Clinical Pharmacokinetics

Pharmacokinetics, Pharmacodynamics and Tolerability of Opicapone, a Novel Catechol-O-Methyltransferase Inhibitor, in Healthy Subjects

Prediction of Slow Enzyme–Inhibitor Complex Dissociation of a Short-Living and Very Long-Acting Inhibitor

Luis Almeida, José Francisco Rocha, Amilcar Falcão, P. Nuno Palma, Ana I. Loureiro, Roberto Pinto, Maria João Bonifácio, Lyndon C. Wright, Teresa Nunes, Patrício Soares-da-Silva

E-mail: psoares.silva@bial.com

Electronic Supplementary Material
Method of assay of opicapone and its metabolites in plasma and urine

Plasma and urine concentrations of opicapone and metabolites were determined using previously validated methods. The following analytes were assayed in plasma and urine: opicapone (parent) and metabolites BIA 9-1100 (methylated, inactive) and BIA 9-1101 (methylated, inactive), BIA 9-1079 (formed by reduction, and which was found to be active in non-clinical studies), BIA 9-1103 (sulphated, inactive) and BIA 9-1106 (glucuronide, inactive). The metabolites to be assayed were chosen on the basis of the results found in non-clinical studies with opicapone.

Assay of plasma concentration of opicapone and its metabolites

Plasma concentrations of opicapone and its metabolites BIA 9-1079, BIA 9-1100, BIA 9-1101, BIA 9-1103 and BIA 9-1106 were determined by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Lower and upper limits of quantification were 10 and 2500 ng/mL, respectively. For the preparation of calibration (CAL) samples, opicapone, BIA 9-1079, BIA 9-1100, BIA 9-1101, BIA 9-1103 and BIA 9-1106 were dissolved in 50/50 (v/v) mixture of acetonitrile and DMSO to a concentration of 1.50 mg/mL each. Equal volumes of the solutions were mixed to a final concentration of 0.250 mg/mL. For the preparation of quality control (QC) samples, a second set of stock solutions were prepared at a concentration of 1.50 mg/mL each. Equal volumes of the solutions were mixed to a final concentration of 0.250 mg/mL. Further dilutions were done in 50/50 (v/v) mixture of acetonitrile and DMSO. For CAL and QC samples, eight and three different concentrations of all analytes spiked into human plasma were used, respectively. The concentrations were calculated under consideration of purity and salt factor respectively. For preparation of the CAL and QC samples, working solutions in a 50/50 (v/v) mixture of acetonitrile and DMSO were added to human plasma using a ratio of 2/98 (v/v). The stock solutions, CALs and QCs were stored at -25°C±5°C. For the preparation of the ISTD solution, nimodipine was dissolved in MeOH to a concentration of 1.00 mg/mL and was diluted in acetonitrile to 2000 ng/mL. The concentration was
calculated under consideration of purity. Plasma samples were vortexed and centrifuged for 20 minutes at approximately 4000 rpm (3362 g) and approximately 8 ºC after unassisted thawing at room temperature. To an aliquot of 100-μL plasma, 200 μL of acetonitrile containing a concentration of 2000 ng/mL of the ISTD were added. After protein precipitation at room temperature, plasma samples were centrifuged for 20 minutes at approximately 4000 rpm (3360 g) and approximately 8 ºC. An aliquot of 120 μL of the supernatant was diluted with 200 μL water. An aliquot of 5 μL for opicapone and BIA 9-1079 (active method) was injected onto the HPLC column. An aliquot of 10 μL for BIA 9-1100, BIA 9-1101, BIA 9-1103 and BIA 9-1106 (metabolites method) was injected onto the HPLC column. The samples were stored in an autosampler tray at approximately 8ºC±5ºC. The analysis was done by separation with an injection volume of 5 μL (10 μL in a 5 μL sample pool) in reversed phase chromatography (column temperature of 50 ºC) followed by detection with triple-stage quadrupole with a negative ion mode in the selected reaction monitoring mode.

The concentration of the analyte was calculated by the internal standardization method. A quadratic regression model (for BIA 9-1100, BIA 9-1101 and BIA 91103) and a linear regression model (for opicapone, BIA 9-1079 and BIA 91106) were used. The area ratio of analytes to internal standards against the concentration of calibration samples was used for quantification. A weighting factor, l/x^2 for opicapone, BIA 9-1079 and BIA 9-1103 and l/x for BIA 91100, BIA 9-1101 and BIA 9-1106 was used for quantification. Calculation of results was done using Xcalibur 1.4 and 2.0 and LCquan 2.0 and 2.5 Software.

For opicapone and its active metabolite BIA 9-1079, the imprecision, as determined from the analysis of QC samples, was respectively ≤7.4% and ≤8.2%, and the accuracy range was respectively 98.5%-100.5% and 100.0%-105.0%. For BIA 9-1100, BIA 9-1101, BIA 9-1103 and BIA 9-1106, the imprecision was respectively ≤7.2%, ≤7.0%, ≤6.3% and ≤8.2%, respectively, and the accuracy range was respectively 101.5%-106.5, 100.5%-102.0%, 101.5%-105.0% and 102.0%-104.5%. Opicapone, BIA 9-1079, BIA 9-1100, BIA 9-1101, BIA 9-1103 and BIA 9-1106 were supplied by BIAL (Laboratory of Chemistry, S. Mamede do Coronado, Portugal). The reference standard
(nimodipine) was supplied by Sigma-Aldrich (St. Louis, Missouri, USA).

