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Determination of imatinib (Gleevec®) in human plasma by solid-phase extraction–liquid chromatography–ultraviolet absorbance detection

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Abstract

A sensitive HPLC method has been developed for the assay of imatinib in human plasma, by off-line solid-phase extraction followed by HPLC coupled with UV-Diode Array Detection. Plasma (750 µl), with clozapine added as internal standard, is diluted $3 + 1$ with water and subjected to a solid-phase extraction on a C18 cartridge. After matrix components elimination with 2000 µl of water (in two aliquots of 1000 µl), imatinib is eluted with 3×500 µl MeOH. The resulting eluate is evaporated under nitrogen at room temperature and is reconstituted in 180 µl 50% methanol. A 50 µl volume is injected onto a Nucleosil 100–5 µm C18 AB column. Imatinib is analyzed using a gradient elution program with solvent mixture constituted of methanol and water containing both 0.05% ammonium acetate. Imatinib is detected by UV at 261 nm. The calibration curves are linear between 0.1 and 10 µg/ml. The limit of quantification and detection are 0.05 and 0.01 µg/ml, respectively. The mean absolute recovery of imatinib is 96%. The method is precise with mean inter-day CVs within 1.1–2.4%, and accurate (range of inter-day deviations -0.6 to $+0.7\%$). The method has been validated and is currently being applied in a clinical study assessing the imatinib plasma concentration variability in a population of chronic myeloid leukemia- and gastro-intestinal stromal tumor-patients.
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1. Introduction

Imatinib mesylate (Gleevec®) (Fig. 1) has revolutionized the treatment of chronic myeloid leukemia (CML) and gastro-intestinal stromal tumor (GIST). Imatinib, previously known as STI571, was rationally designed to inhibit the Bcr-Abl tyrosine kinase, the fusion oncoprotein resulting from the $t(9, 22)$ translocation which gives rise to the Philadelphia chromosome, the hallmark of CML [1]. Imatinib was also found to inhibit potently the autophosphorylation of two additional tyrosine kinases: c-Kit involved in the oncogenesis of GIST [2], and platelet-derived growth factor receptor (PDGFR), involved in the pathogenesis of the hypereosinophilic syndrome [3].

Gleevec® was approved by the FDA for CML in an unprecedented time of 72 days because of the impressive results obtained during the initial phases I and II clinical trials [4]. A recent phase III trial confirmed that Gleevec® induces a very high percentage of hematologic and cytogenetic responses in chronic phase CML [5]. These studies showed however less impressive responses with patients in accelerated phase and blastic crisis [1]. Other clinical trials have also showed an important anti-tumor activity of Gleevec® in GIST and in the hypereosinophilic syndrome [2,3].

Resistance to Gleevec® develops in variable proportions of patients, especially in CML in accelerated or blastic phase [1,6]. Cellular mechanisms of resistance include point mutations at the protein Bcr-Abl, or alternately, *BCR-ABL* gene amplification, or activation of survival signaling pathways. Resistance can also be caused by an increase in efflux of imatinib, mediated by the drug transporter P-glycoprotein (P-gp), the gene product of *MDR1* [7,8]. As far as the
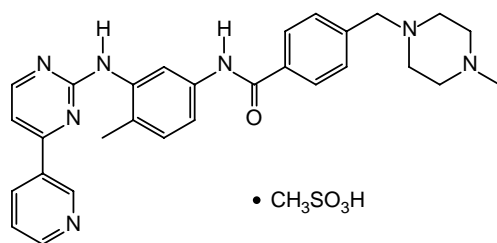
Fig. 1. Chemical structure of imatinib mesylate.

influence of *MDR1* is concerned, we have recently shown a marked impact of Pgp expression on imatinib intracellular concentrations [9].

Other mechanisms of resistance have also been incriminated [1,10] including host-dependent factors such as modulation of imatinib α1-glycoprotein binding and/or enhanced drug metabolism [6]. Imatinib is mainly metabolized by the cytochrome P4503A4 (CYP3A4) [4], whose enzymatic activity presents a large inter-individual variability and is susceptible to induction or inhibition by numerous co-medication, environmental and dietary constituents.

