Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 791 (2003) 39–44
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Liquid chromatographic–mass spectrometric assay for quantitation of imatinib and its main metabolite (CGP 74588) in plasma

Robert A. Parise[a], Ramesh K. Ramanathan[b], Michael J. Hayes[c], Merrill J. Egorin[a,b,d,*]

[a]*Program of Molecular Therapeutics and Drug Discovery, University of Pittsburgh Cancer Institute, Biomedical Science Tower, E-1040, 200 Lothrop Street, Pittsburgh, PA 15213, USA*
[b]*Division of Hematology/Oncology, Department of Medicine, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA*
[c]*Department of Preclinical Safety, Novartis Institute for Biomedical Research, Hanover, NJ 07936, USA*
[d]*Department of Pharmacology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA*

Received 16 July 2002; received in revised form 8 January 2003; accepted 21 February 2003

Abstract

Imatinib mesylate (Gleevec, Glivec, STI571) is a targeted, small molecule inhibitor of the oncogenes, BCR/ABL and c-KIT, and has striking antitumor activity in patients with chronic myelogenous leukemia or gastrointestinal stromal tumors. We have developed a liquid chromatographic–electrospray ionization mass spectrometric (LC–MS) method for quantifying imatinib and its main metabolite (CGP 74588) in plasma. The assay uses deuterated imatinib as the internal standard; acetonitrile deproteination; a Phenomenex Luna C_{18}(2) (5 μm, 50×4.6 mm) reversed-phase analytical column; a gradient mobile phase of 0.1% formic acid in methanol and water; and mass spectrometric detection using electrospray positive mode electron ionization. The assay has a lower limit of quantitation (LLOQ) of 30 ng/ml and is linear between 30 and 10 000 ng/ml for both imatinib and CGP 74588. We demonstrated the suitability of this assay for imatinib using it to quantify the concentrations of imatinib and CGP 74588 in plasma of a patient given a 200-mg dose of imatinib orally. We believe that this LC–MS assay should be an important tool for future pharmacokinetic studies of imatinib.

Keywords: Imatinib; CGP 74588

1. Introduction

Imatinib mesylate [1], also known as Gleevec, Glivec, STI571, is a targeted, small molecule inhibitor of the receptor kinases c-KIT [2,3]and PDGF-R [2,4], the non-receptor kinases c-ABL and c-ARG, and the related fusion oncogene BCR/ABL [5–7]. Imatinib has striking activity in patients with BCR/ABL-positive chronic myelogenous leukemia (CML) [1,8–12] or gastrointestinal stromal tumors (GIST) that harbor c-KIT mutations [13–20]. Based on its activity against CML and GIST, imatinib is undergoing extensive evaluation for its activity against other tumor types [21,22] and its utility when combined with other agents. As a result of these activities, there is an ongoing, and in fact increasing, need for evaluation of imatinib pharmacokinetics so that issues such as pharmacokinetic/pharmacodynamic relationships, drug–drug interactions, and patient adherence to daily oral therapy can be evaluated. To

*Corresponding author. Tel.: +1-412-624-9272; fax: +1-412-648-9856.
E-mail address: egorinmj@msx.upmc.edu (M.J. Egorin).

1570-0232/03/$ – see front matter
doi:10.1016/S1570-0232(03)00206-X

date, two assays for imatinib have been published [23,24]. While these assays provided the sensitivity and speed that were necessary for initial dose escalation studies and pivotal registration trials of imatinib, each uses LC–MS–MS technology, which is unfortunately not widely available at sites likely to be performing the imatinib studies described above. Furthermore, LC assays with absorbance detection have proven insufficiently sensitive for application to clinical pharmacology studies of imatinib. The LC–MS assay for imatinib that is described in the current article was developed based on: (1) recognition of the great need to generate imatinib pharmacokinetic data; (2) understanding that such a need would require a facile, sensitive assay for imatinib and its main metabolite, CGP 74588; and (3) recognition of the need for such an assay to use less expensive and more widely accessible instrumentation than that required in previously published assays.

