

Effects of vercirnon on the activity of CYP3A4, CYP2C19 and CYP2C8 enzymes and BCRP and OATP1B1 transporters using probe substrates

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Concise Title: Effect of vercirnon on cytochrome P450 enzymes and BCRP/OATP1B1 transporter activity

ONLINE RESOURCE 1

SUPPLEMENTAL METHODS

Study assessments and procedures

Sample collection

Blood samples were obtained via an indwelling cannula or by direct venipuncture, collected into K₂EDTA tubes (vercirnon, pioglitazone), sodium heparinised tubes (midazolam, omeprazole) or K₃EDTA (rosuvastatin) tubes which were inverted several times and immediately placed in ice water before undergoing centrifugation at 1500 g for 15 minutes at 4°C. Supernatant plasma was transferred to 1.8 mL polypropylene tubes and stored at -70°C until shipping. Samples were shipped frozen on dry ice to the bioanalytical site (PPD, Inc. Richmond, VA or Middleton, WI) for determination of plasma concentration using commercially-validated analytical methods.

Bioanalytical methods

Plasma concentrations for pioglitazone, omeprazole, and 5-OH omeprazole (an omeprazole metabolite) were determined by PPD, Inc. Middleton, WI, while those of midazolam, rosuvastatin, vercirnon and its metabolites were determined by PPD, Inc. Richmond, VA using validated analytical methods. Quality controls for run acceptance were prepared and analysed with each batch of samples against separately prepared calibration standards to assess the day-to-day performance of the assay. For the analysis to be acceptable, no more than one-third of the quality control results were to deviate from the nominal concentration by more than 15%, with at least 50% of the quality control results acceptable at each concentration. Quality control results for all analytes in this study met these acceptance criteria.

Probe sample analysis

Midazolam

Plasma concentrations of midazolam were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (PPD Method LCMSC 97 Version 1.00). All plasma samples were received frozen on dry ice and in acceptable condition and were stored at approximately -20°C.

Midazolam and its internal standard, midazolam-d₄, were extracted from 250 µL human plasma through liquid-liquid extraction using 1.0 mL of methyl t-butyl ether. The organic solvent was transferred and then evaporated under a nitrogen stream at approximately 40°C, and the remaining residue was reconstituted with 250 µL of 0.25:50:50 1.0 M ammonium acetate/water/methanol,

v/v/v. The final extract was analysed via HPLC with column-switching and MS/MS detection using positive ion electrospray. The assay was validated over the midazolam concentration range of 0.100 to 100 ng/mL in human sodium heparin plasma. Precision and accuracy were evaluated by replicate analyses of human plasma quality control (QC) pools prepared at five concentrations spanning the calibration range. Precision (measured as the percent coefficient of variation for each set of concentration values [%CV]) ranged from 1.75% to 3.89%. Accuracy (percent difference of the mean value for each pool from the theoretical concentration [%diff]) ranged from -6.24% to 3.35%.

Pioglitazone

Plasma pioglitazone concentrations were determined by PPD, Inc., Middleton, Wisconsin. Human plasma samples, collected from whole blood in dipotassium EDTA, were analysed using a validated method (PPD Method LCMS P1085.00). All plasma samples were received frozen on dry ice and in acceptable condition and were stored at approximately -20°C.

Pioglitazone and the internal standard, pioglitazone-d₄, were extracted from human plasma with acetonitrile. The eluate was diluted with 0.02% TFA in acetonitrile:water (40:60). The final extract was analysed via HPLC with MS/MS detection using positive ion electrospray. The assay was validated over the pioglitazone concentration range of 25.0 to 2500 ng/mL in human dipotassium EDTA plasma. Precision and accuracy were evaluated by replicate analyses of human plasma QC pools prepared at five concentrations spanning the calibration range. Precision (%CV) ranged from 2.31% to 3.51%. Accuracy (%diff) ranged from -1.84% to 1.97%.

