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Quantification of the anticancer agent STI-571 in erythrocytes and plasma by measurement of sediment technology and liquid chromatography–tandem mass spectrometry

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Abstract

An isocratic high-performance liquid chromatographic method coupled to tandem mass spectrometry for the quantification of the revolutionary and promising anticancer agent STI-571 (tradenames Gleevec, Glivec, Imatinib) in blood plasma and red blood cells (RBCs) is described. The method involves measurement of sediment technology for RBCs and a subsequent single protein precipitation step by the addition of acetonitrile to both the RBC isolate and plasma. The sample mixture was centrifuged (10 min, 3600 *g*), and the supernatant filtered through a HPLC filter (0.45 μm). The analytes of interest, STI-571 and the internal standard [$^2H_8$]STI-571 were eluted on a Waters Symmetry C_{18} column (50×2.1 mm I.D., 3.5 μm particle size) using a methanol–0.05% ammonium acetate (72:28, v/v) mixture. STI-571 and [$^2H_8$]STI-571 were detected by electrospray tandem mass spectrometry in the positive mode, and monitored in the multiple reaction monitoring transitions 494>394 and 502<394, respectively. The lower limit of quantitation of STI-571 was 2.1 ng/ml in RBCs and 1.8 ng/ml in plasma. The recovery from both plasma and RBCs was between 65 and 70%. The method proved to be robust, allowing simultaneous quantification of STI-571 in RBCs and plasma with sufficient precision, accuracy and sensitivity and is useful in monitoring the fate of this signal transduction inhibitor in whole blood of cancer patients.

Keywords: Measurement of sediment technology; STI-571; Gleevec; Glivec; Imatinib

1. Introduction

The pathological activation of the Abelson tyrosine kinase (Abl) causes chronic myelogenous leukaemia (CML). A hallmark of CML is a reciprocal chromosomal translocation involving the long arms of chromosomes 9 and 22. This somatic mutation fuses a segment of the *bcr* gene, from chromosome 9, to

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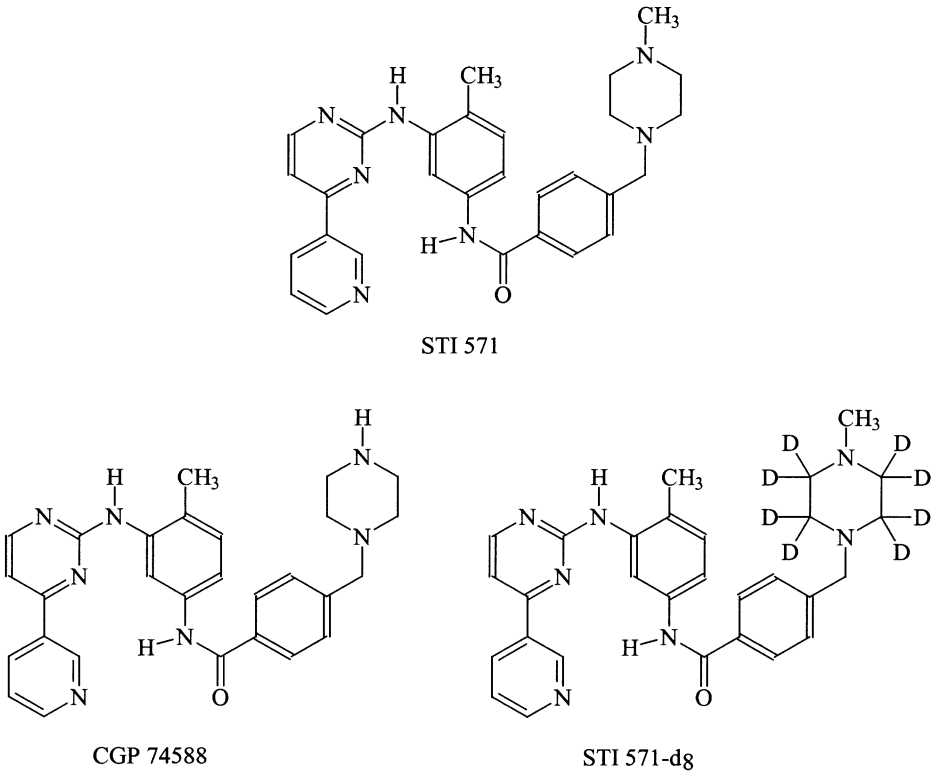