2. Experimental

2.1. Materials

Imatinib, imatinib-D_8, and CGP 74588 were supplied by Novartis Pharmaceuticals (East Hanover, NJ, USA). Methanol (Optima grade) and acetonitrile (Optima grade) were purchased from Fisher (Fair Lawn, NJ, USA). Formic acid (minimum 95%) was purchased from Sigma (St Louis, MO, USA). Control human plasma was prepared by centrifugation of outdated, citrate-anticoagulated blood obtained from the Central Blood Blank (Pittsburgh, PA, USA). Medical grade nitrogen and liquid nitrogen were purchased from Praxair (Pittsburgh, PA, USA).

2.2. Procedure

2.2.1. Sample preparation

Three triple standard curves were prepared as follows. Initially 1, 0.1, 0.01, and 0.001 mg/ml solutions of both imatinib and CGP 74588 were prepared in methanol–water (50:50, v/v). The appropriate amount of these solutions was then added to 0.2 ml of human plasma, in 1.5-ml microcentrifuge tubes, to produce final concentrations of 30, 100, 300, 1000, 3000, or 10 000 ng/ml imatinib and CGP 74588. As a result, samples containing 30, 300, or 3000 ng/ml included 1.5% methanol, and samples containing 100, 1000, or 10 000 ng/ml included 0.5% methanol. Ten microliters of a 10 μg/ml solution of internal standard (imatinib-D_8) in methanol were added to each tube. One milliliter of acetonitrile was then added to each tube, and the tubes were then vortexed briefly and centrifuged at 12 000 *g* for 5 min. One-milliliter aliquots of the resulting supernatants were transferred to 12×75 mm, borosilicate glass tubes and evaporated to dryness under a stream of nitrogen at 27 °C. Each dried residue was redissolved in 100 μl of methanol/distilled water (20:80, v/v), vortexed briefly, transferred to HPLC autosampler vials, and 3 μl were injected into the LC–MS system.

2.2.2. LC–MS system

The LC system consisted of an Agilent model 1100 Autosampler (Agilent Technologies, Palo Alto, CA, USA), an Agilent 1100 Quaternary pump, and a Phenomenex Luna C_{18}(2) (5 μm, 50×4.6 mm) reversed-phase analytical column (Phenomenex, Torrance, CA, USA). The mobile phase was a linear gradient consisting of 0.1% formic acid in both methanol and water with the following program:

Time (min)	Flow rate (ml/min)	Methanol (%)	Water (%)
0	1	20	80
6	1	60	40
7	2	100	0
9	2	100	0
10	2	20	80
13	2	20	80
13.1	1	20	80

Overall run time was 14 min. Column eluate was then split by a P-470 micro-splitter valve (Upchurch, Oak Harbor, WA, USA) so that 10% of the flow entered the mass spectrometer. Column eluate was analyzed with a ThermoFinnigan aQa Mass Spectrometer (ThermoQuest, San Jose, CA, USA) operating in electrospray, positive-single-ion mode to monitor 493.7 *m/z* for imatinib, 479.7 *m/z* for CGP 74588 and 501.7 *m/z* for the internal standard. The

insert probe temperature was set at 250 °C with 5000 V applied as the ion spray voltage and 10 V as the orifice voltage. Nitrogen gas flow was fixed by the tank head unit set at 75 p.s.i. (520 kPa). The system was operated with ThermoFinnigan Excalibur Software installed on a Gateway 2000 (N. Sioux City, SD, USA) Pentium II 300 MHz computer. The I.S. ratio was calculated for each standard by dividing the analyte peak area by the peak area of the internal standard. Standard curves of imatinib and CGP 74588 were constructed by plotting the I.S. ratio versus the known concentration of analyte in each sample. Standard curves were fit by linear regression with weighting by $1/y^2$, followed by back calculation of concentrations.

