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Quantification of the anti-leukemia drug STI571 (Gleevec™) and its metabolite (CGP 74588) in monkey plasma using a semi-automated solid phase extraction procedure and liquid chromatography-tandem mass spectrometry

R. Bakhtiar *, L. Khemani, M. Hayes, T. Bedman, F. Tse

Department of Drug Metabolism and Pharmacokinetics, Novartis Institute for Biomedical Research, East Hanover, NJ 07936, USA

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Abstract

Signal Transduction Inhibitor 571 (STI571, formerly known as CGP 57148B) or Gleevec™ received fast track approval by the US Food and Drug Administration (FDA) for treatment of chronic myeloid leukemia (CML). STI571 (Gleevec™) is a revolutionary and promising new oral therapy for CML, which functions at the molecular level with high specificity. The dramatic improvement in efficacy compared with existing treatments prompted an equally profound increase in the pace of development of Gleevec™. The duration from first dose in man to completion of the New Drug Application (NDA) filing was less than 3 years. In addition, recently, FDA approved Gleevec™ for the treatment of gastrointestinal stromal tumor (GIST). In order to support all toxicokinetic (TK) studies with sufficient speed to meet various target dates, a semi-automated procedure using solid phase extraction (SPE) was developed and validated. A Packard Multi-Probe I and a SPE step in a 96-well plate format were utilized. A 3M Empore octyl (C_8)-standard density 96-well plate was used for plasma sample extraction. A Sciex API 3000 triple quadrupole mass spectrometer with an atmospheric pressure chemical ionization (APCI) interface operated in positive ion mode was used for detection. Lower limits of quantification of 1.00 and 2.00 ng/ml were attained for STI571 and its metabolite, CGP 74588, respectively. The method proved to be rugged and allowed the simultaneous quantification of STI571 and CGP 74588 in monkey plasma. Herein, assay development, validation, and representative concentration–time profiles obtained from TK studies are presented.

Keywords: Gleevec™; STI571; Leukemia; Bcr-Abl; CGP 74588; Imatinib mesylate

1. Introduction

Gleevec™ or imatinib mesylate, designated chemically as 4-[(4-Methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]

* Corresponding author. Present address: Mail Stop: RY80L-109, 126 E. Lincoln Avenue, Merck and Co., Rahway, NJ 07065, USA. Tel.: +1-732-594-1454; fax: +1-732-594-4390.

E-mail address: ray_bakhtiar@merck.com (R. Bakhtiar).

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amino]-phenyl]benzamide methanesulfonate, is a protein-tyrosine kinase (PTK) inhibitor which potently inhibits the Abelson (Abl) tyrosine kinase in in vitro [1–3] and in vivo studies [4–11].

Protein-tyrosine kinases (PTKs) are enzymes that can transfer the terminal phosphate of an adenosine triphosphate (ATP) molecule to a tyrosine residue of a cytoplasmic protein substrate. PTKs are key modulators of cellular signal-transduction pathways [12,13]. If for any reason these signaling proteins are subjected to oncogenic mutation(s), a cellular deregulation may occur, yielding an imbalance between cell proliferation, cell growth, and cell death (apoptosis). Hence, PTKs have emerged as important therapeutic targets for intervention in cancer [14]. The insights gained from these studies are now giving rise to novel forms of chemotherapy treatments.

The Philadelphia (Ph) chromosome is the consequence of a reciprocal translocation between chromosomes 9 and 22 yielding a fusion oncoprotein referred to as Bcr-Abl (~ 210 kDa). This molecular consequence leads to an elevated catalytic activity of Bcr-Abl resulting in a resistance to apoptosis, cell transformation, and malignancy. A cytogenetic hallmark of chronic myeloid leukemia (CML), a clonal hematopoetic stem cell disorder, is the Ph chromosome and high activity of Bcr-Abl tyrosine kinase [15–17]. The clinical chemistry manifestation of CML is elevated levels of white blood cells (e.g. $> 20 \times 10^9$/l) and in some patients increase in platelet counts (e.g. $> 450 \times 10^9$/l). Therapeutic options for CML includes allogeneic stem cell transplantation, interferon-alpha treatment, and chemotherapy with hydroxyurea or busulfan [6,18,19]. Allogeneic stem cell transplantation requires the availability of a suitable donor and presents a risk of mortality in older patients. Chemotherapy based methods often do not provide a cure, present toxic side effects, and lead to intolerability and/or resistance to the treatment. In addition, none of the agents used for CML is known to target the underlying cause of the disease.

Recently, signal transduction inhibitor 571 (STI571 or Gleevec™) was approved by FDA in a record time [20] for the treatment of patients at any of the three stages of CML: myeloid blast crisis, accelerated phase, and chronic phase after failure of interferon-alpha therapy. Gleevec™ has been referred to as a milestone for the drug development in cancer and an ideal targeted drug at the molecular level [20,21]. With high specificity, it competitively inhibits the binding of ATP to the kinase activation domain of Bcr-Abl (Fig. 1) [22]. As a result, the activation loop of Bcr-Abl, which modulates the catalytic activity of this enzyme by switching between different states in a phosphorylation fashion, is blocked from interaction with cellular ATP molecules.