Fig. 1. Chemical structures of STI-571 (a), STI-571-d_8 (b) and the metabolite CGP 74588 (c).

a region upstream of the second exon of the *c-abl* gene from chromosome 22. *c-abl* encodes a non receptor tyrosine kinase that is strictly controlled in normal cells. However, Bcr-Abl fusion proteins have a constitutive catalytic activity in the transformation and further malignant development of cells. A series of inhibitors, based on the 2-phenylaminopyrimidine class of pharmacophores, exhibits intrinsic affinity and specificity for Abl. The small molecule inhibitor STI-571 (Fig. 1) is one of the most potent, and has proved effective in the treatment of CML, and also several solid tumors [1], particularly gastrointestinal stromal tumors (GISTs). In the latter type of tumors, STI-571 anticancer activity is based on interaction with c-kit, which is amongst key regulators in cellular transduction.

In order to support pharmacokinetic studies with sufficient speed, suitable analytical procedures are required. Methods of quantification in plasma have been described very recently [2,3]. A semi-automated solid-phase extraction procedure with liquid chromatography–tandem mass spectrometry (LC–MS–MS) was an initial development [2], but with the extension of clinical studies of this promising agent, a high-throughput system is needed. The assay was adapted to meet the demand, and has proved robust, allowing the simultaneous quantification of STI-571 and its main metabolite (CGP 74588) in human plasma at high rates of throughput [3].

However, it has been demonstrated during the past decade that plasma monitoring alone misses important information since blood cells [red blood cells (RBCs), erythrocytes], and sometimes other blood cells are not analyzed [4,5]. We therefore developed a device, based on measurement of sediment (MESED) technology (Fig. 2), to quantify substances of interest in sediments, such as RBCs [5].

In the case of blood, RBCs are by far the most important transport cells, as they have the largest theoretical volume and surface. It has been shown that RBCs

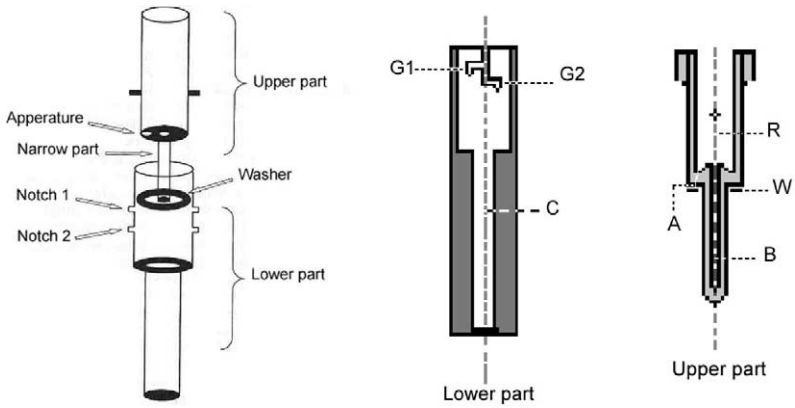

Fig. 2. The MESED tube has two parts. The left one is the reservoir R in which an aliquot of the sample is introduced. R fits in the right part, the container C, on two levels. At the low level the aperture A of R is closed by washer W. In the first centrifugation the sample is spun down into the chamber of known volume B and into the broad lower part of R above B. At the high level the aperture A is set free and in a second centrifugation the surplus of the sample is vented from R into c, leaving 100 μl of the sample in B behind. Finally, reservoir R was separated from C, inverted and inserted in a centrifuge tube. After centrifugation for a third time, erythrocytes were collected in the centrifuge tube. After hemolysis of the RBCs with distilled water and centrifugation, the supernatant was used for STI analysis.

can carry high quantities of anticancer moieties, such as the mustard species originating from the oxazaphosphorines (i.e., cyclophosphamide and ifosfamide), as well as other anticancer agents [6–16]. MESED is of particular interest in RBC analysis: it not only provides an opportunity for simultaneous RBC and plasma analysis, but also an opportunity for easy and fast mass analysis. A direct relation between mass and volume can than be obtained. In this report, the data of MESED technology coupled to LC–MS–MS analysis of STI-571 and possibly its metabolite(s) is presented for the first time. MESED–LC–MS–MS is routinely applied to monitor STI-571 and calculate the RBC versus plasma partition of the substance in patients blood.