To examine whether the resistance to imatinib treatment could be explained in some patients by alterations in imatinib pharmacokinetics, a prospective observational study has been initiated for assessing the variability of imatinib plasma levels in CML and GIST patients. For that purpose, we had to set-up a robust assay for measuring imatinib on a semi-routine basis.

Two methods to quantify imatinib in human or primate plasma have been published last year [11,12], using liquid chromatography coupled to tandem mass spectrometry, preceded either by protein precipitation [11] or solid-phase extraction [12]. Since MS/MS facilities are not always available in standard hospital laboratories, a LC method with single-quadrupole MS has also been recently proposed [13], under the assumption that UV detection was not sensitive enough for its application to clinical studies. Just recently however, an HPLC-UV method has been briefly mentioned as a part of an imatinib pharmacokinetic study [14]. To the best of our knowledge, however, no report has yet been published describing the validation of an HPLC-UV method for the assay of imatinib in biological fluids.

We report here a sensitive method for the assay of imatinib in human plasma, by off-line solid-phase extraction followed by HPLC coupled with UV-Diode Array Detection. This assay reaches the required level of sensitivity and reproducibility for routine clinical application.

2. Materials and methods

2.1. Chemicals

Imatinib mesylate was kindly provided by Novartis (Basel, Switzerland). Clozapine (Internal Standard, I.S.)

stock solution (250 μg/ml) in MeOH was obtained by extraction with MeOH of Leponex® (Novartis, Basel, Switzerland) tablet. This solution was diluted before use. Methanol (MeOH) for chromatography LiChrosolv®, and ammonium acetate (NH₄Ac) GR for analysis were from E. Merck (Darmstadt, Germany). All other chemicals were of analytical grade and used as received. Ultrapure water was obtained from a Milli-Q® UF-Plus apparatus (Millipore).

2.2. Chromatographic system

The chromatographic system consisted of a Hewlett-Packard 1050 (Agilent, formerly Hewlett-Packard, Germany) pump connected to a spectrophotometric UV-Vis DAD 1050 detector set at 261 nm. This wavelength corresponds to the maximal absorption of imatinib in the chromatographic analysis conditions. The separation was performed at room temperature (RT) on a ChromCart® cartridge column (125 mm × 4 mm i.d.) filled with Nucleosil 100–5 μm C18 *AB* (Macherey-Nagel, Düren, Germany) equipped with a guard column (8 mm × 4 mm i.d.) filled with the same packing material. The injection volume was 50 μl.

The HP-ChemStation A.06.03 software was used to pilot the HPLC instrument and to process the data. Baselines were visually inspected and were manually adjusted using peak start and end features of the HP-ChemStation software.

2.3. Mobile phase solutions

Solvent A consisted of water containing 0.05% (w/v) of NH₄Ac and solvent B was methanol containing also 0.05% (w/v) of NH₄Ac. The mobile phase was delivered at 1 ml/min and the gradient program conditions are given in Table 1.

2.4. Stock solution, working solution, plasma calibration and control samples

Stock solution of imatinib mesylate was prepared at a concentration of 1 mg/ml (calculated as base) in 100% methanol and was appropriately diluted with 50% methanol for the preparation of working solutions at concentrations of 2–200 μg/ml. Plasma calibration samples at 0.1, 0.3, 0.9, 2.5, 5.0, 10.0 μg/ml, together with plasma control samples

Table 1
Gradient elution program

Time (min)	Solvent A (%)	Solvent B (%)	Flow (ml/min)
0	80	20	1.0
20	50	50	1.0
36	40	60	1.0
36.5[a]	0	100	2.0
40.5[a]	0	100	2.0
41[a]	80	20	1.0
45[a]	80	20	1.0

[a] Rinsing/re-equilibration program.

at 0.2, 4.5, 9.0 µg/ml, were prepared by 1:20 dilution of the respective working solution to blank plasma (total added volume ≤10% of the biological sample volume), in accordance with the recommendations on bioanalytical method validation [15,16].

The working solutions were stored at −20 °C and the calibration and control samples were prepared on the day of the analysis.

The I.S. stock solution at 250 µg/ml was diluted to 105 µg/ml with 50% methanol prior use.

