232.0	31	57	14
D8-DAS	497.0 → 406.0	31	41	34
D8-DAS	497.0 → 237.0	31	57	14
LAP	580.9 → 365.0	36	53	32
LAP	580.9 → 350.0	36	53	28
D4-LAP	586.1 → 366.0	36	47	26
D4-LAP	586.1 → 351.9	36	61	24

2.5. Tandem mass spectrometry

All the experiments were performed on a QTRAP 5500 triple quadrupole instrument (AB Sciex, Foster City, CA, USA). Mass spectrometric detection of the analytical run was monitored in positive MRM and MRM^3 modes. The dwell times of two MRM transitions between the precursors and product ions for each compound and each appropriate internal standard were set to 30 ms. The parameters of the ion source were optimized to the following settings: an ionization spray voltage of 5500 V, curtain gas of 30 psi, heater gas of 40 psi, turbo ion spray gas of 40 psi, a source temperature of 350 °C, and entrance potential of 10 V. High-purity nitrogen was used as the collision gas. The gas pressure for collision-activated dissociation was adjusted to "medium settings". Declustering potential, collision energy, and collision cell exit potential were optimized on standards of the analytes under study in a methanolic solution of 0.1% formic acid. All the parameters are detailed in Table 1. Both quadrupoles (Q1 and Q3) were set to unit resolution. The Analyst 1.5.1 software (AB Sciex, USA) was used for tuning the mass spectrometry parameters and data evaluation and quantification on the basis of the ratio of the corrected peak area of the compound and its deuterated internal standard. In order to exclude possible interferences the ratios of the two m/z transitions for each analyte and its deuterated standard were determined.

Due to low signal-to-noise ratio in MRM mode of DAS the MRM^3 mode was used to improve sensitivity and the selectivity of the method. In order to achieve secondary fragmentation of first product ion, the third quadrupole operated in the linear ion trap (LIT) mode. In this experiment, the protonated DAS (m/z 488.0) was isolated as a first precursor in the first quadrupole and fragmented in collision cell (the second quadrupole) to a first product using collision gas flow at the medium instrument setting, a collision energy of 41 V, and a declustering potential of 50 V. The first product was subsequently trapped in the third quadrupole (working in LIT mode) using a dynamic LIT fill time of up to 250 ms and an excitation time of 25 ms and fragmented under an excitation energy of 0.13 V, giving the most intensive second product ion at m/z 232.0. Finally, the MRM^3 ion transition of 488.0 → 401.0 → 232.0 was monitored for the quantification of DAS. LIT was set to perform a mass scan centered at m/z 232.0 with a mass window of 0.5 Da (231.75–232.25 Da).

2.6. Method validation

The method validation was based on the recommendations published by the Food and Drug Administration (FDA) [34], and European Medicine Agency [35]. Validation procedure was performed for all inhibitors in one method by the use of 0.3 and 0.03 mL/min flow rate. In order to obtain maximum speed, finally we tested multiinjection method with flow rate of 0.5 mL/min and compared it with LC–MS/MS method.

2.6.1. Linearity, limit of detection, limit of quantification

The plasma of healthy volunteers, with the addition of 10, 30, 100, 300, 1000, 3000, and 10,000 ng/mL of IMA and NIL, 3, 10, 30, 100, 300, and 1000 ng/mL of DAS, and 25, 50, 250, 500, 2500, 5000, and 10,000 of LAP in triplicates was used for the construction of the calibration curves. For the quantification and calculation of the linear regression model (least-square method) fitted by $1/x$ weighting, the concentration as dependence between the peak area ratio of the TKI/internal standard and concentration was used. The linearity slope, intercept, correlation coefficient, and standard deviation were calculated. The sensitivity of the method was determined on blank plasma with addition of low TKI standard concentration: 30 and 10 ng/mL for IMA and NIL, 10 and 5 ng/mL for DAS, and 25 and 10 ng/mL for LAP to reflect the lowest clinically relevant concentrations based on previously published pharmacokinetics data [36]. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as a signal-to-noise ratio of 3 and 10, respectively; the bias and coefficient of variation were determined on LOD/LOQ concentration levels (n = 10, spiked plasma).

2.6.2. Imprecision, recovery, accuracy

Intra- and inter-day imprecision and recoveries were measured using plasma samples added to 3000, 1000, and 300 ng/mL for IMA and NIL, 300, 100 and 30 ng/mL for DAS, and 5000, 2500, and 500 ng/mL for LAP in six replicates within one day and consecutive six days, respectively. Means and standard deviations were calculated by the ANOVA method; recovery was expressed as a percentage of target value on IQC samples. Accuracy was measured using IQC for IMA, NIL, DAS on two concentration levels, and EQC in 15 samples for IMA.