2. Experimental

2.1. Reagents and materials

High-purity solvents were obtained from Acros Organics (Geel, Belgium). All other chemicals were commercially available and of analytical grade. Blank RBCs and plasma samples were obtained from volunteers and, as an individual control, from patients immediately before STI-571 treatment. All water used in the analyses was HPLC grade and obtained from Merck (Darmstadt, Germany). STI-571 and the internal standard [$^2H_8$]STI-571 (STI-571-d_8) were synthesized at Novartis (Basel, Switzerland). Filtration filters [polyvinylidene difluoride (PVDF) Acrodisc, Waters] were used to filter the supernatant obtained from RBCs and plasma. MESED tubes were employed in the clean-up procedure of RBC samples (Fabre, Kelmis, Belgium).

2.2. Mass spectrometry

A Micromass Quattro Ultima triple quadrupole mass spectrometer (Micromass, Manchester, UK) with electrospray ionization (ESI) in the positive ion mode was used for detection. The mass spectrometer was operated in the multiple-reaction monitoring (MRM) mode. The ESI desolvation temperature was 320 °C. The capillary voltage was 3.2 kV and the cone voltage 80 V. The cone gas flow was 71 l/h and the desolvation gas flow was 627 l/h. The MS collision gas was argon at $30{\cdot}10^{-3}$ mbar and the collision energy was 25 eV. In order to establish the appropriate MRM conditions for STI-571 and its deuterated analogue, solutions of the standards (0.1 mg/l in HPLC mobile phase) were infused into the mass spectrometer and the cone voltage (CV) optimized to maximize the intensity of the protonated molecular species

$[M+H]^+$. Collision-induced dissociation (CID) of each protonated molecule was performed. STI-571 and STI-571-d_8 were monitored in the MRM transitions 494 > 394 and 502 > 394, respectively.

2.3. Data processing

Quantitation was performed by integration of the area under the specific MRM chromatograms. The STI-571 concentration was calculated with reference to the integrated area of the respective deuterated analogue. The calibration curves were generated using linear least squares regression analysis of the data of calibration samples according to the equation $y = a + bx$ (where y is the peak area substance/peak area internal standard; x is the concentration of analyte in the calibration sample; a is the intercept; b is the slope of the regression line). A weighted (b/x^2) linear least-squares regression analysis was applied. Data of the quality control (QC) samples were derived from the regression equation of the calibration curve.

2.4. Chromatography

A C_{18} 50×2.1 mm (I.D.), 3.5 μm particle size Waters Symmetry column was preferred for RBC sediment analyses. A precolumn was not necessary. The LC system consisted of a Waters Alliance 2695 HPLC system, with a liquid chromatography pump using methanol–0.05% ammonium acetate (72:28, v/v) at 300 μl/min. The system operated at ambient temperature, and the injection volume was 10 μl. The autosampler syringe and its six-port valve were each rinsed twice with 100 μl of methanol–water (50:50) times. Degassing and synchronization of the HPLC and autosampler systems are integrated in the Waters Alliance system.

2.5. Assay procedure

STI-571 stock solutions for the calibration standards and QC samples were prepared separately in methanol, and stored at 4 °C. STI-571 concentrations in human RBC and plasma solutions were 1.00, 5.00, 20.0, 100, 200, 500, 1000, 5000 and 10 000 ng/ml. Reference solutions of STI-571 at a concentration of 50 μg/ml were prepared in methanol. The calibration standards were freshly prepared on each day by adding an appropriate aliquot of the spiking stock solution to 250 μl of blank human RBCs and plasma. The QC concentrations for STI-571 in human RBCs and plasma were 1.00, 2.0, 10.0, 50.0, 200 and 1000 ng/ml. Five batches of QC samples with different lot numbers were prepared as a pool, separated and stored at −20 °C pending analysis; five other batches were used for stability studies. Stock solutions of STI-571-d_8, the internal standard, were prepared in methanol yielding concentrations of 0.10 μg/ml and stored at +4 °C. Method validation was carried out over a period of 3 days.

Blood collected from patients and volunteers was prepared by MESED [4,5,8,14]. Fig. 3 shows the procedures for RBC and plasma sample pre-treatment.

