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Short communication

Liquid chromatography–tandem mass spectrometric assay for the VEGFR inhibitor cediranib and its primary human metabolite cediranib-N$^+$-glucuronide in plasma

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ABSTRACT

A quantitative bioanalytical assay for cediranib and its N$^+$-glucuronide metabolite was developed and validated. Human plasma samples were pre-treated using protein precipitation with acetonitrile containing erlotinib and CYT-387 as internal standards for the glucuronide metabolite and parent compound, respectively. The extract was diluted with water and injected into the chromatographic system. This system consisted of sub-2 μm particles, a trifunctional bonded octadecyl silica column with gradient elution using 0.005% (v/v) of formic acid in a mixture of water and methanol. The eluate was transferred into the electrospray interface with positive ionization and the analytes were detected in the selected reaction monitoring mode of a triple quadrupole mass spectrometer. The assay was validated in a 1–290 ng/ml calibration range for cediranib and 0.2–52 ng/ml for its glucuronide metabolite. The lowest levels of these ranges corresponded to the lower limits of quantification for both compounds. Within day precisions were 4.0–6.7% for cediranib and 4.1–11.9% for its glucuronide, between day precisions were 4.2–10.2 and 4.8–14.4% and accuracies were between 99 and 106 and 84 and 94% for cediranib and its metabolite, respectively. Stabilities of both compounds were sufficient under all relevant conditions. Finally, the assay was successfully used to assess drug levels in a pharmacokinetic mouse study.

1. Introduction

Cediranib (AZD 2171, Fig. 1A) is a novel, potent and orally bioavailable inhibitor of the vascular endothelial growth factor receptor (VEGFR) and other tyrosine kinases. The highest activity was initially observed for VEGFR-2 [1]. Drug development resulted in three currently ongoing Phase III clinical studies in recurrent glioblastoma, untreated metastatic colorectal cancer and non-small cell lung cancer, respectively [2]. In addition to oxidative *in vitro* hepatic biotransformation of cediranib, a major metabolite, cediranib-N$^+$-glucuronide (Fig. 1B), was observed in human microsomal and hepatocyte preparations but not in hepatic preparations of 8 different animal species [3]. This compound was identified as the major circulating metabolite in human and demonstrated to be formed by uridine glucuronosyltransferase 1A4 [4]. No clinical levels of this metabolite have been reported yet.

For pharmacokinetic evaluation of this drug a bioanalytical assay is required. An LC-MS/MS assay for cediranib was reported by Mitchel et al. [5], but details were not given except the lower limit of quantification (1 ng/ml), and very recently also by Wang et al. [6]. The latter method was validated for cediranib in mouse plasma and brain homogenate in the 2.5–2500 ng/ml range using liquid–liquid extraction. For future human studies simultaneous determination of the main metabolite is preferred. A new bioanalytical assay using LC–MS/MS for determination of cediranib and its glucuronide in plasma was therefore developed and validated. The use of MS/MS detection in combination with an LC system using sub-2 μm particles was expected to result in a high sensitivity and selectivity allowing the analysis to be executed with a simple sample pre-treatment and short analytical run times. The usefulness of the assay was demonstrated for cediranib in a pharmacokinetic mouse study.

Abbreviations: HESI, heated electrospray ionization; LLOQ, lower limit of quantification; SRM, selected reaction monitoring; QC, quality control; VEGFR, vascular endothelial growth factor receptor.

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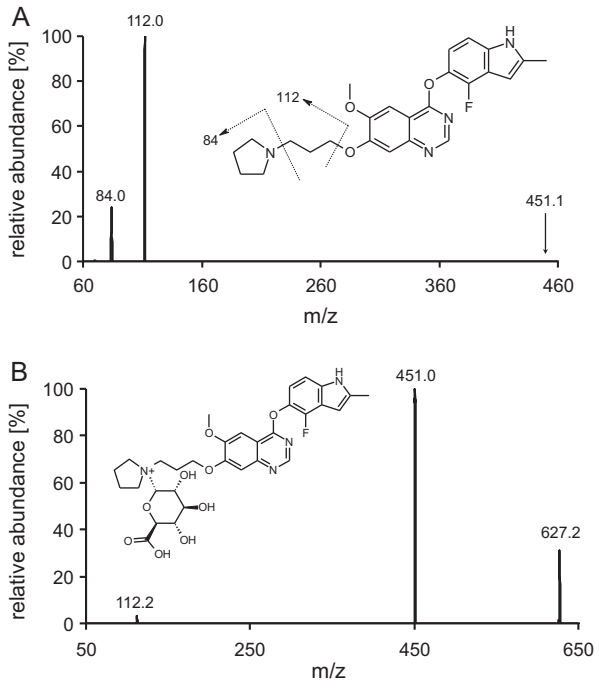

Fig. 1. Product mass spectra of (A) cediranib ([M+H]$^+$: m/z 451.10@-40 V) and (B) cediranib-N$^+$-glucuronide ([M+H]$^+$: m/z 627.20@-15 V).

