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Development of a liquid chromatography–mass spectrometry (LC/MS) assay method for the quantification of PSC 833 (Valspodar) in rat plasma

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ABSTRACT

A liquid chromatography–mass spectrometry (LC/MS) assay method was developed for the quantification of PSC 833 in rat plasma, using amiodarone as internal standard (IS). Separation was achieved using a C_8 3.5 μm (2.1 mm × 50 mm) column heated to 60 °C with a mobile phase consisting of acetonitrile–ammonium hydroxide 0.2% (90:10 v/v) pumped at a rate of 0.2 mL/min. Detection was accomplished by mass spectrometer using selected ion monitoring (SIM) in positive mode. An excellent linear relationship was present between peak height ratios and rat plasma concentrations of PSC 833 ranging from 10 to 5000 ng/mL ($R^2 > 0.99$). Intra-day and inter-day coefficients of variation (CV%) were less than 15%, and mean error was less than 10% for the concentrations above the limit of quantification. The validated limit of quantification of the assay was 10 ng/mL based on 0.1 mL rat plasma. The method limit of detection, based on an average signal-to-noise (S/N) ratio of 3, was found to be 2.5 ng/mL. The assay was capable of measuring the plasma concentrations of PSC 833 in rats injected with a single dose of 5 mg/kg of the drug. PSC 833 and IS eluted within 4 min, free of interfering peaks. The method was found to be fast, sensitive, and specific for the quantification of PSC 833 in rat plasma.

1. Introduction

Chemotherapy is one of the most effective approaches in the management of cancer. However, its effectiveness in the cure of cancer is partly hampered by inherent or acquired multi-drug resistance (MDR), where cancer cells become resistant simultaneously to pharmacologically and structurally diverse drugs [1,2]. One of the major underlying mechanisms for MDR is the over-expression of membrane efflux pumps, such as P-glycoprotein (P-gp), in the tumor cells that exports its substrates out of the cell [3–6]. The group of Victor Ling was the first to identify P-gp as being responsible for reduced drug accumulation in MDR Chinese hamster ovary cells in 1976 [7]. Since then, a growing body of literature has linked the failure of certain anticancer agents to the over-expression of P-gp in various types of tumorigenic cancer [6,8–10]. To date, many anticancer agents have been shown to be substrates of P-gp including *anthracyclines* (doxorubicin, daunorubicin and epirubicin), *Vinca alkaloids* (vincristine and vinblastine), *epipodophyllotoxins* (etoposide and teniposide), and *taxanes* (paclitaxel and docetaxel) [11,12].

Three generations of P-gp inhibitors have arisen from drug discovery efforts, each subsequent generation displaying greater specificity for P-gp [4]. PSC 833 (6-[[R-(E)]-6,7-didehydro-*N*,4-dimethyl-3-oxo-L-2-aminooctanoic acid]-7-L-valine; Fig. 1A) is a noncompetitive, second generation inhibitor of P-gp widely used in *in vitro* studies, preclinical and clinical trials for overcoming MDR to various anticancer drugs [13]. PSC 833 is a derivative of the cyclic polypeptide, cyclosporine A (CyA), but displays no evidence of nephrotoxicity or immunosuppressive activity [13–17]. It has shown to significantly prolong the survival rates of MDR-P388 tumor bearing mice and dogs with canine osteosarcoma when combined with doxorubicin [18,19]. It has increased the sensitivity towards etoposide of human carcinoma xenografts in nude mice [20]. Furthermore, clinical treatment with PSC 833 resulted in an increased intracellular accumulation of doxorubicin and vincristine in P-gp-positive myeloma cells [21]. However, PSC 833 interferes with the metabolism of anticancer drugs, slowing their clearance and increasing their toxicity to healthy tissues [22–26]. This is mediated by the ability of PSC 833 to potently inhibit CYP3A [22,24,27,28]. For this reason, control of PSC 833 plasma concentration is of great importance in each of the PSC 833 trials.

The quantification of PSC 833 in blood or plasma is usually performed either by radio-immunoassays (RIA) [29–32] or by

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high-performance liquid chromatography (HPLC) [33–36]. However, some of these RIA kits are beset by cross-reactivity with PSC 833 metabolites, which can lead to an over-estimation of the parent drug concentration [37]. HPLC assays are capable of separating intact cyclosporine analogues from their metabolites, although use of conventional HPLC is itself not without limitations. It has proved difficult for HPLC methods to elute PSC 833 from the columns as a single peak [33,35]. Moreover, the lack of a suitable chromophore in PSC 833 for UV-absorbance necessitates the use of short wavelength (e.g., 210 nm) for detection. Because numerous molecular species absorb energy in this wavelength, sample preparation usually is complex and involves several steps including protein precipitation followed by solid-phase extraction in order to remove potentially interfering compounds. The reported PSC 833 recoveries from different extraction procedures were relatively low (~ 50%) which restricts the limit of quantification (LOQ) to concentrations above 100 ng/mL [33,35]. In addition, to our knowledge, all the HPLC methods used for PSC 833 require a relatively long run time of analysis.

The main purpose of this study was to develop and validate a reliable, sensitive, and fast method for the quantification of PSC 833 in small volumes of plasma based on liquid chromatography–mass spectrometry (LC/MS). The method we developed exhibited excellent reliability, sensitivity, short run time of analysis (10 min/sample), and simplicity of sample preparation. The application of this assay method was also demonstrated after a single i.v. dose of PSC 833 administered to rats.