In recent years, numerous laboratories have reported the use of high-throughput bioanalytical

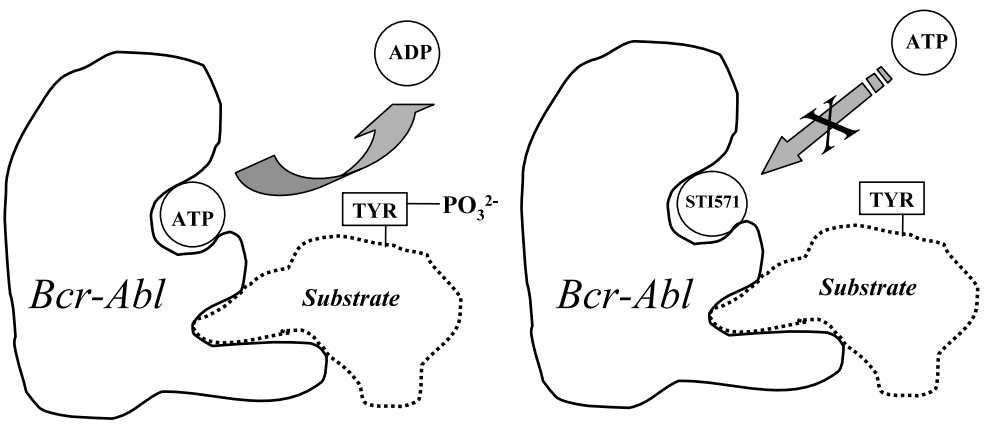

Fig. 1. A simplified representation illustrating the likely mode of action of STI571 or Gleevec™. STI571 inhibits the binding of ATP to the kinase activation domain of Bcr-Abl oncoprotein.

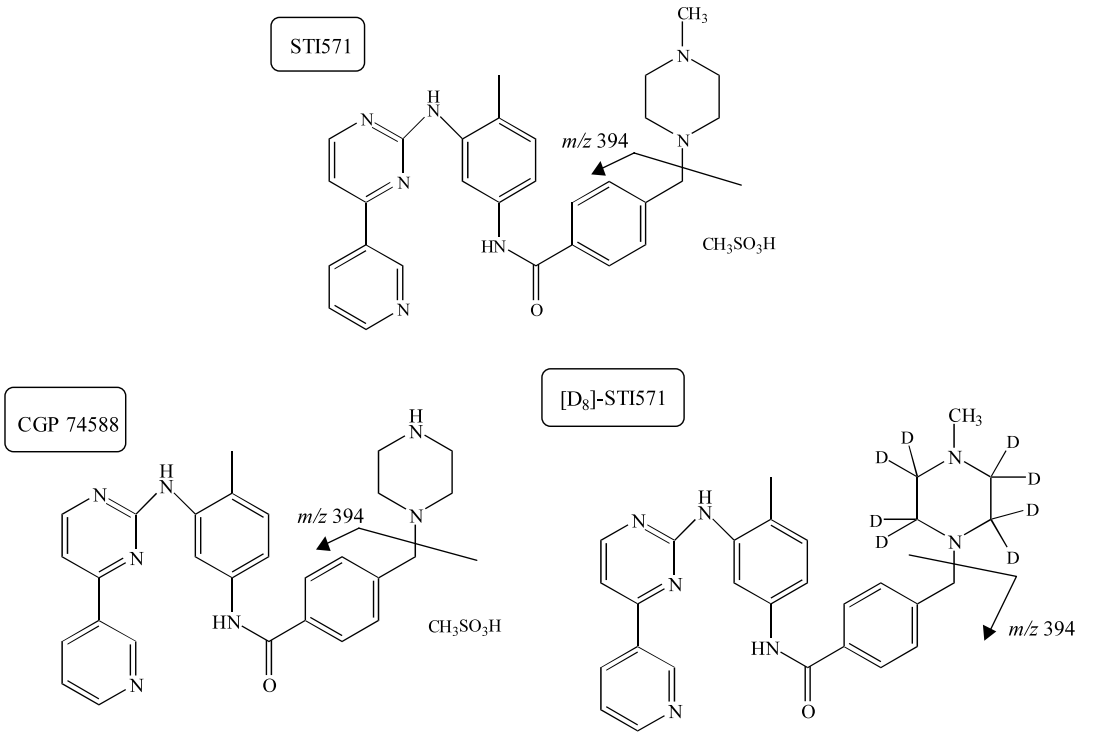

Fig. 2. Structure of STI571, CGP 74588, and the internal standard. Arrows indicate the product-ion that was selected for the multiple-reaction monitoring experiment.

procedures using liquid chromatography-tandem mass spectrometry (LC-MS-MS) [23–44]. Herein, we describe the validation of a semi-automated solid phase extraction (SPE) method using a Packard Multiprobe I in a 96-well plate format. This method proved to be rugged and allowed the simultaneous quantification of STI571 and its metabolite, CGP 74588, in monkey plasma. Consequently, this method was successfully used in the analysis of plasma samples during toxicokinetic (TK) studies, where monkeys were orally dosed with STI571.