The erythrocyte concentration results do not discriminate between adsorbed and incorporated species and the partition RBCs/plasma calculated have to be treated as minimum values [4,8]. Although an incom-

RBC sample pretreatment	**plasma sample pretreatment**
100μl RBC was collected by MESED	250 μl plasma
⇓	⇓
100 μl of erythrocytes	addition of 50 μl STI-571-d8 (25μg/ml)
⇓	⇓
addition of 400 μl of distilled water (cell lysis)	addition of 250 μl cold acetonitrile (protein precipitation)
⇓	⇓
addition of 50 μl STI-571-d8 (25μg/ml)	vortex
⇓	⇓
vortex	centrifugation of the precipitated proteins (10 min, 3600 g)
⇓	⇓
centrifugation of the cell debris (10 min, 3600 g)	filtration of the supernatant over a PVDF filter (0.45 μm)
⇓	⇓
further work-up of the supernatant as plasma work-up (with omission of addition of STI-571-d8)	LC-MS/MS analysis

Fig. 3. Procedures for RBC and plasma sample pretreatment.

plete recovery of active compounds from the RBCs cannot be excluded in vivo, repeated extractions of STI-571 from RBC lysates did not yield increased recoveries. RBC lysates and plasma can be frozen after the first centrifugation. Frozen samples were defrosted to room temperature, and centrifuged for 10 min at 14 000 rpm before processing.

2.6. Assay validation

For optimal comparisons with previously published data similar assay procedures to those followed by Bakhtiar and co-workers [2,3] were employed.

2.6.1. Linearity

The linearity of the method was evaluated over the concentration range of 1.00–10 000 ng/ml for STI-571 in RBCs and plasma. Calibration standards were prepared freshly in duplicate on each day of validation. The assay acceptance criterion for each back-calculated standard concentration was 20% deviation from the nominal. The level of acceptance of correlation coefficient for calibration curves was set at $\geq$0.95. Comparisons of the slope, intercept and correlation coefficient were performed for the 5-day validation process.

2.6.2. Intra- and inter-day accuracy and precision

The intra- and inter-day accuracy and precision of the assay was tested by analyzing six RBC and plasma QC samples in replicates of at least five on 5 separate days. Precision was expressed as the relative standard deviation (RSD). The intra-day accuracy and precision was calculated as the mean of all QC samples analyzed during a single analysis run (a minimum of five replicates of each QC concentration). The values were calculated for each day of validation separately. The inter-day accuracy and precision was calculated as the mean of all the QC samples analyzed during a 5-day validation (replicates of 16 for each QC concentration).

2.6.3. Recovery

The recoveries of the sample preparation methods were assessed by comparing the peak areas obtained from the analysis of unprocessed reference solutions and of processed human RBC and plasma samples. The recoveries of STI-571 from RBCs and plasma were examined at 1.00, 10.00, 100, 1000 and 2000 ng/ml.

2.6.4. Specificity

The specificity of the assay was investigated by obtaining ion-chromatograms of blank pooled human RBCs and plasma samples and blank samples spiked only with internal standard. Blank samples were prepared from both volunteer human blood and pretreatment blood samples from patients due to receive STI-571. They were analyzed in duplicate on each validation.

2.6.5. Freeze and thaw stability

RBC and plasma samples spiked with five different STI-571 concentrations were subjected to five unassisted freeze–thaw cycles and were analyzed in duplicate by the present LC–MS–MS assay.

2.6.6. Stability

The stability of five different concentrations of STI-571 in RBCs and plasma, and standard stock calibration solutions (methanol) stored at -20 and 4 °C, was evaluated over 52 weeks in triplicate.