2. Experimental

2.1. Chemicals and reagents

PSC 833 was a kind gift from Novartis (Basel, Switzerland). Amiodarone was obtained from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile, ammonium hydroxide, methanol, diethyl ether, and water were all HPLC grades and were purchased from Caledon Laboratories (Georgetown, ON, Canada).

2.2. Liquid chromatography/mass spectrometry conditions

LC/MS analyses were done using a Waters Micromass ZQ™ 4000 mass spectrometer coupled to a Waters 2795 separations module with an autosampler (Milford, Ma, USA). The mass spectrometer was operated in positive ion mode with selected ion recording (SIR) acquisition mode. The nebulizing gas was obtained from an in house high purity nitrogen source. The temperature of the source was set at 150 °C and the voltage of the capillary and cone were 3.1 KV and 30 V, respectively. The gas flow of desolvation and the cone were set at 550 and 80 L/h, respectively. Chromatographic separation was achieved using a C_8 3.5 μm (2.1 mm × 50 mm) column as the stationary phase (Agilent® Eclipse XDB-C8, USA). Mobile phase was pumped as an isocratic acetonitrile: ammonium hydroxide 0.2% at a ratio of 90:10 v/v, respectively. Total analytical run time was 10 min. A constant flow rate of 0.2 mL/min was used throughout the run. The column was heated to 60 °C during the chromatographic run. Amiodarone was used as internal standard (IS).

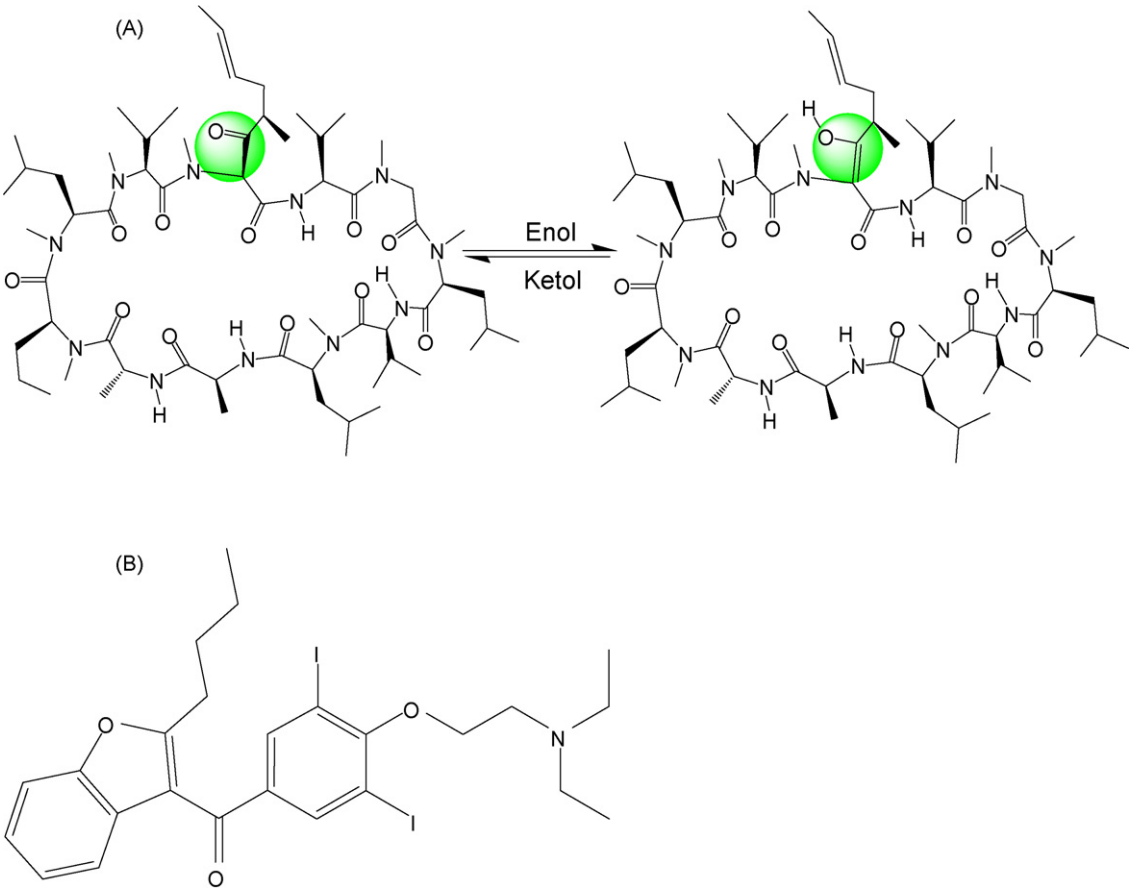

Fig. 1. Chemical structures of (A) PSC 833 in keto-enol tautomerism and (B) amiodarone (IS).

The mixture of PSC 833 and IS was analyzed on the mass spectrometer using flow injection in scan mode to determine optimal fragmentation for each compound and establishment of the mass-to-charge ratio (m/z) values of the molecular ions. The analysis was carried out using SIM at the protonated molecular ions m/z 1214.81 (PSC 833) and 645.84 (IS).