2. Experimental

2.1. Reagents and materials

High purity solvents were purchased from Fisher Scientific (Springfield, NJ). All other chemicals used were commercially available (Aldrich, Milwaukee WI or Sigma, St. Louis, MO) and of analytical grade. Blank plasma samples containing heparin were obtained from untreated monkeys in-house. Water was de-ionized and purified using a Nanopure system (Barnstead-Thermolyne Corporation, Dubuque, IA). The internal standard and STI571 (Fig. 2) were synthesized at Novartis (Basel, Switzerland and East Hanover, NJ).

2.2. Mass spectrometry

A Sciex API 3000 triple quadrupole mass spectrometer (PE-Sciex, Toronto, Canada) with atmospheric pressure chemical ionization (APCI) in positive ion mode was used for detection. The mass spectrometer was operated in the multiple-reaction monitoring (MRM) mode. The APCI source was operated at a temperature of 450 °C with a corona discharge current of 4 μA. Nitrogen was used as the curtain (setting = 10), nebulizing

(setting = 4), and collision gas (setting = 6). The collision energy (Q0-RO2) was set at 35.0 eV (laboratory frame). During the MRM experiments, the nitrogen pressure in the second quadrupole was measured at 3.0×10^{-5} Torr (1 Torr = 133.322 Pa). The instrument was operated in unit resolution. The orifice (OR) and ring (RNG) voltages were set at 55 and 350 V, respectively. Following HPLC separation, the peak areas corresponding to the m/z 494.5 → 394.0 reaction (dwell-time = 600 ms) for STI571 and m/z 480.4 → 394.0 reaction (dwell-time = 600 ms) for CGP 74588 were measured relative to that of the m/z 502.4 → 394.0 reaction (dwell-time = 100 ms) of the internal standard (Fig. 2).

Table 1
Summary of automated preparative steps performed by the Packard Multi-Probe I (Model 204) platform using a SPE procedure

Description	Step
Sorbent conditioning	200 μl of methanol 200 μl of water
Addition to sample	250 μl of internal standard (0.10 ng/μl)
Sample loading to the 96-well plate	500 μl of sample
Mix (performed twice)	Aspiration/dispensing (×2)
Wash	200 μl of 5% (v/v) methanol in water
Elution	200 μl of 1% (v/v) 1 N HCl in methanol

Table 2
Daily STI571 variation of calibration parameters in monkey plasma

Analysis day	Slope	y-Intercept	Correlation coefficient (r)
Day 1	0.00815	0.000260	0.994
Day 2	0.00872	−0.00136	0.995
Day 3	0.00908	0.000820	0.995
Mean	0.00865	−0.0000933	0.995
S.D.	0.000469	0.00113	0.0006

Table 3
Daily CGP 74588 variation of calibration parameters in monkey plasma

Analysis day	Slope	y-Intercept	Correlation coefficient (r)
Day 1	0.0106	0.00062	0.996
Day 2	0.0107	0.00245	0.996
Day 3	0.0103	0.00015	0.994
Mean	0.0105	0.0011	0.996
S.D.	0.000180	0.0012	0.0009

2.3. Data processing

A Power Macintosh G3 workstation was used for data acquisition and processing. SAMPLE CONTROL (version 1.4), TURBOQUAN (version 1.0), and MICROSOFT EXCEL (version 6.0) were used for data processing and statistical analysis. A 3-point smoothing algorithm was applied to all ion-chromatograms and viewed using MULTIVIEW (version 1.4) software. The calibration curve was generated using the results of the calibration samples by linear least-squares regression analysis according to the equation $y = a + bx$, where y was the peak-area ratio of substance to internal standard, x was the concentration of analyte in the calibration sample, a was the intercept, and b was the slope of the regression line. A weighted ($1/x^2$) linear least-squares regression was used. Subsequently, concentrations of the quality control (QC) samples were calculated from the regression equation of the calibration curve.