2.6.3. Ion suppression

Quantitative determination of matrix effects was performed on six different blank plasma samples with the addition of TKIs to the same final concentration as for imprecision evaluation. Ion suppression was calculated as the ratio of signal intensities of a plasma sample supplemented by TKIs to the final concentration equal to the standard solution and the standard solution of TKIs dissolved in methanol.

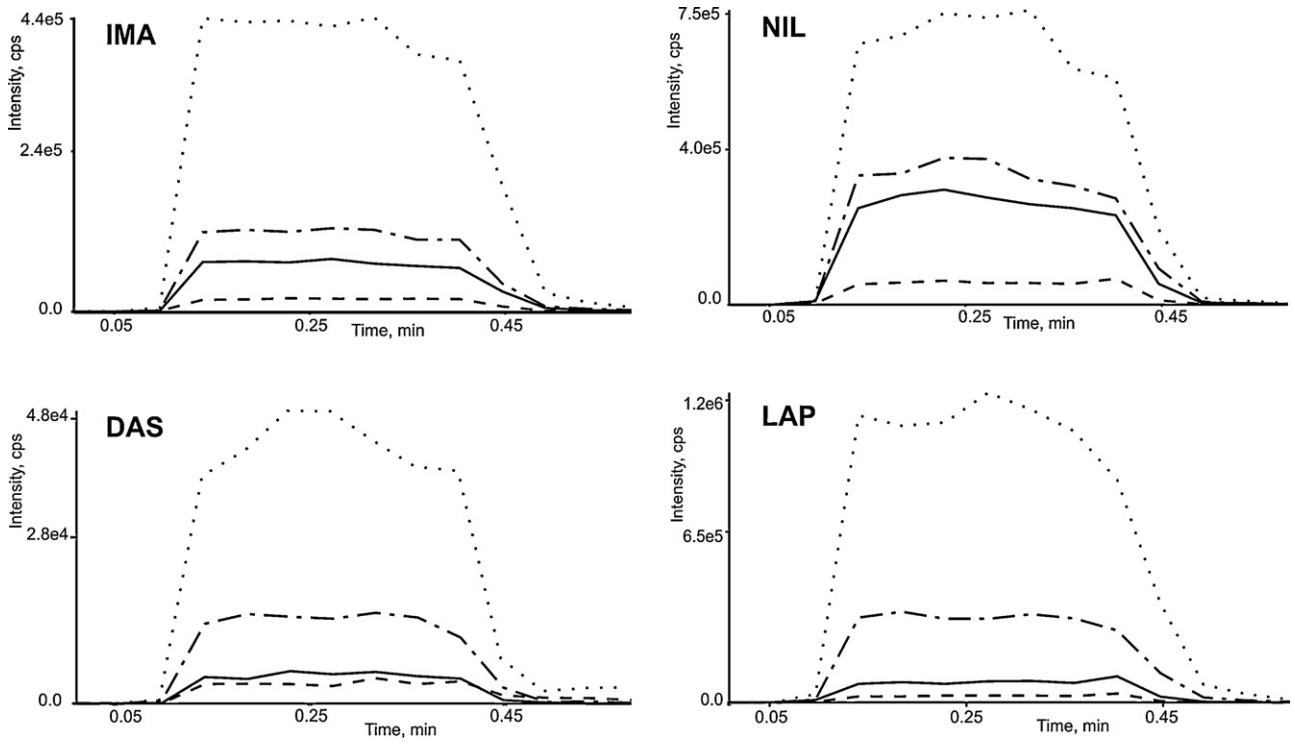

Fig. 1. Representative direct injection analysis-data of IQC (IMA, $c = 1911$ ng/mL; NIL, $c = 1184$ ng/mL; DAS, $c = 252$ ng/mL) and added plasma (LAP, $c = 2500$ ng/mL) samples measured in MRM mode. Dotted, dot-dashed, full and dashed lines correspond to first and second transitions of analytes and deuterated internal standards, respectively (see Table 1).

2.6.4. Comparison of the direct injection method with liquid chromatography

Developed method was compared with our previously published LC–MS/MS method [17,37]. Plasma samples ($n = 28$) were analyzed and the results were evaluated by Bland–Altman plot and regression analysis. The equation of linear regression, correlation coefficient, mean difference from zero, and standard deviation were calculated.