2.5. Plasma collection

According to a study protocol previously approved by the Ethics Committee of the University Hospital, blood samples were obtained from CML and GIST patients on their scheduled visits. Blood samples (5 ml) were collected in Monovettes® (Sarstedt, Nümbrecht, Germany) with K-EDTA as anticoagulant. Samples were centrifuged at 1850 × g (3000 rpm) for 10 min at +4 °C (Beckmann Centrifuge, Model J-6B), plasma separated and transferred into polypropylene test tubes before being stored at −20 °C up to the time of analysis.

2.6. Sample preparation

Samples of 750 µl of plasma (calibration, control) were diluted with 200 µl of H_2O and 50 µl of diluted I.S. solution (providing thus a clozapine plasma concentration of 7.0 µg/ml) in an Eppendorf microvial. The resulting solution was vortexed and centrifuged for 10.0 min on a benchtop centrifuge at 20,000 × g (14,000 rpm) (Hettich® Benchtop Universal 16R centrifuge, Bäch, Switzerland). Plasma samples from patients were processed by the same manner.

The clean up procedure of diluted plasma samples was performed by solid-phase extraction (SPE) using a 24 tubes vacuum manifold Macherey-Nagel (Düren, Germany). The C18 cartridges of 100 mg packing with 40–45 µm diameter and 60 Å porosity (Supelclean LC-18, Supelco) were conditioned with 2 ml of methanol (in two aliquots of 1 ml) followed by 2 × 1 ml water. An aliquot (900 µl) of the diluted plasma sample was applied onto the cartridge and drawn through completely under vacuum (ca. 2.5 mmHg).

The cartridge was washed twice with 1000 µl of H_2O and a light vacuum (about 10 mmHg) was applied thereafter for 5 min. Imatinib and clozapine were subsequently eluted with 1500 µl of methanol (in three aliquots of 500 µl), followed by a final drying step (about 10 mmHg vacuum). The eluted solutions were evaporated to dryness under nitrogen steam at room temperature for approximately 45 min, and the residue was reconstituted in 180 µl of 50% methanol. The resulting solutions were carefully vortexed and centrifuged at 20,000 × g for 10 min. Aliquots (80 µl) of the supernatants were introduced into 200 µl HPLC microvials (Hewlett-Packard, Germany) and a volume of 50 µl was injected onto the HPLC column.

2.7. Calibration curves

Quantitative analysis of imatinib was performed using the Internal Standard method.

The calibration curves were obtained by $1/x$ weighted least-squares linear regression of the peak-area ratio of imatinib to clozapine (I.S.), versus the ratio of the injected amount of imatinib to I.S., in each standard samples. The calibration was established over the range 0.1–10.0 µg/ml, where the response ratio/amount ratio was linear.

Clozapine was chosen as internal standard because it shares some structural similarity with imatinib (heterocyclic base with a piperazinyl group). It has already been used as I.S. for the dosage of other basic drugs [17,18].

2.8. Analytical method validation

The validation of the method was based on the recommendations published as a Conference Report of the Washington Conference on "Analytical methods validation: Bioavailability, Bioequivalence and Pharmacokinetic studies" [15] and of the Arlington Workshop "Bioanalytical Methods Validation—A revisit with a Decade of Progress" [16]. Recent SFSTP (*Société Française des Sciences et Techniques Pharmaceutiques*) recommendations were also considered [19].

Each level of the calibration curve was measured with two sets of calibrators: one set at the beginning and a second set at the end of the HPLC run. When series of patient analysis were performed, control samples at three concentrations levels (low, medium and high (i.e. 0.2, 4.5 and 9.0 µg/ml)) were assayed every seven samples.

Quality control samples were used for the precision and accuracy determination, the precision being calculated as the coefficient of variation (C.V.%) within a single run (intra-assay) and between different assays (inter-assays), and the accuracy (i.e. in fact, the inaccuracy, or bias) as the percentage of deviation between nominal and measured concentration.

The limit of quantitation (LOQ) in plasma was experimentally chosen as the minimal concentration in plasma samples which could be confidently determined in accordance with the Arlington Workshop [16], recommending that the deviation between measured and nominal concentration at LOQ should not deviate more than ±20%.

The limit of detection (LOD) was considered as the concentration of imatinib that provides a signal corresponding to three times the HPLC background signal.