2.4. Chromatography

A 50 × 4.6 mm (i.d.), 3.5-μm, Waters Symmetry Shield™-RP_8 column (Milford, MA), packed with C_8 bonded silica particles was used. The pre-column was a Phenomenex (Torrance, California), C_8 4.0 × 3.0 mm (i.d.). The LC system consisted of a LC-10AD *VP* pump (Shimadzu, Columbia, MD) and an SCL-10A *VP* controller. The autosampler was a CTC HTS-PAL unit (LEAP Technologies, Carrboro, NC) equipped with a 96-well plate stack set at room temperature. Subsequent to each injection, the autosampler syringe and its 6-port valve were each washed with 100 μl of methanol–water (80:20, v/v) three times. The HPLC flow rate was 1.0 ml/min. An on-line degasser, Degassit Model 6324

Table 4
Percent individual, mean accuracy, and precision of back-calculated concentrations of calibration samples for STI571 in monkey plasma

Analysis day	Nominal concentration (ng/ml)					
	1	2	5	20	100	1000
Day 1	106	86.5	92.6	95.0	104	120
	106	95.0	95.8	97.0	96.3	105
Day 2	104	98.0	94.0	99.0	90.2	107
	102	96.5	93.4	92.0	118	106
Day 3	91.0	89.0	93.8	100	94.7	110
	111	110	98.8	97.5	95.3	109
Mean	103	95.7	94.7	96.8	99.8	110
S.D.	6.74	9.22	2.25	2.89	10.0	5.47
CV (%)	6.54	9.63	2.38	2.99	10.0	4.99

Table 5
Percent individual, mean accuracy, and precision of back-calculated concentrations of calibration samples for CGP 74588 in monkey plasma

Analysis day	Nominal concentration (ng/ml)				
	2	5	20	100	1000
Day 1	88.0	93.0	96.0	103	116
	100	97.8	95.5	97.6	105
Day 2	98.0	94.0	99.0	90.2	107
	96.5	93.4	92.0	118	106
Day 3	96.0	92.8	92.0	92.3	107
	120	104	95.0	97.1	111
Mean	99.7	95.8	94.9	99.7	109
S.D.	10.5	4.26	2.65	10.0	4.13
CV (%)	10.6	4.45	2.80	10.0	3.80

(MetaChem Technologies, Inc., Torrance, CA), was used. The mobile phase was composed of methanol–water (72:28, v/v) containing 0.05% (by weight) ammonium acetate. Separation was performed at ambient temperature. Sample injection volume was 10 μl. The HPLC and autosampler systems were synchronized via the Power Macintosh G3 workstation (SAMPLE CONTROL 1.4 software).

2.5. Assay procedure

Stock solutions of STI571 and CGP 74588 for calibration standards and QC samples were separately prepared in methanol–water (50:50, v/v) and stored at 2–8 °C. For the standard curve, the concentrations of STI571 and CGP 74588 (both calculated as the free base) in monkey plasma were as follows: 1.00 (only for STI571), 2.00, 5.00, 20.0, 100, and 1000 ng/ml. Reference solutions of STI571 and CGP 74588 at a concentration of 50 ng/μl were prepared in methanol–water (50:50, v/v). The calibration standards were freshly prepared on each analysis day by adding an appropriate aliquot of the spiking stock solution to 250 μl of blank monkey plasma. The QC concentrations for STI571 and CGP 74588 in monkey plasma were 1.00 (only for STI571), 2.00, 10.0, 50.0, 200, and 1000 ng/ml. Six batches of QC samples with varying lot numbers were prepared as a pool, portioned, and stored at −20 °C pending analysis. A stock solution of the internal

standard, STI571-D_8, was prepared in methanol–water (50:50, v/v) yielding a concentration of 0.10 ng/μl and stored at 2–8 °C. Method validation was carried out over a period of 3 days.

Frozen plasma samples were defrosted to room temperature and centrifuged for 10 min at 2000 rpm before processing. In a 10×75 mm glass disposable tube, 250 μl of monkey plasma was added. The glass tubes containing plasma samples were placed in a custom-made rack and placed in a Packard Multi-Probe I Model 204 automated sample handling system (Packard Instrument Company, Meriden, CT). The Packard Multi-Probe I was programmed to perform all the subsequent preparative steps as outlined in Table 1. A 96-well plate 3M Empore C_8-S.D. containing 20 mg of sorbent (Varian Associates Inc., Harbor City, CA) was used for the SPE procedure.

2.6. Assay validation

2.6.1. Linearity

The linearity of the method was evaluated over the concentration range of 1.00–1000 and 2.00–1000 ng/ml for STI571 and CGP 74588 in monkey plasma, respectively. Calibration standards were freshly prepared in duplicate on each day of validation. The assay acceptance criterion for each back-calculated standard concentration was 20% deviation from the nominal. The calibration curve had to demonstrate a correlation coefficient of 0.95 or higher. Comparison of the slope, intercept, and correlation coefficient was made for the 3-day validation process.