2. Experimental

2.1. Chemicals

Cediranib, erlotinib and CYT-387·H_2SO_4 (*N*-(cyanomethyl)-4-[2-[[4-(4-morpholinyl)phenyl]amino]-4-pyrimidinyl]-benzamide·H_2SO_4) were obtained from Sequoia Research Products (Pangbourne, UK). Water (LC–MS grade), methanol (HPLC grade) and acetonitrile (HPLC-S grade) were from Biosolve (Valkenswaard, The Netherlands). Water not used as eluent was home purified by reversed osmosis on a multi-laboratory scale. Formic acid was of analytical grade and originated from Merck (Darmstadt, Germany) and analytical grade dimethyl sulfoxide (DMSO) from Acros Organics (Geel, Belgium). Pooled human EDTA-disodium plasma was supplied by Seralab Laboratories International (Haywards Heath, UK), plasma from individual human donors by Innovative Research (Southfield, MI, USA).

The analytical standard of cediranib-N$^+$-glucuronide was prepared by human liver microsomal incubations of cediranib using a procedure (Supplemental Method 1) based on Brandon et al. [7]. Concentrations of both compounds in incubation mixtures produced were assessed using an LC–UV method at 320 nm (Supplemental Method 2), assuming the molar absorptivities at this wavelength are identical for cediranib and its glucuronidated metabolite. Cediranib-N$^+$-glucuronide concentrations reported are based on the molecular mass of the parent compound without correction for the glucuronide moiety.

2.2. LC–MS/MS

The LC–MS/MS equipment consisted of an Accela pump and auto-injector and a TSQ Quantum Ultra triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) with heated electrospray ionization (HESI), operated using the Thermo Fisher Xcalibur software (version 2.07).

Injections (10 μl) were made on an Acquity UPLC® BEH C18 column (30 mm × 2.1 mm, d_p = 1.7 μm, Waters, Milford, USA), protected by the corresponding VanGuard pre-column (Waters, 5 mm × 2.1 mm). The column temperature was maintained at 40 °C and the samples at 4 °C. The eluent comprised a mixture of 0.1% (v/v) formic acid in water (5%, v/v) and a water-methanol gradient from 45 to 50% (v/v) methanol during the first minute after injection. Next, the column was flushed with 95% (v/v) acetonitrile for 0.5 min and reconditioned at the starting conditions for 0.8 min. The whole eluate (0.6 ml/min) was transferred into the electrospray probe, starting at 0.5 min after injection by switching the MS divert valve until 1.6 min after injection. The method used a 3000 V spray voltage, a 350 °C capillary temperature, a 367 °C vaporizer temperature, and the nitrogen sheath, ion sweep and auxiliary gasses set at 50, 0 and 5 arbitrary units, respectively; the skimmer voltage was set off. The SRM mode was used with argon as the collision gas at 1.5 mTorr. Mass resolutions were set at 0.7 full width at half height (unit resolution) for both separating quadrupoles and settings for individual compounds are shown in Table 1. The electromultiplier was set at 5×10^6.

2.3. Sample pre-treatment

To a volume of 50 μl of human plasma, pipetted into a 1.5 ml polypropylene tube, 75 μl of 8 ng/ml erlotinib and 100 ng/ml CYT-387·H_2SO_4 in acetonitrile was added. The tube was then closed and shaken vigorously for 5–10 s using vortex-mixing. After centrifugation of the sample at $10 \times 10^3 \times g$ at 20 °C for 1 min, 100 μl of the supernatant was pipetted into a 250 μl glass insert placed in an auto-injector vial. Before closing the vial, 100 μl of water was added to obtain sufficient hydrophobicity of the sample for the chromatographic system.

2.4. Validation

A laboratory scheme based on international guidelines was used for the validation procedures [8–10].