2.3. Standard and stock solutions

The stock solution of PSC 833 (200 μg/mL) was prepared by dissolving 20 mg of PSC 833 in 100 mL of methanol. A 50 mg/mL stock solution of PSC 833 in a Cremophor EL/ethanol mixture (containing 600 mg Cremophor EL qs to 1 mL with ethanol) was used as the standard i.v. formulation for PSC 833. This solution was diluted in saline to a final concentration of 5 mg/mL for PSC 833 prior to use [38]. Amiodarone (IS) stock solution (200 μg/mL) was prepared by dissolving 5 mg of amiodarone powder in 25 mL of methanol. Standard solutions were freshly prepared each day by serial dilutions in methanol. All of the stock solutions were stored at 4 °C between uses. Standard samples were prepared by spiking appropriate amounts of PSC 833 in 100 μL of rat plasma at a concentration range of 10–5000 ng/mL. Blank plasma for the preparation of standard solutions was collected from drug-free Sprague–Dawley rats.

2.4. Extraction procedure

A published method (originally used for the extraction of CyA) was used for the extraction of PSC 833, with a minor modification [39]. To each 100 μL plasma sample in a glass tube, 500 μL HPLC water, 100 μL sodium hydroxide (1 M), and 50 μL of IS (0.25 μg/mL) were added. PSC 833 and IS were then extracted into 4 mL of an ether/methanol (95:5) solution by vortex mixing for 30 s. After centrifugation at 3000 × g for 5 min, the organic layer was transferred to new glass tubes and evaporated in vacuum (ISS 110 Speedvac system, Thermosavant). The residues were reconstituted using 0.25 mL of methanol. Aliquots of 10 μL from this solution were injected into the LC/MS system.

To determine the recovery of PSC 833 after extraction from plasma, the peak height obtained from extracts of spiked plasma samples was compared to that obtained from direct injection of known amounts of drug using standard PSC 833 solutions. The recovery was assessed at PSC 833 concentrations of 50, 1000, and 5000 ng/mL, using four replicates for each concentration.

2.5. Calibration, accuracy and validation

Complete validation assessment was undertaken using drug-spiked rat plasma. Calibration samples of 100 μL containing PSC 833 and IS were constructed over the concentration range of 10–5000 ng/mL. The sample to standard solution ratio was constantly 1:2.5 (100 uL plasma and 250 uL of varying standard solution). The ratios of PSC 833 to IS peak height were calculated and plotted versus nominal PSC 833 concentrations. Due to the wide range of concentrations, data for calibration curves was weighted by a factor of 1/concentration.

Intra-day accuracy and precision of the assay were determined using five different concentrations of PSC 833 in rat plasma; namely, 10, 25, 100, 500, 1000 ng/mL. Each concentration was prepared in five replicates. To assess the inter-day accuracy and precision, the assay was repeated on three separate days. For each daily run, a set of calibration samples separate from the validation samples was prepared to allow quantification of the peak height of PSC 833 to IS ratios. Precision was assessed by percentage coefficient of variation (CV%). Bias was assessed by determining mean intra-day and inter-day percentage error.

Table 1
The intra-day (n = 5) and inter-day (n = 3) accuracy and precision of the developed LC/MS method in rat plasma

Nominal concentration (ng/mL)	Intra-day mean ± S.D. measured concentrations (ng/mL) (intra-day CV%)			Inter-day mean ± S.D. measured concentrations (ng/mL)	Inter-day CV%	Inter-day mean error (%)
10	10.24 ± 1.78 (17.38)	12.86 ± 2.04 (15.85)	12.22 ± 2.23 (18.28)	11.78 ± 1.37	17.17	17.76
25	23.52 ± 2.11 (8.97)	23.00 ± 1.88 (8.19)	25.17 ± 1.16 (4.60)	23.90 ± 1.13	7.25	−4.41
100	95.39 ± 4.97 (5.21)	97.44 ± 13.35 (13.70)	105.57 ± 2.65 (2.51)	99.47 ± 5.38	7.14	−0.83
500	510.43 ± 13.43 (2.63)	495.01 ± 49.25 (9.95)	535.08 ± 20.96 (3.92)	513.51 ± 20.21	5.50	2.7
1000	1099.24 ± 159.69 (14.53)	970.40 ± 28.78 (2.97)	1091.57 ± 67.48 (6.18)	1053.73 ± 72.27	7.89	5.37

2.6. Animal study

To demonstrate the applicability of the developed method *in vivo*, the right jugular vein of three male Sprague–Dawley rats (280–350 g) were cannulated with Micro-Renathane tubing (Braintree Scientific, Braintree, MA, USA) under isofluorane anesthesia as previously described [40]. The protocol of the animal study was approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee. The day after surgery, the standard PSC 833 i.v. formulation (PSC 833, 50 mg; Cremophor EL, 600 mg; ethanol qs to 1 mL) was diluted in saline to a final concentration of 5 mg/mL and a 5 mg/kg dose was injected into the catheter. Blood samples (150–250 μL) were collected from each implanted cannula at 5, 20, and 40 min, then 1, 2, 4, 6, 9, 12, and 24 h after drug admin-