2.2.3. Demonstration of applicability to biological samples

To demonstrate the applicability of this LC–MS method to pharmacokinetic samples, it was used to quantify concentrations of imatinib and CGP 74588 in the plasma of a patient who received a 200-mg dose of imatinib orally. Prior to treatment, this patient gave written, informed consent as approved by the Institutional Review Board. Blood samples were collected into heparinized tubes before imatinib delivery and at 0.5, 1, 2, 3, 4, 8, 12, 24, 36, 48 and 72 h after administration of imatinib. Each sample was centrifuged at approximately 1000 *g* for 10 min, and the resulting plasma layer was stored at −70 °C until analyzed with the procedure described above. Imatinib and CGP 74588 concentrations were calculated by comparing the I.S. ratio measured for each sample to the weighted, linear function derived from the standard curve that related the I.S. ratio to imatinib or CGP 74588 concentration.

3. Results

With the chromatographic conditions described, imatinib, CGP 74588 and imatinib-D_8 eluted at approximately 4.0, 3.7, and 3.9 min, respectively (Fig. 1). There was baseline separation of imatinib and CGP 74588, and no endogenous materials interfered with measurement of any compound of interest. To assess the recovery of imatinib and CGP 74588 from plasma, three identical samples containing 100 or 10 000 ng/ml of each compound were analyzed as described above. When compared to three direct injections of an equivalent amount of either compound in mobile phase, the recovery of imatinib was 99±1% (mean±SD) whereas that for CGP 74588 was 85±1%. The assay also proved suitable for use with an autosampler as there was no

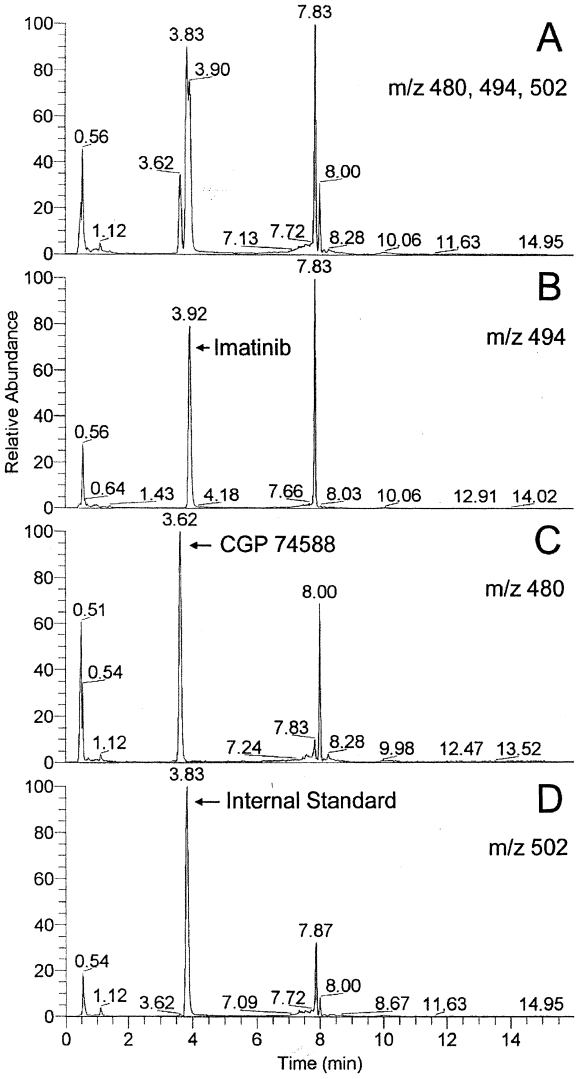

Fig. 1. Chromatogram of 1000 ng/ml imatinib, 1000 ng/ml CGP 74588, and 500 ng/ml imatinib-D_8 internal standard extracted from control, human plasma. (A) Total ion current monitoring *m/z* 480, 494, and 502; (B) monitoring *m/z* 494 (imatinib); (C) monitoring *m/z* 480 (CGP 74588); (D) monitoring *m/z* 502 (imatinib-D_8 internal standard).

decay in peak shape or area of imatinib, CGP 74588 or imatinib-D_8 solutions that were prepared in mobile phase and monitored during a 24-h incubation at room temperature. In addition, gleevec and CGP 74588 proved to be stable over 38 weeks stored at −20 °C and repetitive freeze–thaw cycles (24).