2.9. Stability of imatinib

Stability studies of imatinib included:

(a) Stability of plasma samples kept frozen at −20 °C: imatinib levels of aliquoted quality control plasma samples (i.e. at 0.2, 4.5 and 9.0 µg/ml) were measured each

month during 12 months. The variations of imatinib concentrations were expressed in percentage of the starting levels measured on the first day of storage.

(b) Stability of imatinib in *plasma* samples at room temperature: the concentration of imatinib in QC plasma samples were measured immediately after preparation and after being left at room temperature for 24 and 48 h.

(c) Stability of imatinib in *blood* samples left at room temperature: anticoagulated (citrate or EDTA) blood samples spiked with 0.2, 4.5 and 9.0 µg/ml of imatinib were left at room temperature. Aliquots (2 ml) were taken at 0, 24, 48, 72 and 96 h, and immediately centrifuged at $1850 \times g$ for 10 min at $+4\,^\circ$C. The plasma was collected and analyzed for imatinib according to the procedure described above.

(d) Stability of *plasma* samples after multiple freeze-thaw cycles: aliquots of plasma spiked with 0.2, 4.5 and 9.0 µg/ml of imatinib, underwent three freeze-thaw cycles: frozen samples were allowed to thaw at ambient temperature for 2 h and were subsequently refrozen during approximately 24 h. The imatinib levels were measured in aliquots from the three consecutive freeze-thaw cycles. The variations of imatinib concentrations were expressed in percentage of the starting levels measured at the beginning of the stability study.

(e) Stability of plasma extracts into HPLC vials at room temperature: processed samples (i.e. reconstituted in 50% methanol) containing imatinib at low, medium and high concentration were analyzed immediately after preparation and after being left 24, 48 and 72 h at room temperature in the autosampler rack.

2.10. Recovery

The efficiency of the solid-phase extraction was determined with control samples at 0.2, 4.5 and 9.0 µg/ml of imatinib. The absolute recovery of imatinib from plasma was obtained as the peak-area response of imatinib in the processed samples, expressed as a percentage of the response of the same amount of imatinib (contained in the 50 µl-injection volume) directly injected onto the HPLC column. The absolute recovery was similarly calculated for the internal standard clozapine.

2.11. Selectivity

The selectivity of our analytical method was determined by injecting onto the HPLC column 22 drugs currently prescribed to CML- or GIST-patients at our hospital: acetylsalicylic acid (Aspirin[®]), diclofenac (Voltaren[®]), acetaminophen (Panadol[®]), tramadol (Tramal[®]), tizanidin (Sirdalud[®]), diltiazem (Dilzem[®]), verapamil (Isoptin[®]), atenonol (Tenormin[®]), torasemide (Torem[®]), acenocoumarol (Sintrom[®]), ranitidin (Zantic[®]), omeprazol (Antra[®]), metoclopramid (Primperan[®]), fluconazole (Diflucan[®]), voriconazole (Vfend[®]), prednisone (Prednisone[®]), fluoxetine (Fluctine[®]), citalopram (Seropram[®]), lorazepam (Temesta[®]), oxazepam (Seresta[®]), zolpidem (Stilnox[®]), allopurinol (Zyloric[®]).

The selectivity was also ascertained with the above-mentioned extraction procedure using blank plasma samples from six healthy subjects [15] to ascertain that no endogenous peak would interfere with imatinib signal.

2.12. Application of the HPLC method

This method is currently used in a research protocol approved by the local Ethics Committee aimed at assessing the interindividual and residual intraindividual variability of imatinib in CML- or GIST-patients, using a population pharmacokinetics approach, and at determining the relation between imatinib plasma concentration and the clinical outcome (relapses, initial/secondary failure, side effects).

3. Results and discussion

3.1. Chromatograms

The proposed HPLC method enables the measurement of imatinib in plasma with UV detection set at 261 nm. With the gradient program used (Table 1), the retention times for imatinib and clozapine are 23 and 33 min, respectively. The gradient elution program yields sharp peaks without any significant drift of the baseline.