2.4.1. Calibration

Stock solutions of 1 mg/ml cediranib and 2 mg/ml CYT-387·H_2SO_4 (IS for cediranib) were prepared in methanol, 4 mg/ml erlotinib (IS for cediranib-N$^+$-glucuronide) was prepared in DMSO. All stock solutions were stored at −30 °C. Stock solutions of cediranib were treated in microsomal incubations to obtain mixtures containing both cediranib and its N$^+$-glucuronide to be used as standards for the assay. One of the mixtures, containing 7250 ng/ml cediranib and 1310 ng/ml cediranib-N$^+$-glucuronide was diluted with blank pooled human plasma to obtain a calibration sample, stored in a polypropylene tube at −30 °C, containing

Table 1
Mass spectrometric settings for individual compounds in the LC–MS/MS assay.

	Cediranib	Cediranib-N$^+$-glucuronide	CYT-387	Erlotinib
m/z (parent)	451.2	627.2	415.15	394.1
Tube lens off set (V)	118	130	118	106
m/z (product)	84.05; 112.1	112.05; 541.2	244.1; 245.1	278.1
Collision energy (V)	−44; −31	−33; −15	−77; −59	−30
Dwell time (ms)	20; 20	100; 100	40; 40	20

290 ng/ml cediranib and 52.4 ng/ml of the glucuronide. Additional calibration samples were prepared daily at 116, 29, 11.6, 2.9 and 1.16 ng/ml cediranib and 20.96, 5.24, 2.096, 0.524 and 0.210 ng/ml cediranib-N^+-glucuronide, respectively, by dilution with blank pooled plasma. The highest and two lowest calibration samples were processed in duplicate for each daily calibration, whereas the levels in between were processed only once. Least-squares linear regression was employed to define the calibration curves using the ratios of the peaks of cediranib, its glucuronide and their corresponding ISs CYT-387 and erlotinib, respectively.

2.4.2. Precision and accuracy

A second incubation product mixture, prepared from a second stock solution, containing 5250 ng/ml cediranib and 1000 ng/ml cediranib-N^+-glucuronide, was used to obtain validation (quality control; QC) samples in pooled human plasma at 210; 40 (QC-high), 21; 4 (QC-med), 2.625; 0.5 (QC-low) and 1.05; 0.2 ng/ml (QC-LLOQ) for parent and metabolic compounds, respectively. The QC samples were stored in polypropylene tubes at −30 °C. Precisions and accuracies were determined by sextuple analysis of each QC in three analytical runs on three separate days for all QCs (total: $n = 18$ for each QC). Relative standard deviations were calculated for both the within day precision and the between day precision.

2.4.3. Selectivity

Six individual human plasma samples were processed to test the selectivity of the assay. The samples were processed without analytes and ISs and with cediranib and its metabolite at the LLOQ levels (1 and 0.2 ng/ml, respectively), supplemented with the ISs.

2.4.4. Recovery and matrix effect

The extraction efficiency (recovery) was determined in quadruplicate by comparing processed samples (QC-high, -med and -low) with extracts of drug-free human EDTA plasma spiked with the analytes at these levels. Ionization efficiency (matrix effect; ion suppression) was assessed by comparing LC–MS/MS responses of the spiked blank extracts with reference material solutions in water–methanol–acetonitrile (63/10/27; v/v/v) at the three validation levels. The extraction and ionization efficiencies of the ISs were assessed using identical procedures at the erlotinib and CYT-387 concentrations used in the assay.

2.4.5. Stability

The stability of cediranib and its glucuronide was investigated ($n = 4$) in QC-high and -low plasma samples after storage at ambient temperature for 24 h, three additional freeze–thaw cycles (thawing at ambient temperature during *ca.* 1 h and freezing again at −30 °C for at least one day) and storage at −30 °C for 10.5 weeks, respectively. Furthermore, validation runs were re-injected after additional storage of the extracts at 4 °C for 1, 2 and 4 days. Finally, the stability of cediranib in the stock solutions and of both compounds in the incubation mixtures was investigated.

2.5. Mouse samples

Wild-type (FVB; $n = 5$) mice were housed and handled as reported previously [11] and were treated with 6 mg/kg cediranib in Tween 80/ethanol/water (20/13/67; v/v/v) orally. Blood samples were collected in tubes containing potassium-EDTA *via* the tail vein at 0.5, 1, 2 and 4 h after administration of the drug. At 8 h, plasma was collected in sodium-EDTA tubes by cardiac puncture. After centrifugation at 2100 × *g* for 6 min at 4 °C, plasma samples were stored at −30 °C. Mouse sodium-EDTA plasma samples (10 μl) were diluted with 40 μl pooled human EDTA plasma before analysis, potassium-EDTA samples (10 μl) were diluted to 200 μl with the human plasma before processing the samples to remain within the validated calibration range. QC samples (high and med levels) in mouse EDTA plasma were processed analogously ($n = 6$) to assess the applicability of the mouse plasma dilution.