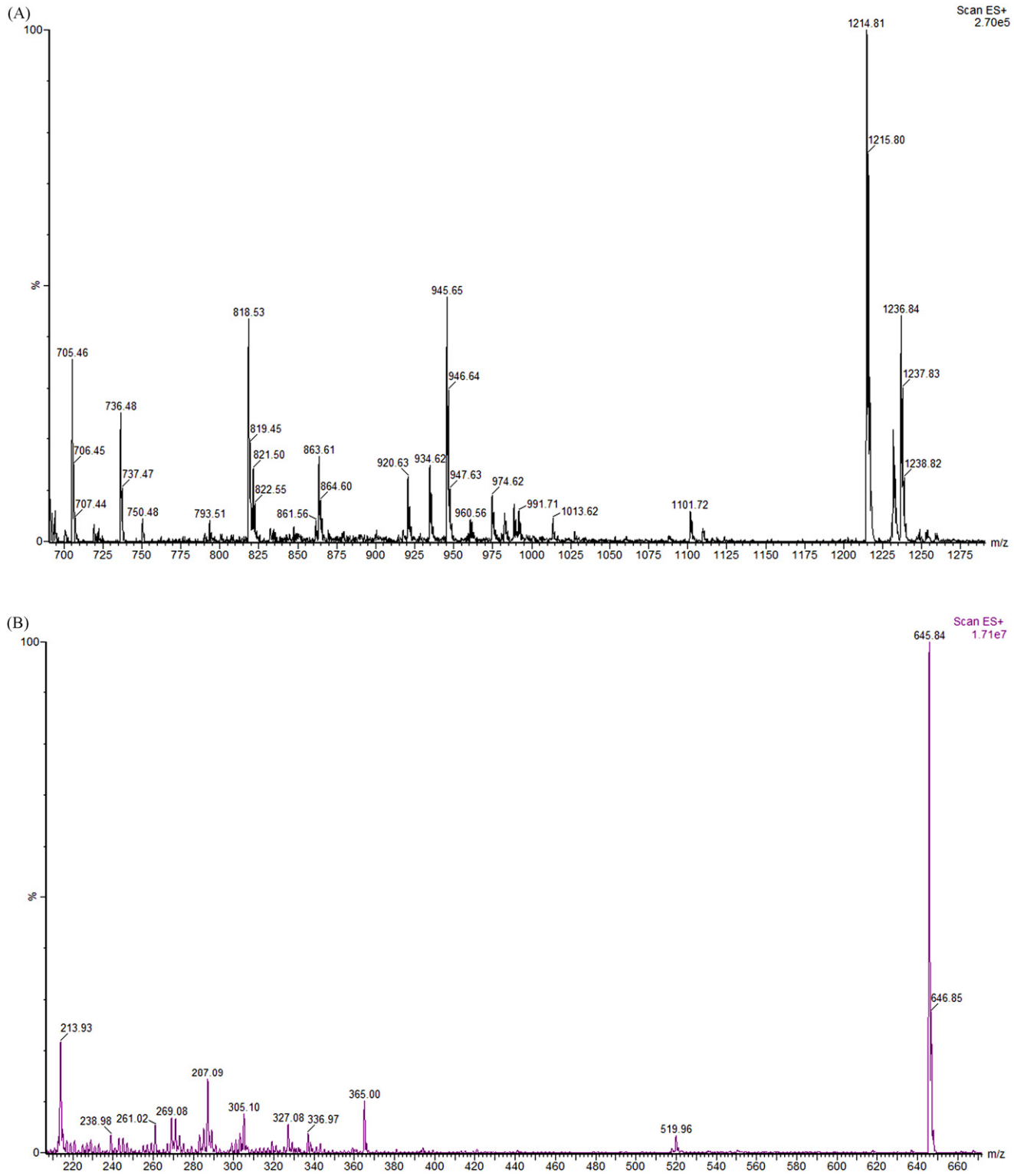

Fig. 2. Positive ion mass spectra of (A) PSC 833 and (B) amiodarone (IS).

istration. Between sampling, 0.2 mL of heparin (100 U/mL) solution was used to maintain the patency of the cannula. Blood samples were immediately centrifuged for 3 min; plasma was separated and stored at −20 °C until analysis. The plasma concentrations of PSC 833 were analyzed by the developed LC/MS method and the plasma concentration versus time curve was profiled. The elimination rate constant (λ_z) was estimated by linear regression of the plasma concentrations in the log–linear terminal phase and the corresponding half-life ($t_{1/2}$) was calculated by dividing 0.693 by λ_z. In order to estimate the initial plasma concentration (Cp_0) immediately after i.v. injection, the linear regression of the log-linear initial state going through the first three time points was extrapolated to the time zero. The estimated Cp_0 was then used with the actual measured plasma concentrations to determine the area under the plasma concentration–time curve from the time of dosing to the last sampling time ($AUC_{0-24\,h}$). The $AUC_{0-\infty}$ was calculated using the combined log-linear trapezoidal rule for data from time of dosing to the last measured concentration (0–24 h), plus the quotient of the last measured concentration divided by λ_z. Non-compartmental pharmacokinetic methods were used to calculate mean residence time (MRT) by dividing area under the first moment curve ($AUMC_{0-\infty}$) by $AUC_{0-\infty}$, clearance (CL) by dividing dose by $AUC_{0-\infty}$, and volume of distribution at steady-state (Vd_{ss}) by multiplying CL by MRT.