Fig. 2 shows the chromatogram of a blank plasma, using the gradient program reported in Table 1, at the same scale as Fig. 4. Fig. 3 presents the chromatogram of the same blank plasma spiked with 2.5 µg/ml of imatinib and 7.0 µg/ml of clozapine (I.S.), which represent a concentration commonly encountered in our patient population. Finally, Fig. 4 shows the chromatographic profile of another blank plasma spiked with 0.2 µg/ml of imatinib and 7.0 µg/ml of clozapine (I.S.), corresponding to the lowest quality control concentration. Even at this low concentration the peak has a sharp shape and is easily detectable.

A HPLC run of 45 min duration (including the rinsing and re-equilibration step) is necessary to achieve a satisfactory separation of imatinib from signals arising from
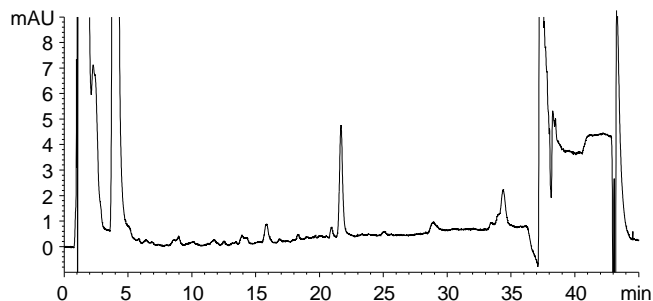

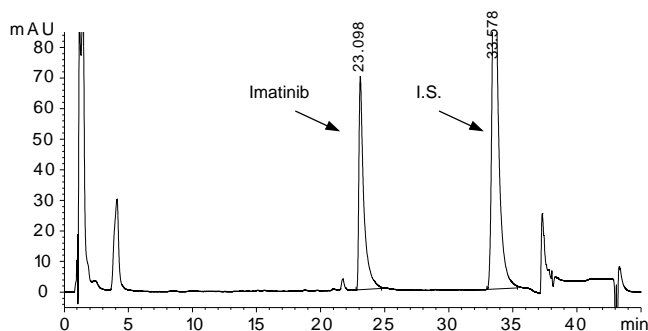Fig. 2. Chromatographic profile of a blank plasma.

Fig. 3. Chromatographic profile of a blank plasma spiked with 2.5 µg/ml of imatinib and 7.0 µg/ml of clozapine.

endogenous plasma components and drugs likely to be taken by this population of patients. Of note, the small peak appearing at 21.5 min is observed in all samples (including blank MeOH/H$_2$O solutions subjected to SPE) and arises from the packing material smearing from SPE cartridge during water rinsing and methanol elution; it does not interfere with the imatinib signal at 23 min.

3.2. Mobile phase composition

Peak tailing is a common phenomenon observed with basic nitrogen-containing molecules eluted on reversed-phase column by high-performance liquid chromatography [20,21]. Thus, a Nucleosil 100–5 µm C18 *AB* column, i.e. a base deactivated column specifically designed for minimizing the peak tailing of base compound [22], was used. Furthermore, ammonium acetate was added to the mobile phase (solvent A and B) to reduce peak tailing by saturation of the stationary phase residual silanols [23].

3.3. Calibration curves

The standard curves for imatinib are satisfactorily described by $1/x$ weighted least-squares linear regression. The slope of the calibration curves is stable, with values averaging 1.25 ($\pm$2.9%) ($n = 6$) during the method validation. Over the concentration range 0.1–10.0 µg/ml,
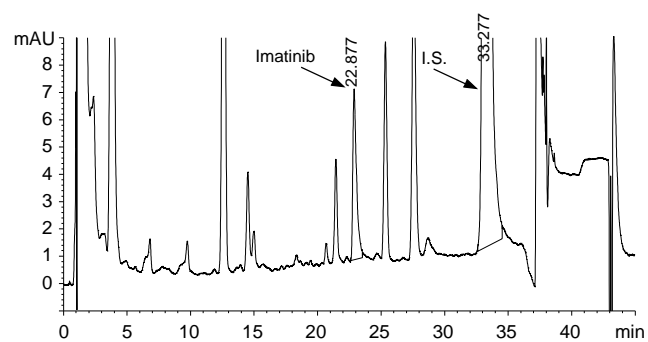
the determination coefficient r^2 of the calibration curves remained always greater than 0.998.