Table 6
Percent accuracy and precision of STI571 QC samples in monkey plasma

Analysis day	Nominal concentration (ng/ml)					
	1	2	10	50	200	1000
Day 1 ($n = 5$)	107	109	102	101	112	100
	87.7	99.9	92.3	97.3	103	97.6
	79.2	100	99.2	103	104	94.5
	86.6	108	105	93.0	105	112
	105	102	108	93.2	101	108
Mean	93.2	104	101	97.4	105	102
S.D.	12.3	4.32	5.98	4.53	4.17	7.30
CV (%)	13.2	4.16	5.91	4.65	3.96	7.12
Day 2 ($n = 6$)	98.2	86.2	85.0	70.4	96.3	86.9
	103	90.8	91.2	85.9	96.6	95.0
	105	92.6	93.1	87.9	97.8	95.7
	107	94.5	94.1	88.8	105	97.8
	111	99.2	96.0	89.0	105	102
	119	112	99.9	90.2	116	103
Mean	107	95.8	93.2	85.4	103	96.8
S.D.	7.16	8.81	5.00	7.46	7.64	5.85
CV (%)	6.69	9.19	5.37	8.74	7.43	6.05
Day 3 ($n = 5$)	96.4	95.3	97.6	110	102	90.4
	102	99.9	87.4	93.8	97.4	103
	98.3	88.0	95.3	104	109	106
	96.9	85.3	93.4	97.2	120	105
	96.8	89.0	86.4	97.6	75.4	97.7
Mean	98.1	91.5	92.0	100	101	100
S.D.	2.31	5.95	4.90	6.43	16.4	6.24
CV (%)	2.36	6.50	5.33	6.43	16.3	6.24
Inter-day ($n = 16$)	99.4	97.0	95.5	94.4	103	99.8
S.D.	7.01	6.30	5.00	7.98	2.25	2.88
CV (%)	7.05	6.50	5.24	8.45	2.18	2.89

Table 7
Percent accuracy and precision of CGP 74588 QC samples in monkey plasma

Analysis day	Nominal concentration (ng/ml)				
	2	10	50	200	1000
Day 1 ($n = 5$)	1023	110	112	114	106
	111	105	110	113	109
	109	115	115	116	103
	114	118	105	116	122
	106	118	106	113	118
Mean	113	113	110	114	112
S.D.	6.50	5.64	4.37	1.60	8.12
CV (%)	5.78	4.97	3.99	1.40	7.27
Day 2 ($n = 6$)	103	104	81.8	112	99.4
	103	104	103	113	109
	110	105	104	113	110
	112	108	105	114	111
	113	109	105	118	118
	119	110	107	121	120
Mean	110	107	101	115	111.3
S.D.	6.19	2.76	9.42	3.76	7.30
CV (%)	5.63	2.59	9.34	3.26	6.56
Day 3 ($n = 5$)	97.3	104	118	108	101
	102	97.6	99.9	105	114
	102	98.2	108	116	114
	100	99.7	101	127	118
	97.0	102	106	82.7	111
Mean	99.5	100	107	108	111
S.D.	2.23	2.64	7.13	16.4	6.49
CV (%)	2.24	2.64	6.69	15.2	5.83
Inter-day Mean ($n = 16$)	107	107	106	112	111
S.D.	6.92	6.53	4.47	4.07	0.204
CV (%)	6.44	6.12	4.23	3.62	0.184

2.6.2. Intra- and inter-day accuracy and precision

The intra- and inter-day accuracy and precision of the assay were tested by analysis of six QC (five QCs concentrations for CGP 74588) sample concentrations in replicates of at least five on 3 separate days. The precision was expressed as the coefficient of variation (%CV). The intra-assay accuracy and precision were calculated as the mean and precision of all individual accuracy of QC samples analyzed during a single analysis run (a minimum of five replicates for each QC concentration). The values were calculated for each day of validation separately. The inter-day accuracy and precision were calculated as the mean and the precision over all the accuracy of QC samples analyzed during a 3-day validation (replicates of 16 for each QC concentration).

2.6.3. Recovery

Recovery of the sample preparation method was assessed by comparison of the peak area obtained from the analysis of neat reference solutions (unprocessed) and of processed monkey plasma samples. The recoveries of STI571 and CGP 74588 were examined at concentrations of 1.00, 100, and 1000 ng/ml.

2.6.4. Specificity

Specificity of the assay was demonstrated by obtaining ion-chromatograms for blank pooled monkey plasma samples as well as blank monkey plasma spiked with only the internal standard. Blank samples were prepared from six different batches of monkey plasma and analyzed in duplicate on each day of validation.

2.6.5. Freeze–thaw stability

In freeze–thaw stability studies, samples of STI571 and CGP 74588 (at three concentrations) were subjected to three freeze–thaw cycles and subsequently analyzed in duplicate. Plasma samples were stored at −20 °C for 24 h and thawed unassisted at room temperature. This cycle of thawing and freezing was repeated two more times followed by LC-MS-MS analysis on the third cycle.

2.6.6. Stability

The stability of STI571 and CGP 74588 in plasma was evaluated over 38 weeks (stored at −20 °C) at three concentrations in duplicate. The average time for a complete analytical run of three 96-well plates was about 16 h. An autosampler stability determination was performed up to 48 h period. The stability of calibration standard stock solutions was also assessed for up to 6 months at 2–8 °C.