3. Results/discussion

Quantitative analysis of PSC 833 in blood or plasma is usually accomplished through analytical methods based on either RIA [29–32] or HPLC [33–36]. Some of the RIA kits are known to detect CyA metabolites especially the major monohydroxylated metabolite (AM9) that also occurs in PSC 833 (M9) [37]. Therefore, application of the RIA assay methods will lead to an over-estimation of PSC 833 concentrations in blood or plasma. Besides cost, several steps involved in sample preparation and more importantly, the safety issues are among several limitations associated with the use of the RIA methods in general [31,32]. Although HPLC analysis methods do not have the limitations of RIA, most of the reported HPLC assays for PSC 833 have failed to produce a single peak. This has resulted in a relatively low sensitivity for PSC 833 analysis by HPLC [33,35]. van Tellingen et al. [35] reported that the poor peak shapes and double peaks markedly reduce the sensitivity of the assay, and they had relatively good chromatographic performance only when columns with NovaPak® Phenyl packing material were used. The presence of two peaks was attributed to the existence of PSC 833 keto-enol tautomerism (Fig. 1A) [33]. Another disadvantage associated with the use of HPLC assays for PSC 833 is the relatively long analytical run times (PSC 833 retention time ≥15 min).

The mass spectra of PSC 833 and IS dissolved in methanol with 0.2% ammonium hydroxide are shown in Fig. 2A and B, respectively. The molecular ion at m/z 1214.81 and 645.84 were selected for quantification of PSC 833 and IS, respectively. Fig. 3A represents the chromatogram of blank rat plasma after the extraction procedure showing no endogenous peaks that might interfere with IS or PSC 833 peaks (Fig. 3B and C, respectively). PSC 833 and IS peaks were well separated with retention times of approximately 2.4 and 3.1 min, respectively. The run time of analysis was 10 min. Under the experimental condition, we have only detected one peak for PSC 833 (Fig. 4).

The average extraction recoveries of PSC 833 from plasma were 102.7, 79, and 73% for 50, 1000, and 5000 ng/mL of PSC 833, respectively. A linear relationship between the peak height ratios and rat plasma concentrations of PSC 833 was observed within the range of 10–5000 ng/mL ($R^2 > 0.99$). The mean slope and intercept from three replicates of calibration curves on different days were calculated to be 0.00015 and −0.0033 for PSC 833, respectively.

The assay CV% for both intra-day and inter-day assessments were less than 15% except for the lowest concentration used in the calibration and validation samples (10 ng/mL), where the values were between 15–19%. Mean error was less than 10% in all the concentrations above 10 ng/mL (Table 1). The low variability in the

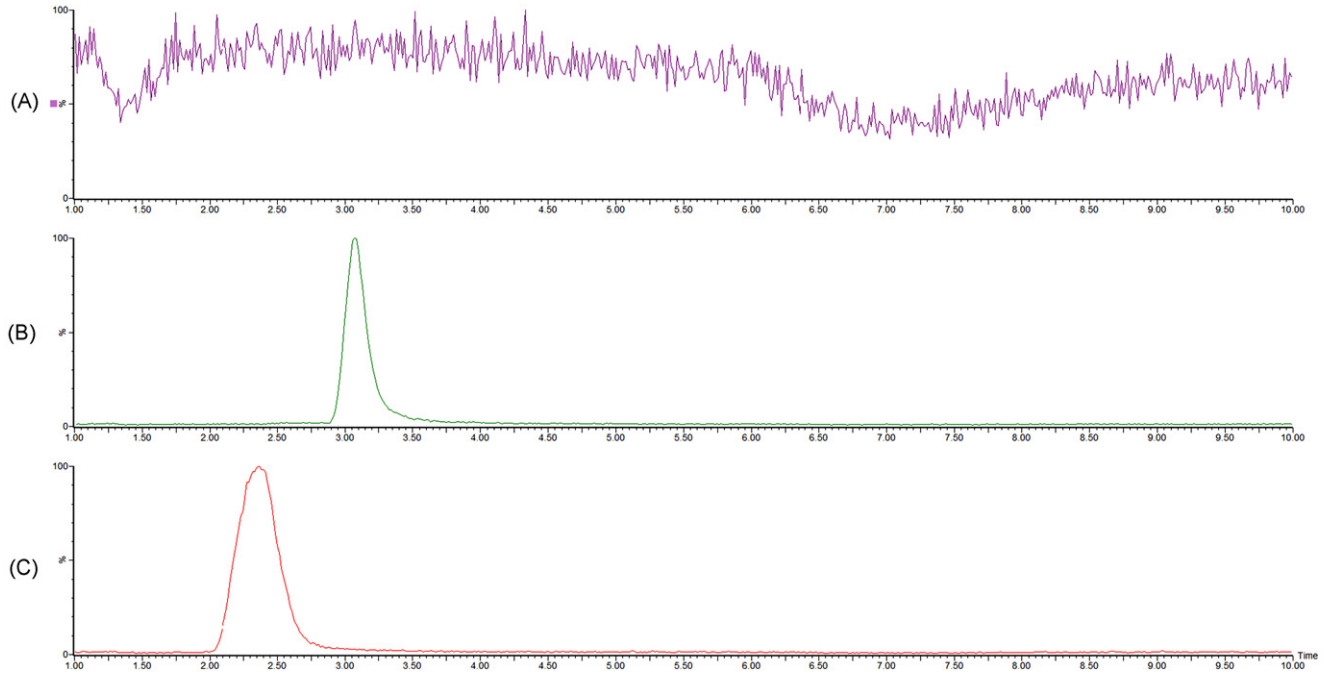

Fig. 3. Representative selected ion recording (SIR) chromatograms of (A) blank plasma, (B) amiodarone (m/z 645.84; 3.05 min), and (C) PSC 833 (m/z 1214.81; 2.37 min) after extraction from rat plasma.