There was originally some concern that the calibration samples prepared with citrated plasma (collected from blood from outdated transfusion bag) may not fully reflect the plasma matrix from CML and GIST patients collected on EDTA Monovettes®. However, getting blood on EDTA from volunteers solely for the purpose of calibration samples preparation is unpracticable and difficult to justify from an ethical point of view. A few reports have been indeed published, showing significant differences in concentrations measured for some drugs, related to the medium (serum versus plasma) or to the anticoagulant used [24–26]. For the sake of validation, the cross-validation has been performed between three series of the three levels of QC and three series of calibration samples (citrate versus EDTA) The results of the head-to-head comparison show that the anticoagulant influences significantly the imatinib results ($P < 0.05$, multi-way ANOVA). This difference was however quantitatively low and highly reproducible at every level (with a mean of 5.0% and a S.D. of 0.04%). Considering this, if the analysis of EDTA samples rather than citrate samples is performed with a citrate calibration, it is thus advised to correct the results accordingly.

3.4. Validation: precision, accuracy and LOQ/LOD

Precision and accuracy determined with the control samples are given in Table 2. The levels of control samples (0.2, 4.5 and 9.0 µg/ml) were selected to encompass the range of concentrations expected in patients' plasma.

The mean intra-assay precision is similar over the whole concentration range and always less than 2.5%. Overall, the mean inter-day precision is good with average CVs within 1.1–2.4%. The intra-assay deviation (bias) from the nominal concentrations of imatinib is between −1.5 and +0.4% and the range of inter-day deviation always lower than 1.0%.

Calibration curves were established up to the highest pharmacologically relevant concentration of imatinib at 10 µg/ml, and at 0.0125, 0.025 and 0.050 µg/ml

Fig. 4. Chromatographic profile of the lowest QC plasma (0.2 µg/ml of imatinib and 7.0 µg/ml of clozapine).

Table 2
Precision and accuracy of the imatinib assay in plasma

Nominal concentration (µg/ml)	Concentration found (µg/ml)	S.D. ($\pm$)	Precision C.V. (%)	Accuracy[a] bias (%)
(A) Intra-assay ($n = 6$)				
0.200	0.199	0.003	1.3	−0.6
4.500	4.433	0.092	2.1	−1.5
9.000	9.034	0.199	2.2	0.4
(B) Inter-assay ($n = 6$)				
0.200	0.201	0.002	1.1	0.7
4.500	4.474	0.098	2.2	−0.6
9.000	9.042	0.217	2.4	0.5

[a] (Found − nominal)/nominal × 100.

for the lowest calibration level. This enables to determine experimentally the lower limit of quantification (LLOQ) at 0.05 μg/ml. With a calibration between 0.05 and 10.0 μg/ml, the precision (C.V.) of the calibration sample at 0.05 μg/ml is 6.4% and the accuracy (i.e. bias, calculated by back-calculation) at this lowest calibration level is 15.5%. Both values are thus comprised within the ±20% limit recommended by the Conference Report [16]. The limit of detection of imatinib with this assay is 0.01 μg/ml, which corresponds to a signal (0.31 mAU) equals to about three times the blank background signal at the retention time of imatinib (0.12 mAU) (Fig. 2).

3.5. Samples stability

3.5.1. Stability at −20 °C

No evidence of imatinib decomposition was found during plasma samples storage in the freezer at −20 °C for at least 12 months. At 12 months, imatinib at low, medium and high quality control levels corresponds to 96.6, 97.4 and 97.2% of their respective initial value (Table 3). This is in good accordance with the good stability observed for imatinib prepared in EDTA plasma [11]. This indicates that patient plasma samples can be confidently stored at –20 °C for a long period of time prior to HPLC analysis.

3.5.2. Stability at room temperature

The stability of *plasma* samples left at room temperature was ascertained up to 48 h. At the concentrations of 0.2, 4.5 and 9.0 μg/ml, the variations over time of imatinib levels were always less than ±5% (Table 4), indicating that at room temperature, the plasma samples are stable for at least 48 h.

The stability of imatinib in *blood* samples left at room temperature for 96 h was checked. The variations of the levels of imatinib, at the concentrations of 0.2, 4.5 and 9.0 μg/ml, respectively, was always less than ±5% (Table 4) for at least 96 h, irrespectively of the anticoagulant used (citrate or EDTA). This is of importance when shipment of blood samples is considered such in the case of multi-centric studies.