The correlation coefficients for three successive imatinib triplicate standard curves were 0.9924, 0.9916, and 0.9943, respectively. Back-calculated values for imatinib varied by 4–15 and 7–11% from the theoretical values of 30 ng/ml and 10 000 ng/ml, respectively. When expressed as percentage coefficient of variation, the within-day variation in imatinib triplicate standards was always <7.2% at all concentrations. Between-day variation in three, successive, triplicate imatinib standard curves was also minimal, as the coefficient of variation of the slopes associated with these repeated standard curves was 1.63%. Also, the coefficients of variation were 5.2 and 2.2% at 100 and 10 000 ng/ml, respectively, when these concentrations of imatinib were added to 2 ml of plasma, and 10 replicate 0.2-ml aliquots were analyzed.

The correlation coefficients for three successive CGP 74588 triplicate standard curves were 0.9914, 0.9927, and 0.9931, respectively. Back-calculated values for CGP 74588 varied by 2–12 and 5–11% from the theoretical values of 30 and 10 000 ng/ml, respectively. When expressed as percentage coefficient of variation, the within-day variation in triplicate CGP 74588 standards was always <9.8% at all concentrations. Between-day variation in three, successive, triplicate CGP 74588 standard curves was also minimal, as the coefficient of variation of the slopes associated with these repeated standard curves was 1.3%. To test the precision and accuracy of the assay, 10 quality control samples containing 100 or 10 000 ng/ml of both imatinib and CGP 74588 were prepared and analyzed. The mean values obtained for imatinib were 105 and 8754 ng/ml, with coefficients of variation of 5.2 and 2.2%, respectively. The mean values obtained for CGP 74588 were 108 and 9019 ng/ml, with coefficients of variation of 3.7 and 2.6%, respectively.

When plasma samples from a patient receiving 200 mg of imatinib orally (Figs. 2 and 3) were analyzed, concentrations of imatinib and CGP 74588 were within the linear range of the assay. Imatinib could be quantified for the entire 72-h pharmacokinetic study period that was planned before daily dosing was initiated, whereas concentrations of CGP 74588 fell below the LLOQ of the assay after 36 h. As indicated, the ability to quantify imatinib and CGP 74588 for this period of time allowed appropriate definition of the terminal phase of both imatinib and CGP 74588 concentration versus time curves.

4. Discussion

Although LC assays for imatinib and CGP 74588 have been published previously [23,24], their suitability for clinical pharmacology studies of imatinib is decreased by the relatively restricted availability of the LC–MS–MS instrumentation on which they rely. LC methodology with absorbance detection has proven insufficiently sensitive for application to clinical pharmacology studies of imatinib. The LC–MS method described in the current manuscript is: sufficiently sensitive; applicable to biological matrices; and capable of being implemented in laboratories with standard LC–MS instrumentation.

The incentive for performing additional clinical pharmacology studies of imatinib is multifactorial. Although imatinib has striking activity against CML [1,8–12] and GIST [13–20] and is licensed for treatment of those diseases, its complete spectrum of activity remains undefined. Numerous studies are actively investigating the utility of imatinib treatment in a variety of solid [21,22] and hematologic malignancies. Additional studies are investigating the potential for combining imatinib with various other antitumor agents as disparate as traditional cytotoxic drugs and monoclonal antibodies. In each of these studies, there is a need to evaluate potential pharmacokinetic/pharmacodynamic relationships and/or potential drug–drug interactions. Of even more importance may be the need to define a number of critical pharmacokinetic issues related to the use of imatinib in treating diseases for which it has documented activity [1,8–20] and is licensed for use. These obvious and overwhelming arguments for generating increased pharmacokinetic data in patients treated with imatinib indicate an equally obvious