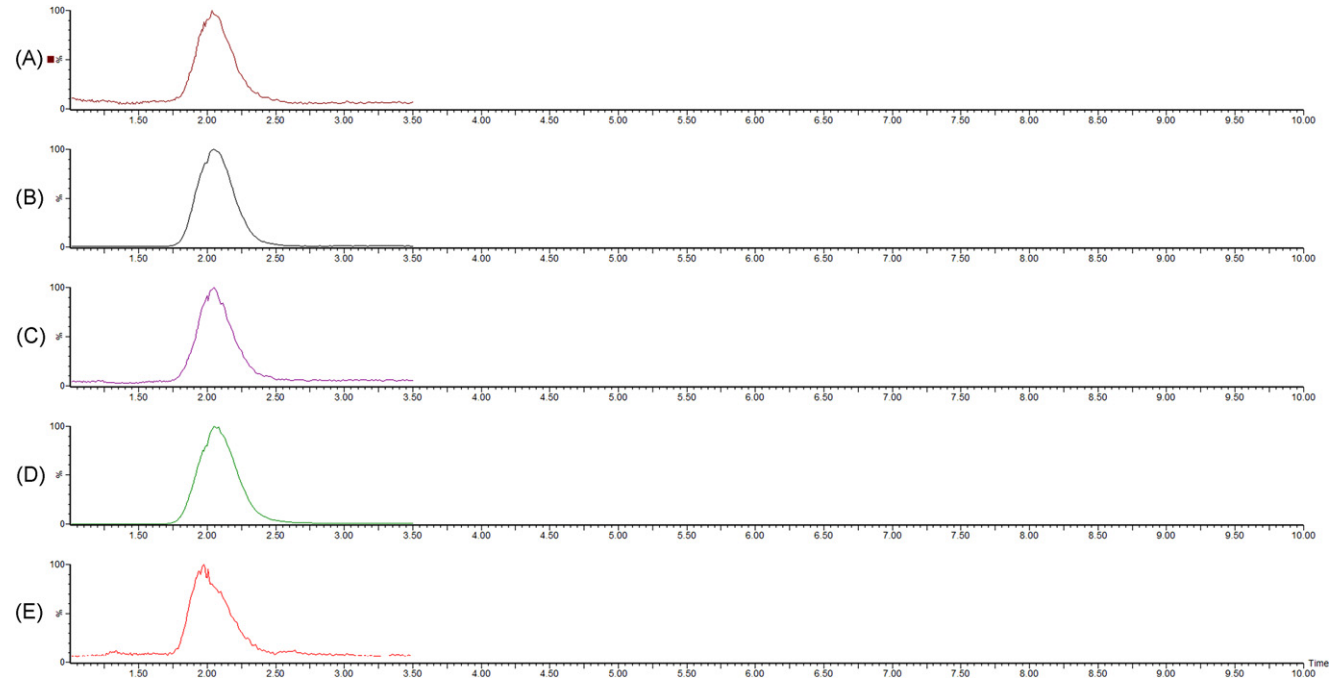

Fig. 4. SIR chromatograms of (A) PSC 833 in methanol (50 ng/mL), (B) PSC 833 in methanol (1000 ng/mL), (C) rat plasma extract of PSC 833 (50 ng/mL), (D) rat plasma extract of PSC 833 (1000 ng/mL), and (E) plasma sample from a rat obtained at 12 h following a single i.v. administration of 5 mg/kg of PSC 833.

validation data demonstrated the accuracy and reproducibility of the developed method. The lower limit of quantification (LOQ) is the lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy [41]. Based on the validation data, the LOQ was set at 10 ng/mL; and with an injection volume of 10 μL, the value translates into an on-column amount of 0.1 ng. The limit of detection (LOD) is the lowest concentration of an analyte that the bioanalytical procedure can reliably differentiate from background noise [41]. The LOD was assessed based on signal-to-noise (S/N) ratio. The concentration of PSC 833 that is associated with an average S/N ratio of 3:1 was considered the LOD. The LOD of this method was found to be 2.5 ng/mL and based on an injection volume of 10 μL, the corresponding amount injected to the system was 0.025 ng (i.e., 25 pg).

Previous studies have shown LOQ values in the range of 37.5–75 and 50–100 ng/mL for RIA and HPLC methods of PSC 833 analysis, respectively [32,34–36]. The lower level of LOQ for the LC/MS method is an indication for the higher sensitivity of this method compared to the reported RIA and HPLC methods. However, the validation of the developed method refers to an individual instrument; this applies in principle for all LC/MS methods. The need for close quality control in further analytical series should be noted.