Table 3
Stability of imatinib in plasma samples kept frozen at −20 °C (concentration variations expressed as the % of the starting levels)

Duration (months)	Nominal concentration (μg/ml)		
	0.2	4.5	9.0
1	−0.8	−3.8	−4.0
2	−1.5	−2.2	−2.1
3	2.5	2.0	4.7
4	−1.6	−2.1	4.1
5	2.8	−2.4	1.5
6	0.4	3.6	0.9
9	0.8	0.5	3.5
12	−3.4	−2.6	−2.8

Table 4
Stability of imatinib in plasma, blood and extracts samples left at RT for 24, 48, 72 or 96 h

Duration (h)	Medium at RT	Nominal concentration (μg/ml)		
		0.2	4.5	9.0
24	Plasma	−1.7	−4.4	−2.3
	Blood (citrated)	−1.8	−4.0	−1.2
	Blood (EDTA)	1.0	0.9	−3.5
	Extract	1.7	1.4	1.0
48	Plasma	−2.8	−4.4	−4.7
	Blood (citrated)	−1.4	−0.9	−2.7
	Blood (EDTA)	2.7	2.1	0.8
	Extract	3.9	1.8	1.1
72	Plasma	NA	NA	NA
	Blood (citrated)	−2.5	0.4	−4.0
	Blood (EDTA)	3.3	2.4	2.0
	Extract	2.9	3.1	3.4
96	Plasma	NA	NA	NA
	Blood (citrated)	−2.3	0.6	−1.9
	Blood (EDTA)	3.3	4.7	4.9
	Extract	NA	NA	NA

Concentrations change expressed as the % of the starting concentration (NA: not available).

3.5.3. Stability after one, two and three freeze-thaw cycles

The variations of imatinib concentrations in QC samples subjected to successive freeze-thaw cycles are reported in Table 5. This indicates that no significant loss of imatinib is to be expected after up to three freeze-thaw cycles.

3.5.4. Stability of extract samples into HPLC vials at room temperature

The stability of plasma extracts (i.e. reconstituted in MeOH/H$_2$O 50:50) submitted to HPLC analysis was checked at room temperature for 72 h and is reported in Table 4. The variations over time of imatinib, expressed as percentage of the initial level, were again within ±5%. These results indicate that, taking into account the analytical variability, the processed samples are stable throughout HPLC analysis performed within up to 3 days.

3.6. Recovery

The mean absolute recovery of imatinib measured with the low, medium and high quality controls are 96.4 ± 2.8, 96.6 ± 2.3 and 96.0 ± 1.9%, respectively. The clean-up

Table 5
Stability of imatinib in plasma samples after multiple freeze-thaw cycles

Cycles	Nominal concentration (μg/ml)		
	0.2	4.5	9.0
1	2.3	−2.6	−4.2
2	−0.6	−2.0	−3.3
3	0.2	−1.7	−2.8

Concentration change expressed as the % of the starting concentration.

procedure by SPE is reliable in eliminating interfering material from plasma, with high absolute recovery and low recovery variability.

The internal standard is fully recovered at the concentration spiked (7.0 μg/ml) and is characterized by a low variability: $101.7 \pm 4.4\%$.

3.7. Selectivity

At 261 nm, six different blank plasmas gave no significant interfering peaks at the retention time of imatinib. The small peak sometimes observed at about 34.5 min, i.e. near the retention time of clozapine (Fig. 2), accounts for only a negligible part of the total area of I.S. (less than 0.5%).

The analysis of 22 drugs prescribed in CML- and GIST-patients at our hospital confirmed the method selectivity. All drugs are eluted at retention times not interfering with imatinib determination, except for three psychotropic drugs (oxazepam, lorazepam and zolpidem) ($\Delta rt < 0.9$ min). For these three potentially interfering compounds, the entire extraction process has also been conducted with plasma samples containing these drugs at their highest concentration (C_{max}) [27]: 1.17 μg/ml, 0.04 μg/ml, 0.14 μg/ml, for oxazepam, lorazepam and zolpidem, respectively. The peak area for these compounds at their C_{max} may not be considered negligible, especially for oxazepam. However, since these drugs are mostly administered in the evening, their dosages quite small by comparison with imatinib, and their concentrations presumably low at time of sampling on next day, they should not interfere to a significant extent with imatinib measurements. More generally however, on-line spectra provided by the diode-array detection should be carefully examined at the time of imatinib elution, to exclude the presence of co-eluting peaks arising from other unusual comedication taken by patients. As far as the potentially interfering drugs are concerned, all samples from patients included in our clinical study are sent to the laboratory with a case report form (CRF) wherein all other drugs taken by patient during the last week are indicated. The three psychotropic drugs of concern -or any unusual drugs- can thus be therefore straightforwardly identified.