Omeprazole and 5-OH omeprazole

Plasma omeprazole and 5-OH-omeprazole concentrations were determined by PPD, Inc., Middleton, Wisconsin. Human plasma samples, collected from whole blood in sodium heparin, were analysed using a validated method (PPD Method LCMS P1009.01). All plasma samples were received frozen on dry ice and in acceptable condition and were stored at approximately -20 °C.

Omeprazole, 5-OH-omeprazole, and their internal standards, omeprazole-d₃ and omeprazole sulfone-d₃, were extracted from human plasma. The analyte was isolated through protein precipitation using 300 µL of (65:35) acetonitrile:methanol. The supernatant was then diluted with 200 µL of 0.25% NH₄OH in water. The final extract was analyzed via HPLC with MS/MS detection using positive ion electrospray. The assay was validated over the omeprazole and 5-OH-omeprazole concentration range of 1.00 to 1000 ng/mL in human sodium heparin plasma. Precision (%CV) ranged from 3.81% to 7.79%. Accuracy (%diff) ranged from -6.10% to -3.76%.

The assay for the omeprazole metabolite, 5-OH-omeprazole, was validated over a concentration range of 1.00 to 1000 ng/mL in human sodium heparin plasma. Inter-assay precision and accuracy was evaluated in the same manner as for omeprazole. Precision (%CV) ranged from 5.87% to 11.6% for 5-OH-omeprazole. Accuracy (%diff) ranged from -5.36% to 0.296% for 5-OH-omeprazole.

Rosuvastatin

Plasma rosuvastatin concentrations were determined by PPD, Inc, Richmond, Virginia. Human plasma samples, collected from whole blood in tripotassium EDTA, were analysed using a validated method (PPD Method LCMSC 393.3 Version 1.00). All plasma samples were received frozen on dry ice and in acceptable condition and were stored at approximately -70°C.

Rosuvastatin and its internal standard, rosuvastatin-d₆, were extracted from 250 µL human plasma through liquid-liquid extraction using 13:37:50 hexane/methylene dichloride/methyl t-butyl ether, v/v/v. The organic layer was evaporated under a nitrogen stream at approximately 35°C, and the remaining residue was reconstituted with 250 µL of 20:80:0.0625 acetonitrile/water/acetic acid, v/v/v. The final extract was analysed via HPLC with MS/MS detection using negative ion electrospray. The assay was validated over the rosuvastatin concentration range of 0.100 to 100 ng/mL in human tripotassium EDTA plasma. Precision (%CV) ranged from 2.59% to 3.72%. Accuracy (%diff) ranged from -3.79% to -2.98%.

Quality control results for rosuvastatin met the acceptance criteria. Since rosuvastatin lactone is known to be present in study samples, an additional QC pool (QC 7) containing 100 ng/mL rosuvastatin lactone was also prepared and analysed with each batch of samples to monitor inter-conversion between the lactone and acid form of rosuvastatin during sample analysis. Two out of three of the lactone QC pools (QC 7) needed to show less than the sum of the baseline value and 5% of the lactone concentration [5.51 ng/mL (baseline) + 5.00 ng/mL (5% of 100 ng/mL) =10.5 ng/mL] for a run to be acceptable. QC results from this study met these acceptance criteria.

Vercirnon and metabolites

Plasma concentrations of vercirnon and its metabolites, GSK2635622 and GSK2656694, were determined using a validated LC-MS/MS method (PPD Method LCMSC 532.4 Version 1.00). The assay was validated over the vercirnon concentration range of 4.00 to 2000 ng/mL in human K₂EDTA plasma. Precision (%CV) ranged from 1.40% to 7.64%. Accuracy (%diff) ranged from -3.81% to 7.79%.

The assay for the vercirnon metabolites, GSK2635622 and GSK2656694, was validated over a concentration range of 1.00 to 500 ng/mL in human K₂EDTA plasma. Inter-assay precision and accuracy was evaluated in the same manner as for vercirnon. Precision (%CV) ranged from 3.09% to 8.85% and from 2.96% to 12.8% for GSK2635622 and GSK2656694, respectively. Accuracy (%diff) ranged from -5.95% to 4.79% and -1.75% to 0.344% for GSK2635622 and GSK2656694, respectively.