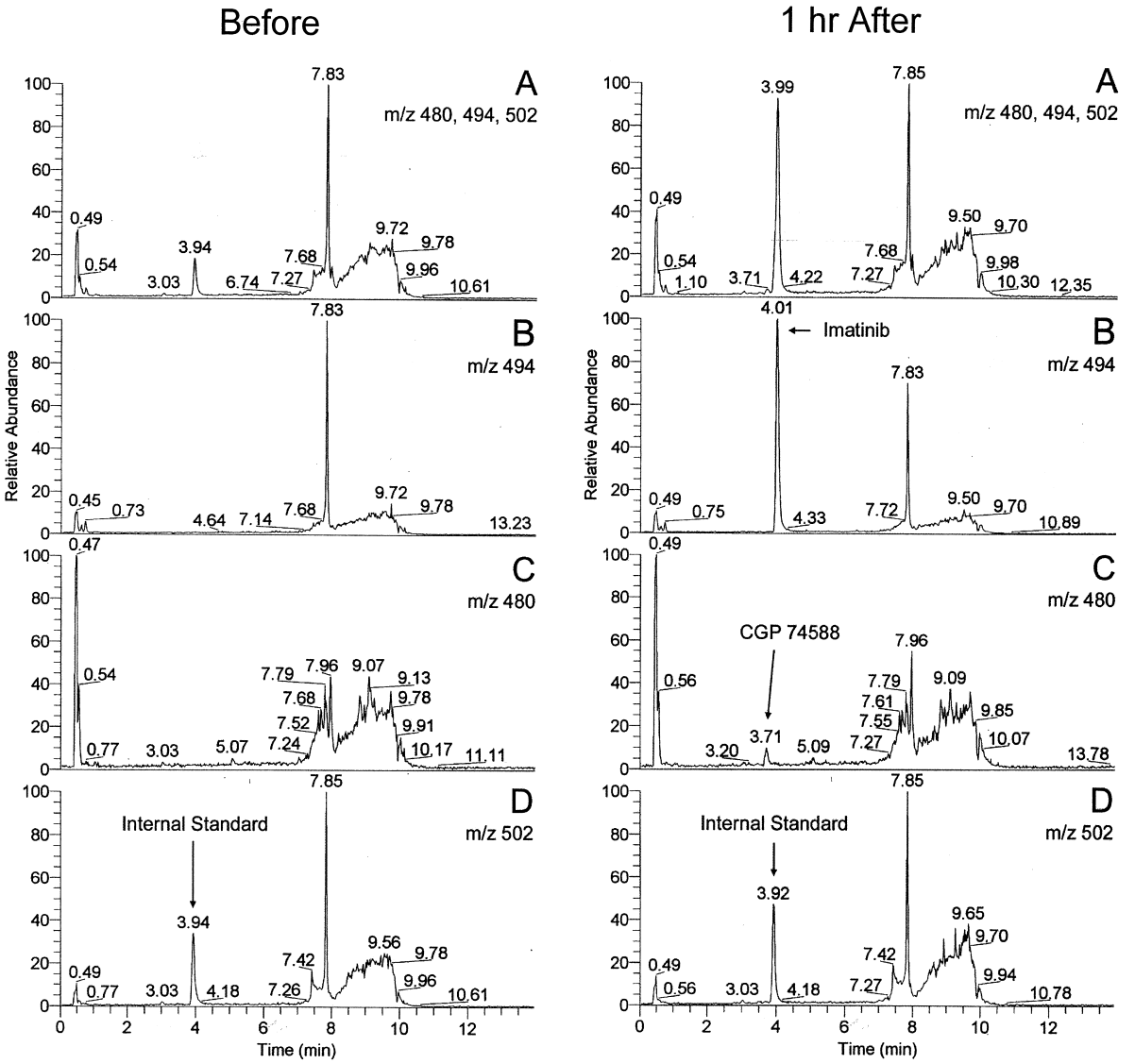

Fig. 2. Chromatograms of plasma samples obtained from a patient before and at 1 h after ingestion of his first dose of 200 mg of imatinib. (A) Total ion current monitoring m/z 480, 494, and 502; (B) monitoring m/z 494 (imatinib); (C) monitoring m/z 480 (CGP 74588); (D) monitoring m/z 502 (imatinib-D_8 internal standard).

need for a widely applicable, sensitive and facile assay for imatinib in biological matrices such as plasma. The LC–MS assay described in this communication fulfills each of these requirements. The overall applicability of this method is evidenced by its currently being employed by us in a variety of clinical trials at our institution and collaborative efforts ongoing with other investigators.