3.8. Clinical application of method

Besides being used for research purposes, for which more sophisticated methods have already been described [11–13], this HPLC assay could be used to guide clinicians when patho-physiological changes or initiation of a new drug treatment could influence circulating levels of imatinib. For example, a 41-year-old male patient suffering from CML in blast crisis was on Gleevec® 400 mg bid for 6 months. He developed a fungal infection and was treated with voriconazole. The patient experienced side effects, such as nausea, anemia, neutropenia and thrombocytopenia. A drug interaction between imatinib and the CYP3A4 inhibitor voriconazole was suspected and imatinib plasma level was measured.
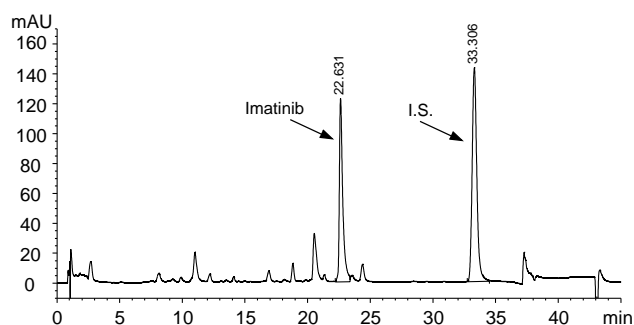

Fig. 5. Plasma chromatogram of a CML-patient obtained at 14 h 30 min after administration of imatinib (400 mg, bid).

Fig. 5 shows the chromatographic profile of the plasma obtained at 14 h 30 min after Gleevec® intake, with an imatinib concentration of 4.1 μg/ml, a particularly high value when compared to the range of concentrations measured over a whole dosing interval at steady state in patients [11]. The imatinib dose was reduced in this patient with a definite relief from ADRs. The causality remains to be formally demonstrated in this case, and it is certainly premature to state that patients may benefit from a formal therapeutic drug monitoring for imatinib. Nevertheless, this example suggests that drug interactions do occur with imatinib and may adversely affect its otherwise good tolerability.

4. Conclusions

This validated HPLC method provides a fairly robust procedure for determining imatinib in patients' plasma. Adapted from previously described MS-methods [11,12], it has been developed using instruments available in conventional hospital laboratories, only necessitating an UV-diode array detector set at 261 nm. We have demonstrated that UV detection provides the required level of sensitivity for measuring pharmacologically relevant concentration of imatinib in patients. The diode array feature is only necessary to exclude any possible interfering peak. To the best of our knowledge, this is the first report describing the validation of an assay of imatinib by HPLC-UV after solid-phase extraction from plasma. Despite the time needed for each analysis and the higher risk of interferences compared to mass spectrometry detection, this method provides a readily available, practicable, cheap and robust method for hospitals not having sophisticated instrumentation of an LC–MS.

Since plasma extract samples are stable at room temperature in the autosampler rack, the duration of the run does not represent a limitation of our method and the assay can be fully automated, requiring no tedious technical supervision. In our studies, batches of samples have been analyzed over 30 h in a row without problems. Using one devoted HPLC apparatus, it is possible to analyze more than 20 patient samples per run sequence.

The method is currently being applied on a semi-routine basis for the analysis of large number of plasma samples collected during an ongoing study on population pharmacokinetics of imatinib. Finally, this HPLC method has also been used, with minor modifications, for the measurement of intracellular levels of imatinib, as part of an ongoing research on the functional consequences of *MDR1* expression on imatinib intracellular concentrations [9].

Note added in proof

While in press, two reports [28,29] have just appeared describing an assay of imatinib in biological fluids, the latter [29] using a valve switching technique and UV detection.

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