3. Results and discussion

The recently available MESED device, enables the collection of an exact volume and mass of RBCs with collection of the associated solvent, i.e., blood plasma. The procedure only requires two centrifugation steps of 3000 rpm for 10 min and allows RBC and plasma analysis in a routine manner [4–12,14–16]. Guidelines for assay validation such as those of the US Food and Drug Administration (FDA), are only available for blood plasma. The assay of STI-571 described in this report is validated in both RBCs and plasma. To support pharmacokinetic studies with the necessary speed to meet target dates, MESED is of interest when concentration–time decays are derived from the cellular fraction of blood with RBCs having by far the largest theoretical volume. Even at low partitions ($\leq$5%), RBC analysis can be important if the agent of interest is combined with others, such as RAD in the recently started phase I study Gleevec (STI-571)+RAD. RAD is another kinase inhibitor, which is harvested to a large extent

by RBCs (~90%), and therefore possibly capable of influencing RBC uptake of STI-571. In the case of single agent STI-571, RBC analysis is of interest since STI-571 is given over prolonged periods. The partition between RBCs and plasma may change over time and influence plasma disappearance profiles. The present method requires an LC–MS–MS run time of about 2 min for both plasma and RBC samples. The difference between the pre-treatment procedures of RBC and plasma samples is negligible: RBCs can be separated easily [5] and subsequently exposed to HPLC water to release STI-571.

3.1. Linearity

The linearity and reproducibility of the STI-571 calibration curves in human RBCs and plasma were satisfactory between 1 and 1000 ng/ml. The mean coefficients of correlation for STI-571 in RBC and plasma over a 5-day validation were 0.999 and 0.998. Intercept values were typically less than 0.003 for both biological matrices. The mean ($n = 5$) calibration slopes for STI-571 in RBCs and plasma were 0.01099 ± 0.00152 and 0.01935 ± 0.00123, respectively. Mean accuracy of standard calibration samples for STI-571 covering the concentration of 1.00–10 000 ng/ml in RBCs ranged between 93.6 and 103% with RSDs of 1.89–6.02%. Mean accuracy of standard calibration samples for STI-571, covering the concentration samples of 1.00–10 000 ng/ml in plasma, ranged between 97.6 and 103%. The lower limit of detection (signal-to-noise ratio 3:1) in RBCs amounted to 680 pg/ml and in plasma 496 pg/m*M*.

3.2. Intra- and inter-day results

The results from QC RBC and plasma samples covering the anticipated dynamic concentration ranges of the method, are shown in Table 1. The mean intra-day accuracies at the lower limit of quantitation (LLOQ) for STI-571 in RBC were between 99.22 and 95.7%, and for plasma were between 99.8 and 102%. Respective RSDs for RBCs and plasma were between 3.78 and 5.02% and 5.74% and 2.39%. Above the LLOQ, the mean intra-day accuracies of QC RBC and plasma samples ranged from 95.7 to 103% and 99.8 to 102%, respectively. The corresponding mean ($n = 5$) inter-day accuracies and RSDs were not essentially different from the inter-day data (data not shown).

Table 1
Percentage accuracy and precision (RSD) of STI-571 QC samples in human RBCs and plasma

	Nominal concentration (ng/ml)						
	1	2	10	50	200	1000	10 000
RBCs							
Mean	99.2	95.7	98.2	103	99.7	102	103
SD	3.56	4.66	5.16	7.59	6.58	5.36	6.58
RSD (%)	3.78	5.02	5.79	8.01	7.68	5.59	6.41
Plasma							
Mean		102	99.8	105	99.5	103	102
SD		5.72	2.38	7.59	4.69	6.55	6.01
RSD (%)		5.74	2.39	6.93	4.58	103	5.78

Nominal concentrations were between 1.00 and 10 000 ng/ml. The means, SDs and RSDs (%) of the data collected per day×5 days are given.

Overall, the assay exhibited similar accuracies and reproducibilities for plasma as reported by Bakhtiar and co-workers [2,3]. For RBCs it can be seen that the data are not essentially different from those of plasma indicating the small influence of the different biological matrix on the positive signal generation and high accuracy of the MESED procedure.

3.3. Recoveries

The estimated recoveries of STI-571 from RBCs and plasma using the present MESED-based assay, compared with the addition of known amounts to the blank biological matrices at the five different concentrations were 65 ± 3.7 and 67 ± 4.2% for RBCs and plasma, respectively.

3.4. Specificity

The specificity of the method was examined by analyzing blank RBCs and plasma extracts of both volunteer blood and those of patients collected immediately before starting STI-571 treatment. As shown in Fig. 4, no significant interferences from endogenous substances in RBCs were traced at the retention times of STI-571 and STI-571-d_8. The signals detected in RBCs were similar to those in plasma and analyte signals observed at the LLOQ in RBCs and plasma were comparable.