3. Results and discussion

3.1. Cediranib-N^+-glucuronide

Lenz et al. [4] demonstrated the formation of cediranib-N^+-glucuronide as the only metabolite during cediranib incubation with human liver microsomes with UDPGA as the co-factor. In the present study, the metabolic product and cediranib showed comparable UV spectra (Supplemental Fig. 1); further, the ESI spectrum of cediranib-N^+-glucuronide showed the same (unexplained) fragment ion (m/z 314.1) as in the work of Lenz et al. [4] and the MS/MS product spectrum of the protonated molecule showed the de-conjugated protonated metabolite and fragments that were also formed by cediranib (Fig. 1). Based on these supportive results the identity of cediranib-N^+-glucuronide was assumed to be correct.

3.2. Method development

A simple pre-treatment procedure using protein precipitation was investigated as the first option because we expected a high selectivity and sensitivity of the MS/MS detection in combination with an LC system using 2-μm particles. Product spectra of both drugs are shown in Fig. 1. The method is a modification of a previous method for the bioanalysis of axitinib [11]. A few essential modifications were made during method development to obtain optimal sensitivity for the metabolite and to avoid long-term ion-suppressing effects.

3.3. Validation

SRM chromatograms of all compounds are depicted in Fig. 2, showing chromatograms of blank and LLQ spiked human plasma samples.

3.3.1. Calibration

A 1–290 ng/ml cediranib range was chosen to assess expected levels up to an average of *ca.* 100 ng/ml for a 45 mg daily regimen [5]. For 6 calibrations (54 samples) the concentrations were back-calculated from the ratios of the peak areas (of analyte and IS; cediranib/CYT-387 and cediranib-N^+-glucuronide/erlotinib) using the linear calibration curves of the run in which they were included. No deviations were observed in the average of each level higher than 7% (data not shown), indicating the suitability of the linear regression model with quadratic weighting for both analytes. The regression coefficients (r^2) were 0.992 ± 0.004 for cediranib and 0.993 ± 0.003 for its metabolite, respectively.

3.3.2. Precision and accuracy

Assay performance data from the validation samples at four concentrations are reported in Table 2. Between day variations and deviations of the accuracy lower than 7% were observed for all levels except at the LLOQ for cediranib and lower than 10% for the glucuronide. Thus, the precision and the accuracy met the required ±15% (±20% for the LLOQ) [8–10].

3.3.3. Selectivity

The analysis of six batches of blank samples showed no interfering peaks in the SRM traces for both compounds and both ISs in human plasma. Blank responses could not be distinguished from the detector noise (Fig. 2) and are therefore <20% of the LLOQ response [12]. The signals of the LLOQ level (1 and 0.2 ng/ml, respectively) were easily distinguishable from blank

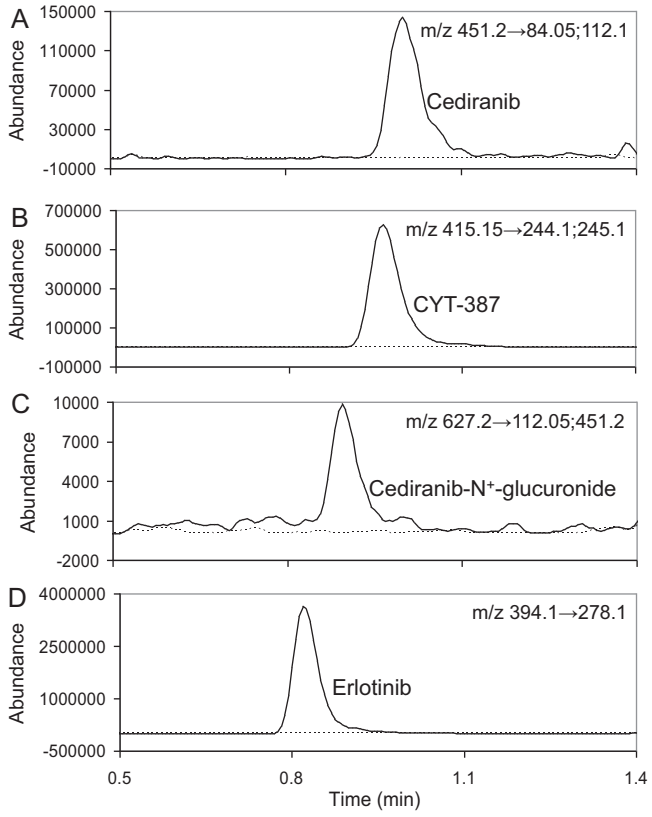

Fig. 2. Chromatograms of blank human plasma (——) and LLOQ spiked plasma (– – – –) for (A) cediranib, (B) CYT-387, (C) cediranib-N⁺-glucuronide and (D) erlotinib.

responses; concentrations found at the LLOQ level ($n = 6$) were 1.11 ± 0.11 ng/ml for cediranib and 0.21 ± 0.03 ng/ml for cediranib-N⁺-glucuronide, respectively; both show the applicability of the investigated LLOQ levels [8–10].