3. Results and discussion

3.1. Sample preparation

On the basis of previously published methods [15,38,39] we chose an easy, cheap, and effective way to prepare the plasma samples for direct injection into a mass spectrometer with high efficiency. This simple sample preparation by protein precipitation using organic solvent without any additional cleaning steps is sufficient and usable for the high-throughput bioanalysis of TKIs in human plasma. In order to remove proteins before analysis deproteination by organic solvents compatible with mass spectrometry were tested. In comparison to acetonitrile tenfold amount of methanol followed by cooling offered homogenous precipitate with clear supernatant. Under these conditions we analyzed more than 1000 plasma samples without any reduction in the mass spectrometer sensitivity.

3.2. Isotope dilution direct injection method

The developed method allows the simultaneous determination of the most commonly used tyrosine kinase inhibitors in chronic

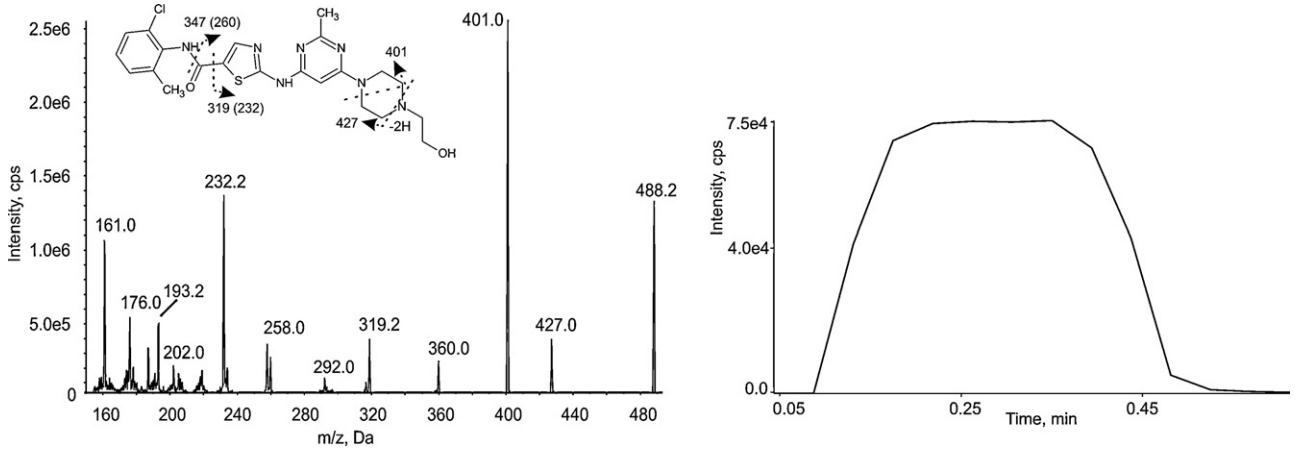

Fig. 2. Mass spectrum and MRM^3 direct injection analysis-data of dasatinib ($c = 252$ ng/mL).

Table 2
Validation – linearity ($n = 3$), LOD ($n = 10$) and LOQ ($n = 10$) of TKIs.

Analyte	Linearity $y = a$ (SD) $x + b$ (SD)	LOD (ng/mL)	LOQ (ng/mL) (bias, CV, %)
IMA	$y = 1.37e^{-4}$ ($1.68e^{-6}$) $x + 8.45e^{-4}$ ($1.64e^{-3}$) ($r = 0.9986$)	1.64	5.45 (−12.58, 24.04)
NIL	$y = 3.39e^{-4}$ ($5.91e^{-6}$) $x + 1.51e^{-3}$ ($1.88e^{-3}$) ($r = 0.9976$)	1.30	4.35 (−5.90, 12.22)
DAS	$y = 1.12e^{-2}$ ($1.22e^{-4}$) $x + 6.75e^{-2}$ ($3.54e^{-3}$) ($r = 0.9990$)	0.29	0.96 (9.59, 12.22)
LAP	$y = 6.24e^{-4}$ ($1.15e^{-5}$) $x + 1.86e^{-2}$ ($3.04e^{-2}$) ($r = 0.9964$)	1.36	4.55 (14.33, 10.67)