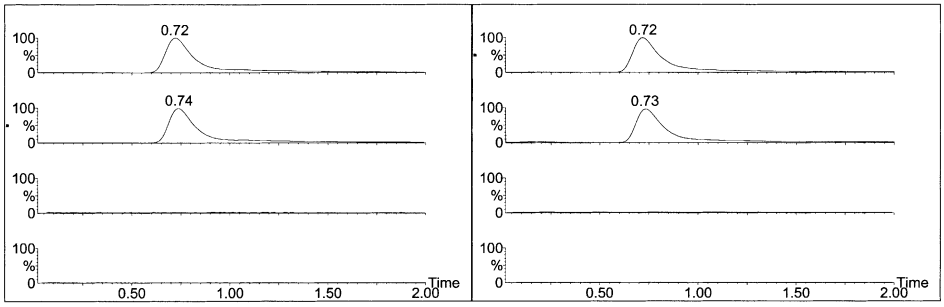

Fig. 4. HPLC–MRM-MS chromatograms for RBCs (left) and plasma (right). Top to bottom: MRM channels of 80 ng/ml STI, 80 ng/ml STI-d_8, blank STI channel, blank STI-d_8 channel. Time scale in minutes.

3.5. Freeze–thaw, room temperature, and storage stability

In the freeze–thaw stability study, RBCs, plasma and methanol–water solutions were subjected to five freeze–thaw cycles and subsequently analyzed in duplicate. STI-571 appeared to be stable in plasma and methanol–water solutions at the concentration ranges explored. However decreases of up to 20% in RBC levels were recorded from the second thawing step onwards.

The benchtop (room temperature) stability was secure for 48 h in RBCs, plasma and methanol–water solutions. There was no significant loss of STI-571 in RBCs, plasma and methanol/water solutions. Stock solutions were stable for 1 year at 4 °C.

Overall, the validation data of plasma and methanol–water solutions agree very well with those reported earlier by Bakhtiar and co-workers [2,3]. RBC validation data are totally new and therefore do not allow comparisons with data of previously published studies.

The validation data for STI-571 in RBCs and plasma underline the supremacy of MESED over alternative approaches to RBC analysis, such as subtraction techniques or raw collection of RBCs by pipetting [4,5]. Moreover, MESED is fast and only requires approximately 400 μl of total blood in delivering blood plasma and RBC (100 μl) [4,5]. The assay was apparently not hindered by the coelution of substances originating from the RBC matrix. We prefer C_{18} material, particularly for RBC analysis, rather than C_8, which has been used in previous reports [2,3]. With the smaller internal diameter used, some gain in sensitivity could be obtained in contrast to 4.6 mm I.D. columns used earlier [2,3]. This in part explains the lower LLOQ reported here for plasma.

3.6. Assay application

The present assay was used routinely to simultaneously monitor STI-571 in the RBCs and plasma of patients treated with this promising new drug. The HPLC column had to be changed every 800 samples (ca. 2000 injections). This is remarkable considering the more complex matrix of RBCs and the fact that no precolumn was used. This is in contrast to previous work [2,3], here a Phenomenex (Torrance, CA, USA), C_8 4.0 × 3.0 mm I.D. column was used. No significant ion-suppression was seen at the lower concentrations.

The LLOQs in plasma reported here are lower than those reported earlier [2,3]. For plasma, lower limits of detection may not be of interest; however, for RBCs lower LLOQs are required as STI-571 can operate in RBCs at much lower levels compared to those encountered in plasma. This can be important in studies of combination therapies, e.g., with the new kinase inhibitor RAD, where interactions can take place, and during long periods of treatment when the intrinsic capacities of RBCs can be changed [4–16].

The MESED device only requires 400 μl blood and with the emerging new nano- and picotechnology in mind, the device can be modified by a volume reduction up to 100-fold [17]. Such a device, only requiring 1–10 μl of blood, is capable of allowing frequent, easy, fingertip blood collections. We have been able to determine over 500 partition ratios of STI RBC/plasma in over 500 human samples using the present MESED–LC–MS–MS analytical tool.

A mean partition ratio of 9.5 ± 3.8% was calculated. In a simulated approach using a newly developed algorithm (paper in preparation), RBC vs. plasma curves were generated using data of our own studies as well as data published earlier [2,3]. The partition ratio varied with time between 0.12 and 0.67. The higher ratios were found at relatively high total blood concentrations. The background of this phenomenon is subject now of extended in vitro studies.