3. Results and discussion

Due to the fast track status of STID571 (Gleevec™) by the US Food and Drug Administration, a dramatic improvement in throughput of bioanalytical method was warranted. The duration from first dose in man to completion of the New Drug Application (NDA) filing was less than 3 years. In order to support the pharmacokinetic

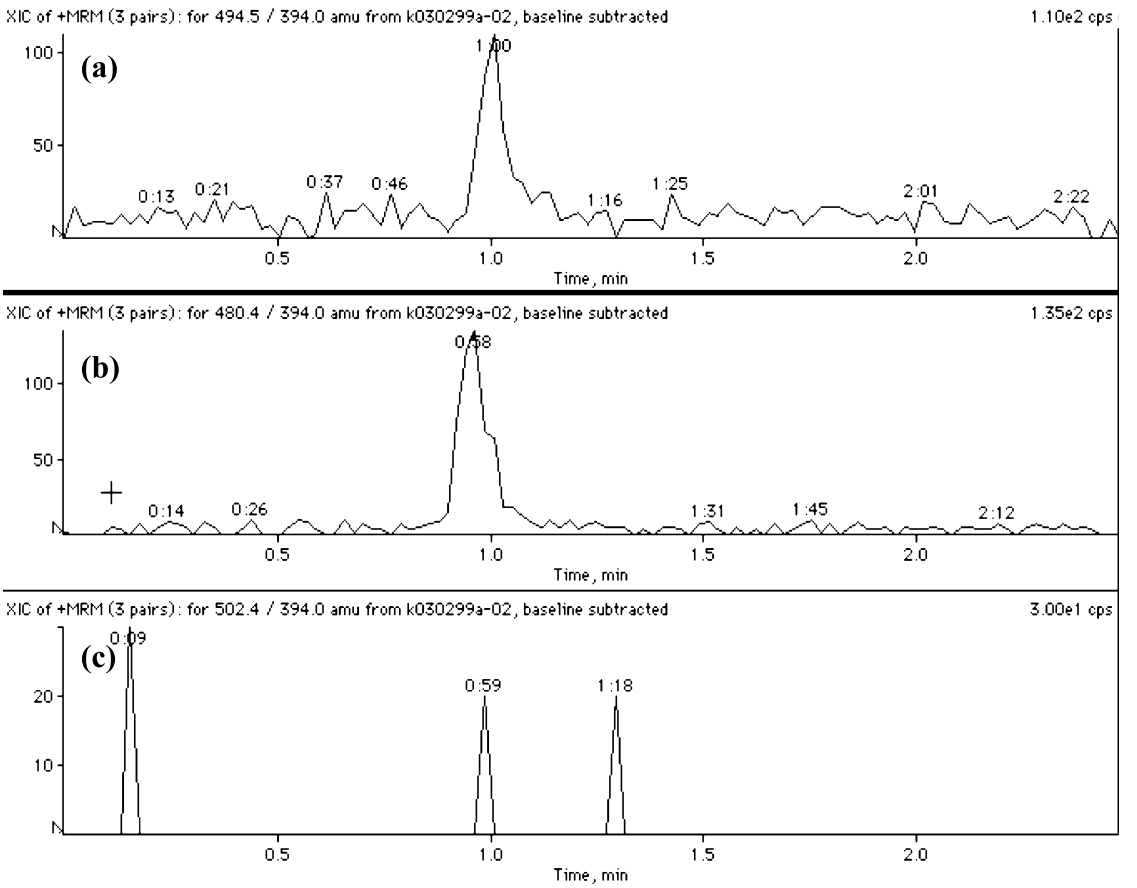

Fig. 3. LC-APCI-MS-MS ion-chromatograms resulting from the analysis of blank (drug and internal standard free) monkey plasma for STI571 (panel A), CGP 74588 (panel B), and STI571-D_8 (panel C).