3.3.4. Recovery and matrix effect

The extraction recoveries showed no losses for all compounds (Supplemental Table 1) and the ionization efficiencies show only small (*ca.* 5–20%) ion suppression effects for all analytes and ISs. For both parameters these data are acceptable for successful validation of the assay [8–10].

Table 2
Assay performance data of cediranib and its glucuronide resulting from 18 validation (QC) samples (human plasma) in 3 analytical runs with additional cediranib data for diluted mouse plasma samples ($n = 6$; diluted levels are given).

Concentrations (ng/ml)	Within day precision [%]	Between day precision [%]	Accuracy [%]
Cediranib			
210	4.0	4.2	99.0
21	4.2	4.7	105.8
2.625	6.6	6.8	99.3
1.05	6.7	10.2	102.3
40.2 (20% mouse sodium-EDTA plasma)	6.2		103.7
4.02 (20% mouse sodium-EDTA plasma)	5.2		110.2
21 (5% mouse potassium-EDTA plasma)	11.2		108.4
Cediranib-N⁺-glucuronide			
40	4.1	4.8	92.3
4	3.2	5.3	93.1
0.5	8.0	9.4	94.1
0.2	11.9	14.4	84.3

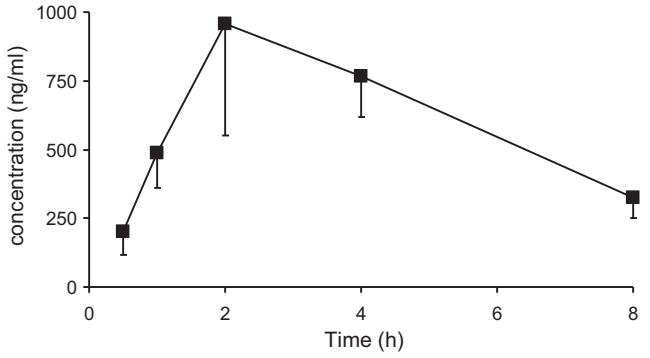

Fig. 3. Pharmacokinetic plot of cediranib, given orally (6 mg/kg) to wild type mice ($n = 5$) mice with standard deviations.

3.3.5. Stability

The stability of cediranib and glucuronide in human EDTA plasma after different storage procedures is shown in Supplemental Table 2. No losses higher than 11.6% were found. Re-injection of calibration and validation samples, after additional storage at 4 °C for 1 day, resulted again in successful performances (less than 33% QC failures (±15%) [8,9,12]). Longer storage under this condition resulted in a too frequent (*ca.* 50% or higher) failure of the accuracy of individual QC samples for cediranib (2 days storage) or both compounds (4 days storage). Recoveries of the analytes in the stock solutions and incubation mixtures were ≥95% for all conditions investigated: 8 h at ambient temperature, 7.5 months at −30 °C (cediranib in stock) and 3 months at −30 °C (both compounds in produced incubation mixture). These stability results were considered sufficient for the validation [8–10].

3.4. Mouse samples

To show the applicability of the new assay after the successful validation procedure, plasma samples from human pharmacokinetic studies were not yet available. Alternatively, the pharmacokinetics of cediranib were investigated in mice. Results are shown in Fig. 3 and Supplemental Fig. 2; cediranib levels in the range 90–1607 ng/ml were observed; cediranib-N⁺-glucuronide was not detected. Diluted mouse plasma QC samples were successfully processed (Table 2).

4. Conclusions

A validated assay for cediranib has now been reported for human plasma samples with simultaneous determination of the main human glucuronide metabolite. The enzymatically synthesized metabolite could be used for quantification in the assay without purification. The LC–MS/MS assay uses a more simple pretreatment method and a faster analytical run than the recently reported cediranib assay for mouse plasma [6]. It also shows values of accuracy, precision, recovery and stability for both analytes allowed by international guidelines [8–10], detection limits for cediranib in the same order as existing methods [5,6] and finally, it can also be used in mouse studies for the parent drug.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2012.03.020.

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