Acknowledgements

We thank Ezekiel Woods for excellent secretarial assistance in preparation of this manuscript. We also thank Drs S. Percy Ivy, Anthony Murgo, Louise Grochow, Sandra Silberman, and Laurie Letvak for encouragement and logistical support of our effort to develop this assay. Supported, in part, by grant NCI

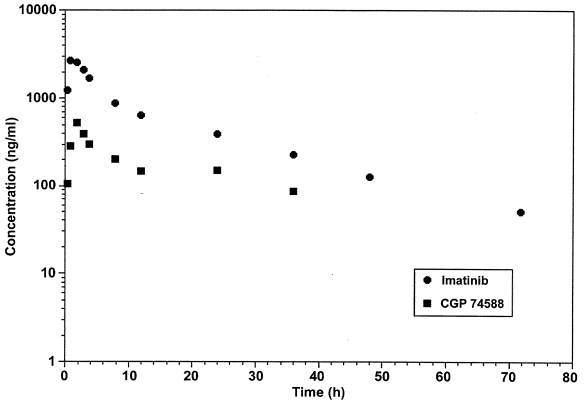

Fig. 3. Time course for imatinib (●) and CGP 74588 (■) in plasma of a patient given 200 mg of imatinib orally.

2P30 CA47904, grant NIH/NCRR/GCRC/ #5M01RR00056, and Grant U01 CA69855.