myeloid leukemia and breast cancer treatment – IMA, NIL, DAS, and LAP in human plasma – by flow injection analysis coupled with tandem mass spectrometry. A representative direct injection analysis data of the plasma samples of particular TKIs is shown in Fig. 1. Transitions of IMA, NIL, and LAP offer a high signal-to-noise ratio and MRM mode is fully suitable for determination in plasma samples from patients. The transition of DAS provides significantly lower ionization and higher noise, which results in insufficient sensitivity in MRM mode (Fig. 1 DAS). In addition, plasma samples from patients on DAS treatment contain concentration levels that are ten times lower as a result of the lower dosage and faster pharmacokinetics. Therefore we applied MRM^3 mode and we obtained excellent sensitivity with high selectivity of DAS. On the other hand, for one transition an MRM^3 period consumes a cycle time of 2.0 s. Together with the MRM period, we obtained 2.6 s per one cycle and 14 data points in one analysis. As a result of time consumption, MRM^3 mode has limited use in fast multianalyte chromatographic analyses where it is necessary to achieve more than ten data points per compound. Mass spectrum and direct injection analysis data for DAS are shown in Fig. 2.

Whereas the analyses for the frequently used LC–MS/MS methods for simultaneous TKI determination take few minutes, the total analysis time of the isotope dilution direct injection method we developed is 55 s. This includes injection of the sample (20 s) and measurement with a washing step (35 s). Signal intensities are one order lower in comparison with liquid chromatography methods as a result of the higher ion suppression. But this has negligible effect on the accuracy and precision of the method because of the utilization of an deuterated internal standard.

In order to obtain more sensitive and accurate results, the flow rate was reduced from 0.3 mL/min to 0.03 mL/min (0.12–0.40 min). This affords longer time and more points for the data acquisition and lower ion suppression. For compounds measured in MRM mode the acceptable ratios of peak areas of two transitions were set to 3.2–3.6, 3.4–4.0, 1.8–2.2, 4.3–4.9, 3.1–3.6, and 2.7–3.3 for IMA, D8-IMA, NIL, D6-NIL, LAP, and D4-LAP, respectively. We found no data ratio of m/z transitions outside the set limits for any of the measured samples. The variation in the peak area of internal standards was in the range 1.4–6.5% (intra-day).

Multiinjection analysis that we developed by the use of higher flow rate of 0.5 mL/min enables measuring of 32 samples during 10 min (Fig. 3). Calculated concentrations were compared with results from routine used UHPLC–MS/MS method by Bland–Altman graph and regression analysis. Calibrators, quality control samples and patient plasma samples on IMA therapy were analyzed by pair test. Comparison by Bland–Altman plot showed mean difference of −4.27 ng/mL and standard deviation of 46.89 ng/mL. Regression analysis provided correlation equation of $y = 1.0144x - 13.003$ and a correlation coefficient of 0.991. High throughput of the method is caused by no delay of new analysis start and direct samples injection in sequence of each 19 s. Dwell time of 150 ms for IMA measurement offers 15 points per peak which is reliable for accurate and precise quantitation.

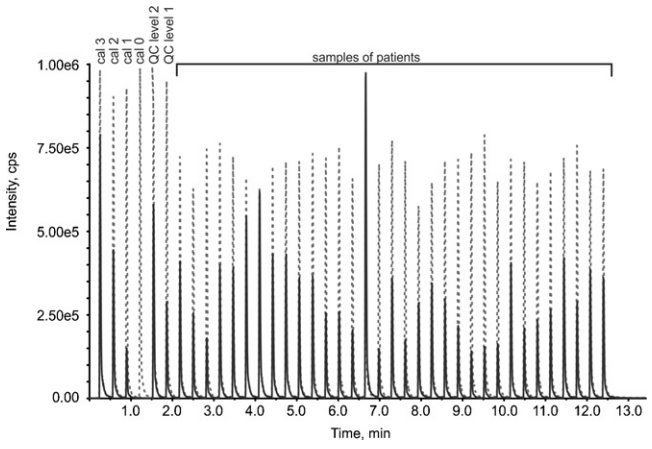

Fig. 3. Flow multipleinjection analysis of IMA plasma samples. Intenzities of dashed line of internal standard correspond to differences in ion suppressions in the plasma samples which is about 25%.

3.3. Validation

In order to validate the linearity of the method, LOD, LOQ, imprecision, analytical recovery, and accuracy were determined. The method offers linear dependences over entire ranges with correlation coefficients $r > 0.994$. The limits of quantification better than 6 ng/ml for analytes measured in MRM mode (IMA, NIL, LAP) are well below the clinically relevant range of concentration encountered in patients (typically > 100 ng/ml). In the case of DAS MRM^3 mode LOQ was five times lower, which corresponds to the requirement of higher sensitivity as a result of lower plasma levels (Table 2). Intra- and inter-day imprecisions and accuracies expressed as variation coefficient and bias are summarized in Table 3. Applied to IQC and EQC blood samples, the method offers excellent bias and standard deviation (Table 4). The stability of TKIs at different conditions was described in previously published studies. All of the analytes are stable in plasma at least five months stored at −20 °C with maximum loss of 10% of the nominal concentration [15,16].