We report here a simple, fast method for the simultaneous quantitation of STI-571 in RBCs and plasma using a measurement of sediment approach by the new MESED device and LC–ESI-MS–MS. RBCs can be isolated quantitatively, with minimum technical difficulties and maintenance. The RBC matrix does not induce technical problems in the procedures, and the amount of organic fluids is reduced to a minimum. MESED–LC–MS–MS can be applied routinely to monitoring STI-571 in clinical sessions. It is an attractive procedure for high-throughput approaches to STI-571 in both the clinic and in animal experiments, were blood samples to be taken should be kept to a minimum.

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References

[1] A.T. van Oosterom, I. Judson, J. Verweij, S. Stroobants, E.D. di Paola, S. Dimitrijevic, M. Martens, A. Webb, R. Sciot, M. Van Glabbeke, S. Silberman, O.S. Nielsen, Lancet 358 (2001) 1421.
[2] R. Bakhtiar, L. Khemani, M. Hayes, T. Bedman, F. Tse, J. Pharm. Biomed. Anal. 28 (2002) 1183.
[3] R. Bakhtiar, J. Lohne, L. Ramos, L. Khemani, M. Hayes, F. Tse, J. Chromatogr. B 768 (2002) 325.
[4] M.S. Highley, E.A. de Bruijn, Pharm. Res. 13 (1996) 186.
[5] O. Driessen, M.S. Highley, P.G. Harper, R.A. Maes, E.A. de Bruijn, Clin. Biochem. 27 (1994) 195.
[6] M.S. Highley, G. Pattyn, H. Lambrechts, E.A. de Bruijn, A.T. van Oosterom, P.G. Harper, Eur. J. Cancer 31A (1995) 969.
[7] M.S. Highley, G. Momerency, K. Van Cauwenberghe, A.T. van Oosterom, D. Schrijvers, E.A. de Bruijn, R.A. Maes, P.G. Harper, Eur. J. Cancer 31A (1995) 942.
[8] G. Momerency, K. Van Cauwenberghe, M.S. Highley, P.G. Harper, A.T. van Oosterom, E.A. de Bruijn, J. Pharm. Sci. 85 (1996) 262.
[9] M.S. Highley, P.G. Harper, P.H. Slee, E.A. de Bruijn, Int. J. Cancer 65 (1996) 711.
[10] M.S. Highley, D. Schrijvers, A.T. van Oosterom, P.G. Harper, G. Momerency, K. Van Cauwenberghe, R.A. Maes, E.A. de Bruijn, M.B. Edelstein, Ann. Oncol. 8 (1997) 1139.
[11] D. Schrijvers, M.S. Highley, E.A. de Bruijn, A.T. van Oosterom, J. Vermorken, Anti-Cancer Drugs 10 (1999) 147.
[12] W.J. Loos, D.M. van Zomeren, H. Gelderboom, J. Verweij, K. Nooter, G. Stoter, A. Sparreboom, J. Chromatogr. B 766 (2002) 99.
[13] T. Kerbusch, V.M.M. Herben, M.J.J. Jeuken, J. Ouwekerk, H.J. Keizer, J.H. Beijnen, Biopharm. Drug Dispos. 22 (2001) 99.
[14] H. Dumez, M.S. Highley, G. Guetens, G. De Boeck, A. Hanauske, A.T. van Oosterom, R.A. Maes, E.A. de Bruijn, P.G. Harper, Semin. Oncol. 28 (2001) 24.
[15] H. Dumez, G. Guetens, G. De Boeck, M.S. Highley, U.R. Tjaden, R.A. Maes, A. Hanauske, A.T. van Oosterom, E.A. de Bruijn, J. Sep. Sci. 24 (2001) 123.
[16] H. Wildiers, G. Guetens, G. De Boeck, W. Landut, E. Verbeken, M.S. Highley, E.A. de Bruijn, A.T. van Oosterom, Int. J. Cancer 99 (2002) 514.
[17] G. Guetens, K. Van Cauwenberghe, G. De Boeck, R.A. Maes, U.R. Tjaden, J. Van Der Greef, E.A. de Bruijn, J. Chromatogr. B 739 (2000) 139.