In order to test the applicability of this method for the *in vivo* analysis of PSC 833, three rats were injected intravenously with a single dose of PSC 833 (5 mg/kg) solubilized with the aid of Cremophor EL and ethanol. The plasma concentration versus time curve was profiled for up to 24 h after drug administration (Fig. 5). To the best of our knowledge, there is no previous report on the pharmacokinetics of PSC 833 in healthy rats in the literature. Most of the published PSC 833 pharmacokinetic studies were those obtained from human subjects in clinical trials. The pharmacokinetics of PSC 833 appears to be similar to that of CyA in humans [34]. Similarly, both drugs seem to have a comparable pharmacokinetic profile in rats (Table 2). When a single i.v. dose of 5 mg/kg was administered, both drugs showed a rapid decline in concentrations in the first two hours which represents a distribution phase. The distribution phase was followed by a terminal elimination phase with an average $t_{1/2}$ of approximately 10 and 8 h for PSC 833 and CyA, respectively [42].

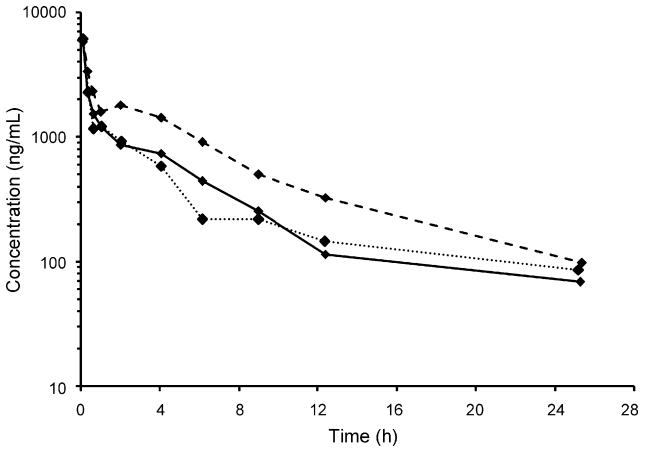

Fig. 5. Plasma concentration vs. time profile of PSC 833 in rats after a single i.v. dose of 5 mg/kg. Data collected from three rats. The PSC 833 level in plasma was quantified by the developed LC/MS method.

Table 2
Non-compartmental pharmacokinetic parameters of PSC 833 ($n = 3$) and CyA ($n = 7$) in rats after a single i.v. dose of 5 mg/kg

Parameter	PSC 833	CyA[a]
$AUC_{0\text{–}24\,h}$ (mg h/L)	11.55 ± 4.38	20.1 ± 9.77
$AUC_{0\text{–}\infty}$ (mg h/L)	12.76 ± 4.20	23.1 ± 12.9
$t_{1/2}$ (h)	10.07 ± 2.99	8.15 ± 1.54
MRT (h)	9.42 ± 1.99	*N/A*
CL (L/kg/h)	0.42 ± 0.12	0.26 ± 0.11
Vd_{ss} (L/kg)	4.06 ± 1.74	1.59 ± 0.66

Data is presented as mean ± S.D.
[a] Data from Ref. [41]. *N/A*: not available.

4. Conclusions

The developed LC/MS method is a simple, quick and reliable method validated for the quantification of PSC 833 in rat plasma that can replace RIA and HPLC methods of PSC 833 analysis for pharmacokinetic studies.