Ion suppression determined using plasma samples added to low, medium, and high concentrations of TKI in six replicates was significantly higher in comparison to liquid chromatography methods as a result of the ionization of many suppressing substances in the

Table 3
Intra- and inter-day imprecision and accuracy ($n = 6$).

Analyte	Concentration (ng/mL)	Imprecision (CV, %)		Accuracy (bias, %)	
		Intra-day	Inter-day	Intra-day	Inter-day
IMA	3000	3.18	2.47	−0.28	1.95
	1000	3.50	4.03	1.12	3.97
	300	3.87	4.93	1.07	−3.11
NIL	3000	4.16	2.48	−1.72	−0.67
	1000	4.16	1.89	5.54	5.67
	300	4.53	6.93	−1.73	−1.81
DAS	300	3.90	3.67	0.17	1.61
	100	4.31	3.33	2.53	1.85
	30	4.36	5.36	1.22	0.06
LAP	5000	3.27	4.01	−0.50	−0.29
	2500	4.17	3.24	1.13	0.69
	500	2.83	4.30	−0.67	2.93

Table 4
Accuracy of IQC and EQC blood samples.

Analyte	Level (ng/mL)	Accuracy	
		(bias, %)	(CV, %)
IMA-iqc	941	2.40	4.29
	1911	1.34	3.68
NIL-iqc	711	0.15	5.58
	1184	−1.00	4.13
DAS-iqc	116	0.93	5.78
	252	2.62	5.59
IMA-eqc	80–6000	1.62	7.37

ion source during the same analysis time. Ion suppressions were 66.5–74.1%, 77.8–86.9%, 54.0–60.0%, and 65.3–84.0% for IMA, NIL, DAS, and LAP, respectively. Thanks to the use of deuterated internal standards, the matrix effects were eliminated.

The direct injection analysis and LC methods were compared by Bland–Altman plots and regression analysis. Quality controls and samples from patients were analyzed by paired test. Bland–Altman plot showed mean difference of −12.5, 0.02, −2.55, −20.00 ng/mL and standard deviation of 39.22, 21.73, 8.36 and 113.24 ng/mL for IMA, NIL, DAS and LAP, respectively. Regression analyses provided a linear correlation with slope of 1.023, 1.005, 0.995, 1.029, intercept of −3.158, −4.395, 2.881, 5.773 and correlation coefficient of 0.997, 0.999, 0.994 and 0.998 for IMA, NIL, DAS and LAP, respectively.

4. Conclusions

Targeted therapy using IMA, NIL, DAS, and LAP, based on the inhibition of protein tyrosine kinases, represents an evolving concept in therapeutic strategies. The monitoring of TKI plasma levels has become an essential tool for the evaluation of response to the treatment and for the management of CML or breast cancer patients. The aim of our study was to develop a high-throughput method for the determination of TKI plasma levels. In comparison with previously published methods using HPLC–MS/MS, our method has a short analysis time of 55 or 19 s in multiple injection settings as a result of the exclusion of the separation step, and sample preparation based on deproteination with an organic solvent is relatively simple. Therefore, the determination of several major anticancer drugs in plasma with the isotope dilution direct injection mass spectrometry method is rapid, sensitive, selective, and high-throughput, requires a small amount of plasma sample, and has a markedly reduced requirement for consumable expenditures.

In addition, we applied MRM^3 mode on the 5500 QTRAP in order to achieve the significantly better selectivity and sensitivity that is necessary for the correct quantification of low-level DAS. MRM^3 mode can be a new approach to the determination of drugs with a low concentration level, where MRM mode detection is not sufficient as a result of the poor ionization or the presence of interferences.

In laboratories this method can be used instead of conventional HPLC–MS/MS because of easier method switching. The criteria for linearity, precision, accuracy, and recovery have been proven to be within the recommendations of the FDA and EMEA guidelines for the validation of bioanalytical methods [33,34]. Consequently, this method is suitable for the use in routine clinical practice and it could be useful for the therapeutic drug monitoring of patients treated with IMA, NIL, DAS, or LAP, especially for the evaluation of patient adherence to daily oral therapy, the efficacy of treatment, severe drug-related adverse events, drug–drug interaction, or the relationship between pharmacokinetics and pharmacodynamics.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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