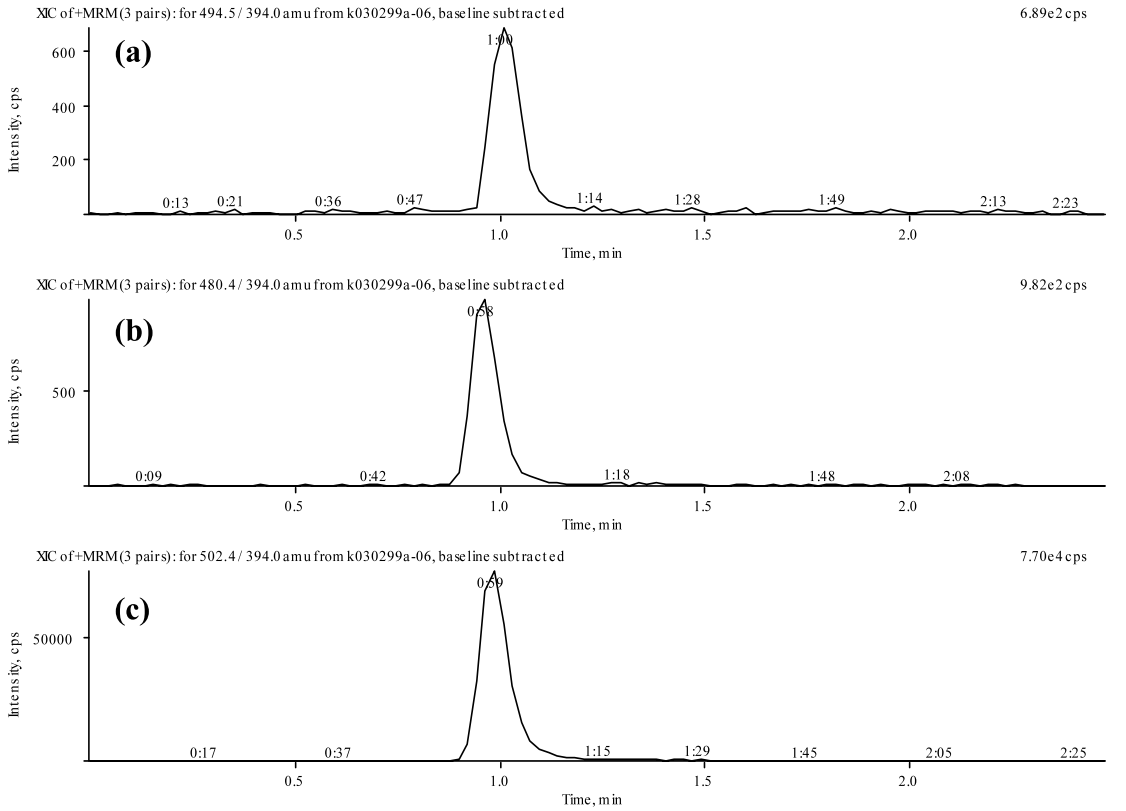

Fig. 4. Representative LC-APCI-MS-MS ion-chromatograms resulting from the analysis of 1.00 ng/ml (LLOQ) of STI571 and 2.00 ng/ml (LLOQ) of CGP 74588 spiked with the internal standard (25 ng per sample). The injection volume was 10 μl. Excellent sensitivity was obtained for ca. 10 and 20 pg/ml of on-column injection for STI571 and CGP 74588, respectively. Panels A, B, and C correspond to the STI571, CGP 74588, and the internal standard, respectively.

studies with sufficient speed to meet various target dates, a semi-automated procedure using a Packard Multi-Probe liquid handling platform in conjunction with a SPE protocol was developed and validated.

The current method required a LC-MS-MS run time of about 3.0 min (injection-to-injection cycle). The typical batch sizes were two to three plates a day. During the analysis of in vivo samples, the absence of adequate chromatography (i.e. co-elution of analyte and possible metabolites) coupled to MS detection may result in interference, which is not easily detected during the validation process. Recently, Jemal and Xia [45] demonstrated the possibility of in-source dissociation of biotransformation products such as phase II metabolites (e.g. glucuronide conjugate of the parent drug) yielding the parent compound. Thus, during the analysis of post-dose biological samples, a clear understanding of the identity and abundance of in vivo biotransformation products is important. In this case, we did not observe any false positives in terms of post-dose STI571 or CGP 74588 concentrations, which could stem from the in-source dissociation of the above compounds yielding identical MRM transitions.

3.1. Linearity

The linearity and reproducibility of calibration curves for STI571 and CGP 74588 in monkey plasma was acceptable between 1.00–1000 and 2.00–1000 ng/ml, respectively (Tables 2 and 3). The mean correlation coefficients obtained for

STI571 and CGP 74588 over a 3-day validation were 0.995 and 0.996, respectively. The percent coefficients of variation for the slopes (Tables 2 and 3) of STI571 and CGP74588 were 5.4 and 1.7%, respectively. Tables 4 and 5 summarize the calibration curve results (observed concentration expressed in percent of the nominal). Mean accuracy of standard calibration samples for STI571 covering the concentration of 1.00–1000 ng/ml ranged between 94.7 and 110% with CVs of 2.38–10.0%. Mean accuracy of standard calibration samples for CGP 74588 covering the concentration of 2.00–1000 ng/ml ranged between 94.9 and 109% with CVs of 2.80–10.6%.

3.2. Intra- and inter-day results

QC samples covering the anticipated dynamic concentration range of the method, a minimum of five replicates for each QC concentration (Tables 6 and 7; observed concentration expressed in percent of the nominal), were analyzed on each validation day. The mean intra-day accuracy of QC samples at LLOQ for STI571 ranged 93.2–107%. The CVs were between 2.36 and 13.2%. The mean intra-day accuracy of QC samples above LLOQ for STI571 ranged 85.4–105%. The CVs were between 3.96 and 16.3%. The mean intra-day accuracy of QC samples at LLOQ for CGP 74588 ranged 99.5–113%. The CVs were between 2.24 and 5.78%. The mean intra-day accuracy of QC samples above LLOQ for CGP 74588 ranged 100–115%. The CVs were between 1.40 and 15.2%.