References

[1] D.G. Savage, K.H. Antman, N. Engl. J. Med. 346 (2002) 683.
[2] E. Buchdunger, C.L. Cioffi, N. Law, D. Stover, S. Ohno-Jones, B.J. Druker, N.B. Lydon, J. Pharmacol. Exp. Ther. 295 (2000) 139.
[3] M.C. Heinrich, D.J. Griffith, B.J. Druker, C.L. Wait, K.A. Ott, A.J. Zigler, Blood 96 (2000) 925.
[4] B.J. Druker, N.B. Lydon, J. Clin. Invest. 105 (2000) 3.
[5] M. Beran, X. Cao, Z. Estrov, S. Jeha, G. Jin, S. O'Brien, M. Talpaz, R.B. Arlinghaus, N.B. Lydon, H. Kantarjian, Clin. Cancer Res. 4 (1998) 1661.
[6] M. Carroll, S. Ohno-Jones, S. Tamura, E. Buchdunger, J. Zimmermann, N.B. Lydon, D.G. Gilliland, B.J. Druker, Blood 90 (1997) 4947.
[7] B.J. Druker, S. Tamura, E. Buchdunger, S. Ohno, G.M. Segal, S. Fanning, J. Zimmermann, N.B. Lydon, Nat. Med. 2 (1996) 561.
[8] J.E. Cortes, M. Talpaz, F.J. Giles, M.B. Rios, D. Thomas, G. Garcia-Manero, S. O'Brien, S. Verstovsek, H. Kantarjian, Proc. Am. Soc. Clin. Oncol 21 (2002) 262a.
[9] B.J. Druker, Proc. Am. Soc. Clin. Oncol. 21 (2002) 1a.
[10] A. Hochhaus, T. Lahaye, S. Kreil, U. Berger, M.C. Müller, K. Merx, P. Paschka, B. Riehm, C. Schoch, P. La Rosée, A. Corbin, H. Gschaidmeier, R. Hehlmann, Proc. Am. Soc. Clin. Oncol. 21 (2002) 262a.
[11] H. Kantarjian, C. Jorge, S. O'Brien, F.J. Giles, D. Thomas, S. Faderl, G. Garcia-Manero, M.B. Rios, L. Letvak, K. Bochinski, J. Shan, M. Talpaz, Proc. Am. Soc. Clin. Oncol. 21 (2002) 261a.
[12] H. Kantarjian, C. Sawyers, A. Hochhaus, F. Guilhot, C. Schiffer, C. Gambacorti-Passerini, D. Niederwieser, D. Resta, R. Capdeville, U. Zoellner, M. Talpaz, B. Druker, N. Engl. J. Med. 346 (2002) 645.
[13] P.G. Casali, J. Verweij, J. Zalcberg, A. le Cesne, P. Reichardt, I. Ray-Coquard, C. Wendtner, I. Judson, E.D. Di Paola, M. Van Glabbeke, R. Bertulli, H. Dhillon, O.S. Nielsen, Proc. Am. Soc. Clin. Oncol. 21 (2002) 413a.
[14] G.D. Demetri, C. Rankin, C. Fletcher, R.S. Benjamin, C. Blanke, M. von Mehren, V. Bramwell, R.G. Maki, R. Blum, K. Antman, L. Baker, E. Borden, Proc. Am. Soc. Clin. Oncol. 21 (2002) 413a.
[15] H. Joensuu, P.J. Roberts, M. Sarlomo-Rikala, L.C. Andersson, P. Tervahartiala, D. Tuveson, S. Silberman, R. Capdeville, S. Dimitrijevic, B. Druker, G.D. Demetri, N. Engl. J. Med. 344 (2001) 1052.
[16] I.R. Judson, J. Verweij, A.T. van Oosterom, J.-Y. Blay, S. Rodenhuis, W. van der Graaf, J.A. Radford, A. le Cesne, P. Hogendoorn, E. Donato di Paola, M. Brown, S. Dimitrijevic, O.S. Nielsen, Proc. Am. Soc. Clin. Oncol. 21 (2002) 403a.
[17] A.T. van Oosterom, I. Judson, J. Verweij, S. Stroobants, D.P. Donato, S. Dimitrijevic, M. Martens, A. Webb, R. Sciot, M. Van Glabbeke, S. Silberman, O.S. Nielsen, Lancet 358 (2001) 1421.
[18] A.T. van Oosterom, I. Judson, J. Verweij, E.D. Di Paola, S. Dimitrijevic, H. Dumez, M. Scurr, R. Sciot, S. Silberman, M. Van Glabbeke, O.S. Nielsen, Proc. Am. Soc. Clin. Oncol. 21 (2002) 82a.
[19] M. von Mehren, C. Blanke, H. Joensuu, M.C. Heinrich, P. Roberts, B. Eisenberg, S. Silberman, S. Dimitrijevic, B. Kiese, J. Fletcher, C. Fletcher, G.D. Demetri, Proc. Am. Soc. Clin. Oncol. 21 (2002) 403a.
[20] C.D. Blanke, M. von Mehren, H. Joensuu, P.J. Roberts, B. Eisenberg, M. Heinrich, B. Druker, D. Tuveson, S. Dimitrijevic, S.L. Silberman, G.D. Demetri, Proc. Am. Soc. Clin. Oncol. 20 (2001) 1a.
[21] B.E. Johnson, B. Fisher, T. Fisher, D. Dumlop, D. Rischin, S. Silberman, M. Kowalski, D. Sayles, C. Fletcher, R. Salgia, C. Delbaldo, T. Le Chevalier, Proc. Am. Soc. Clin. Oncol. 21 (2002) 293a.
[22] P.Y. Wen, W.K. Yung, K. Hess, S. Silberman, M. Hayes, D. Schiff, F. Lieberman, T.F. Cloughesy, L.M. DeAngelis, S.M. Chang, L. Junck, H.A. Fine, K. Fink, H.I. Robins, J.J. Raizer, L.E. Abrey, M.P. Mehta, E.A. Maher, P.M. Black, J. Kuhn, R. Capdeville, R.S. Kaplan, A. Murgo, C. Stiles, M.D. Prados, Proc. Am. Soc. Clin. Oncol. 21 (2002) 73a.
[23] R. Bakhtiar, J. Lohne, L. Ramos, L. Khemani, M. Hayes, F. Tse, J. Chromatogr. B Biomed. Sci. Appl. 768 (2002) 325.
[24] R. Bakhtiar, L. Khemani, M. Hayes, T. Bedman, F. Tse, J. Pharm. Biomed. Anal. 28 (2002) 1183.