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References

[1] J.M. Ford, W.N. Hait, Pharmacol. Rev. 42 (1990) 155.
[2] K.V. Chin, I. Pastan, M.M. Gottesman, Adv. Cancer Res. 60 (1993) 157.
[3] N. Aouali, L. Eddabra, J. Macadre, H. Morjani, Crit. Rev. Oncol. Hematol. 56 (2005) 61.
[4] R. Krishna, L.D. Mayer, Eur. J. Pharm. Sci. 11 (2000) 265.
[5] B.I. Sikic, Oncology (Williston Park) 13 (1999) 183.
[6] B.I. Sikic, G.A. Fisher, B.L. Lum, J. Halsey, L. Beketic-Oreskovic, G. Chen, Cancer Chemother. Pharmacol. 40 (Suppl.) (1997) S13.
[7] R.L. Juliano, V. Ling, Biochim. Biophys. Acta 455 (1976) 152.
[8] M. Hirose, Int. J. Hematol. 76 (Suppl. 2) (2002) 206.
[9] D. Israeli, S. Ziaei, P. Gonin, L. Garcia, J. Theor. Biol. 232 (2005) 41.
[10] N. Kartner, J.R. Riordan, V. Ling, Science 221 (1983) 1285.
[11] A. Sparreboom, K. Nooter, Drug Resist. Updat. 3 (2000) 357.
[12] I.E. Kuppens, P. Breedveld, J.H. Beijnen, J.H. Schellens, Cancer Invest. 23 (2005) 443.
[13] D. Boesch, K. Muller, A. Pourtier-Manzanedo, F. Loor, Exp. Cell Res. 196 (1991) 26.
[14] M. Lemaire, A. Bruelisauer, P. Guntz, H. Sato, Cancer Chemother. Pharmacol. 38 (1996) 481.
[15] N. Kusunoki, K. Takara, Y. Tanigawara, A. Yamauchi, K. Ueda, F. Komada, Y. Ku, Y. Kuroda, Y. Saitoh, K. Okumura, Jpn. J. Cancer Res. 89 (1998) 1220.
[16] T. Watanabe, H. Tsuge, T. Oh-Hara, M. Naito, T. Tsuruo, Acta Oncol. 34 (1995) 235.
[17] M.M. Gottesman, I. Pastan, Annu. Rev. Biochem. 62 (1993) 385.
[18] D. Boesch, C. Gaverieaux, B. Jachez, F. Loor, Cancer Res. 51 (1991) 4226.
[19] E. Cagliero, R. Ferracini, E. Morello, K. Scotlandi, M.C. Manara, P. Buracco, A. Comandone, R. Baroetto Parisi, N. Baldini, Oncol. Rep. 12 (2004) 1023.
[20] R.P. Keller, H.J. Altermatt, P. Donatsch, H. Zihlmann, J.A. Laissue, P.C. Hiestand, Int. J. Cancer 51 (1992) 433.
[21] P. Sonneveld, M. Schoester, K. de Leeuw, J. Clin. Oncol. 12 (1994) 1584.
[22] D.J. Boote, I.F. Dennis, P.R. Twentyman, R.J. Osborne, C. Laburte, S. Hensel, J.F. Smyth, M.H. Brampton, N.M. Bleehen, J. Clin. Oncol. 14 (1996) 610.
[23] B.L. Lum, S. Kaubisch, A.M. Yahanda, K.M. Adler, L. Jew, M.N. Ehsan, N.A. Brophy, J. Halsey, M.P. Gosland, B.I. Sikic, J. Clin. Oncol. 10 (1992) 1635.
[24] I. Chico, M.H. Kang, R. Bergan, J. Abraham, S. Bakke, B. Meadows, A. Rutt, R. Robey, P. Choyke, M. Merino, B. Goldspiel, T. Smith, S. Steinberg, W.D. Figg, T. Fojo, S. Bates, J. Clin. Oncol. 19 (2001) 832.
[25] S.E. Bates, S. Bakke, M. Kang, R.W. Robey, S. Zhai, P. Thambi, C.C. Chen, S. Patil, T. Smith, S.M. Steinberg, M. Merino, B. Goldspiel, B. Meadows, W.D. Stein, P. Choyke, F. Balis, W.D. Figg, T. Fojo, Clin. Cancer Res. 10 (2004) 4724.
[26] G. Giaccone, S.C. Linn, J. Welink, G. Catimel, H. Stieltjes, W.J. van der Vijgh, C. Eeltink, J.B. Vermorken, H.M. Pinedo, Clin. Cancer Res. 3 (1997) 2005.
[27] K.T. Kivisto, H.K. Kroemer, M. Eichelbaum, Br. J. Clin. Pharmacol. 40 (1995) 523.
[28] V. Fischer, A. Rodriguez-Gascon, F. Heitz, R. Tynes, C. Hauck, D. Cohen, A.E. Vickers, Drug Metab. Dispos. 26 (1998) 802.
[29] J.M. Kovarik, E.A. Mueller, F. Richard, W. Tetzloff, J. Clin. Pharmacol. 37 (1997) 1009.
[30] S.M. Kornblau, E. Estey, T. Madden, H.T. Tran, S. Zhao, U. Consoli, V. Snell, G. Sanchez-Williams, H. Kantarjian, M. Keating, R.A. Newman, M. Andreeff, J. Clin. Oncol. 15 (1997) 1796.
[31] S. Bates, M. Kang, B. Meadows, S. Bakke, P. Choyke, M. Merino, B. Goldspiel, I. Chico, T. Smith, C. Chen, R. Robey, R. Bergan, W.D. Figg, T. Fojo, Cancer 92 (2001) 1577.
[32] P.M. Fracasso, P. Westervelt, C.L. Fears, D.M. Rosen, E.G. Zuhowski, L.A. Cazenave, M. Litchman, M.J. Egorin, J. Clin. Oncol. 18 (2000) 1124.
[33] M.G. Scott, K.G. Hock, D.L. Crimmins, P.M. Fracasso, Clin. Chem. 43 (1997) 505.
[34] R.M. Lush, B. Meadows, A.T. Fojo, G. Kalafsky, H.T. Smith, S. Bates, W.D. Figg, J. Clin. Pharmacol. 37 (1997) 123.
[35] O. van Tellingen, M. Kemper, F. Tijssen, J. van Asperen, W.J. Nooijen, J.H. Beijnen, J. Chromatogr. B, Biomed. Sci. Appl. 719 (1998) 251.
[36] E.A. Mueller, J.M. Kovarik, Y. Uresin, S.S. Preisig-Fluckiger, S. Hensel, P.W. Lucker, B. Holt, J. Clin. Pharmacol. 37 (1997) 1001.
[37] R.W. Yatscoff, K.R. Copeland, C.J. Faraci, Clin. Chem. 36 (1990) 1969.
[38] T. Watanabe, Y. Nakayama, M. Naito, T. Oh-hara, Y. Itoh, T. Tsuruo, Anticancer Drugs 7 (1996) 825.
[39] A.P. Chimalakonda, R.B. Shah, R. Mehvar, J. Chromatogr. B, Analyt. Technol. Biomed. Life Sci. 772 (2002) 107.
[40] D.R. Brocks, Biopharm Drug Dispos. 21 (2000) 365.
[41] FDA, U.S. Department of Health and Human Services, Rockville, 2001.
[42] D.R. Brocks, S. Ala, H.M. Aliabadi, Biopharm Drug Dispos. 27 (2006) 7.