The mean ($n = 16$) inter-day accuracy values for QC samples obtained for STI571 and CGP 74588 at LLOQ were 99.4 and 107%, respectively. The CV values for STI571 and CGP 74588 were 7.05 and 6.44%, respectively. At above LLOQ, STI571 QCs yielded mean accuracy values between 94.4 and 103% with CVs of 2.18–8.54%. Likewise, the mean accuracy values for CGP 74588 at above LLOQ spanned from 106 to 112% with CV values of 0.184–6.12%. Overall, the assay exhibited excellent accuracy and reproducibility within the tested concentration range.

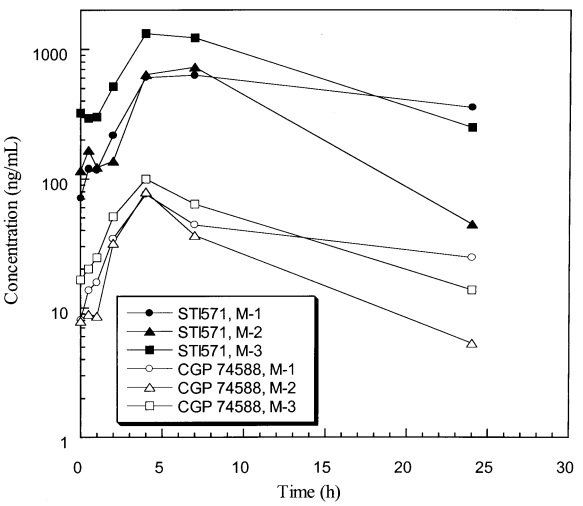

Fig. 5. Steady-state concentration-time profiles for three male cynomolgus monkeys subsequent to oral administration of STI571, 75 mg/kg per day, for 13 consecutive weeks during a TK study. Monkeys are arbitrary denoted as M-1, M-2, and M-3.

3.3. Recovery

The recovery of STI571 and CGP 74588 using this procedure, compared with an aqueous standard solution at 1.00, 100, and 1000 ng/ml, averaged approximately 69.1 and 69.7%, respectively.

3.4. Specificity

The specificity of the method was examined by analyzing blank monkey plasma extract (Fig. 3). As shown in Fig. 3, no significant interference in the blank plasma traces was seen from endogenous substances in drug-free monkey plasma at the retention time of the either analytes. The trace amount of signals detected in blank samples (Fig. 3) corresponded to about 1/6 and 1/7 of the analyte signals observed at LLOQ for STI571 and CGP 74588, respectively. Fig. 4 depicts a representative ion-chromatogram for the LLOQ (1.00 ng/ml for STI571 and 2.00 ng/ml for CGP 74588) of the calibration curve.

Excellent sensitivity was obtained for approximately 10 and 20 pg/ml of on-column injection for STI571 and CGP 74588, respectively.

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

In the freeze–thaw stability study, samples were subjected to three freeze–thaw cycles and subsequently analyzed in duplicate. No evidence of sample degradation was observed at concentrations of 2.0, 50, and 1000 ng/ml for either analytes in plasma. Furthermore, STI571 and CGP 74588 exhibited excellent room temperature (benchtop) stability for at least up to 72 h in plasma. Long term storage (stored at −20 °C) stability for up to 38 weeks led to no discernible loss of STI571 or CGP 74588 in plasma. In addition, a 48 h autosampler stability study did not lead to any STI571 and CGP 74588 degradation in the plasma sample extracts. The stock solutions of both analytes were also found to be stable for at least 7 months at 2–8 °C.

3.6. Assay application

The current assay was utilized successfully in support of several TK studies. The HPLC column was changed after the analysis of every three to four 96-well plates. Although the chromatographic capacity factor was only about 1.0, no significant ion-suppression was observed at the lower concentrations. This is not surprising since ion-suppression has a more dramatic manifestation on electrospray ionization than APCI [46–48].

Although we were able to detect ($S/N > 3$) STI571 and CGP 74588 at 250 pg/ml (data not shown), the method was validated with a LLOQ of 1.00 and 2.00 ng/ml for STI571 and CGP 74588, respectively. The relatively high plasma concentrations of analytes encountered in TK studies did not warrant attempts to perform validation at a greater sensitivity. Fig. 5 depicts a representative mean concentration-time profile (semi-log scale) for three male monkeys subsequent to daily oral administration of STI571 (75/mg per kg per day) for 13 consecutive weeks.

4. Conclusions

We have demonstrated a method for the quantification of STI571 and its metabolite, CGP 74588, in monkey plasma using a semi-automated SPE method and a relatively rapid LC-APCI-MS-MS analysis. The Packard Multi-Probe I performed the steps outlined earlier (Table 1) with minimum technical difficulties and maintenance. The method required no evaporation and reconstitution of the samples with mobile phase prior to injection. Adequate sensitivity allowed the direct injection of a small volume of the elution solvent from the extraction step. The assay was amenable to routine analysis of STI571 and CGP 74588 in TK studies. The simplicity and speed of the semi-automated SPE made it an attractive procedure in high-throughput bioanalysis of Gleevec™.

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