Assay of urinary concentration of opicapone and its metabolites

Urinary concentrations of opicapone and its active metabolite BIA 9-1079 were determined by LC-MS/MS, with lower and upper limits of quantification of 50 and 12500 ng/mL. For the preparation of CAL samples, BIA 9-1100 and BIA 9-1101 were dissolved in a 50/50 (v/v) mixture of acetonitrile and DMSO to a concentration of 1.50 mg/mL each. Equal volumes of the solutions were mixed to a final concentration of 0.750 mg/mL. For the preparation of QC samples, a second set of stock solutions were prepared at a concentration of 1.50 mg/mL each. Equal volumes of the solutions were mixed to a final concentration of 0.750 mg/mL. Further dilutions were done in a 50/50 (v/v) mixture of acetonitrile and DMSO. For CAL and QC samples, eight and three different concentrations of all analytes spiked into human urine were used, respectively. The concentrations were calculated under consideration of purity. For preparation of the CAL and QC samples, working solutions in a 50/50 (v/v) mixture of acetonitrile and DMSO were added to human urine using a ratio of 2/98 (v/v). The stock solutions, CALs and QCs were stored at -25°C±5°C. For the preparation of the ISTD solution, nimodipine was dissolved in MeOH to a concentration of 1.00 mg/mL and was diluted in acetonitrile to a final concentration of 2000 ng/mL. The concentration was calculated under consideration of purity.

Urine samples were vortexed and centrifuged for 20 minutes at approximately 4000 rpm (3362 g) and approximately 8°C after unassisted thawing at room temperature. To an aliquot of 20 μL urine, 80 μL human blank K-EDTA plasma and 200 μL of acetonitrile containing a concentration of 2000 ng/mL of the ISTD were added. After protein precipitation at room temperature and short mixing, urine samples were centrifuged for 20 minutes at approximately 4000 rpm (2770 g) and approximately 8°C. An aliquot of 120 μL of the supernatant was diluted with 200 μL water. An aliquot of 2 μL was injected onto the HPLC column. The samples were stored in an autosampler tray at approximately 8°C±5°C. The analysis was done by separation with an injection volume of 2 μL (10 μL in a 2 μL sample pool) in reversed phase
chromatography (column temperature of 50 °C) followed by detection with triple-stage quadrupole with a negative ion mode in the selected reaction monitoring mode. The concentration of the analyte was calculated by the internal standardization method. A quadratic regression model was used. The area ratio of analytes to internal standards against the concentration of calibration samples was used for quantification. A weighting factor, $1/x^2$ was used for quantification. For opicapone, the imprecision was ≤8.5% and the accuracy ranged from 97.2% to 101.5%. For BIA 9-1079 the imprecision was ≤9.3% and the accuracy ranged from 97.4% to 106.4%.

Urinary concentrations of metabolites BIA 9-1100 and BIA 9-1101 were determined by LC-MS/MS with lower and upper limits of quantification of 50.0 and 12500 ng/mL.

For the preparation of calibration samples, BIA 9-1100 and BIA 9-1101 were dissolved in a 50/50 (v/v) mixture of acetonitrile and DMSO to a concentration of 1.50 mg/mL each. Equal volumes of the solutions were mixed to a final concentration of 0.750 mg/mL. For the preparation of quality control samples, a second set of stock solutions were prepared at a concentration of 1.50 mg/mL each. Equal volumes of the solutions were mixed to a final concentration of 0.750 mg/mL. Further dilutions were done in a 50/50 (v/v) mixture of acetonitrile and DMSO. For CAL and QC samples, eight and three different concentrations of all analytes spiked into human urine were used, respectively. The concentrations were calculated under consideration of purity. For preparation of the CAL and QC samples, working solutions in a 50/50 (v/v) mixture of acetonitrile and DMSO were added to human urine using a ratio of 2/98 (v/v). The stock solutions, CALs and QCs were stored at -25°C±5°C. For the preparation of the ISTD solution, nimodipine was dissolved in MeOH to a concentration of 1.00 mg/mL and was diluted in acetonitrile to a final concentration of 2000 ng/mL. The concentration was calculated under consideration of purity. Samples were vortexed and centrifuged for 20 minutes at approximately 4000 rpm (3362 g) and approximately 8°C after unassisted thawing at room temperature. To an aliquot of 20 μL human urine, 80 μL human blank K-EDTA plasma and 200 μL of acetonitrile containing a concentration of 2000 ng/mL of the ISTD were added. After protein precipitation at room temperature and short mixing, urine samples were centrifuged for 20 minutes at
approximately 4000 rpm (2770 g) and approximately 8°C. An aliquot of 120 μL of the supernatant was diluted with 200 μL water. An aliquot of 10 μL was injected onto the HPLC column. The samples were stored in an autosampler tray at approximately 8°C±5°C. The analysis was done by separation with an injection volume of 10 μl (10 μl in a 20 μl sample pool) in reversed phase chromatography (column temperature of 50 °C) followed by detection with triple-stage quadrupole with a negative ion mode in the selected reaction monitoring mode. The concentration of the analyte was calculated by the internal standardization method. A quadratic regression model was used. The area ratio of analytes to internal standards against the concentration of calibration samples was used for quantification. A weighting factor, 1/x for BIA 9-1100 and 1/x^2 for BIA 9-1101 was used for quantification. For BIA 9-1100, the imprecision was ≤8.6% and the accuracy ranged from 93.2% to 98.3%. For BIA 9-1101, the imprecision was ≤8.6% and the accuracy ranged from 93.3% to 98.0%.

Urinary concentrations of metabolites BIA 9-1103 and BIA 9-1106 were determined by LC-MS/MS, with lower and upper limits of quantification of 50.0 and 12500 ng/mL. For the preparation of CAL samples, BIA 9-1103 and BIA 9-1106 were dissolved in a 50/50 (v/v) mixture of acetonitrile and DMSO to a concentration of 1.50 mg/mL each. Equal volumes of the solutions were mixed to a final concentration of 0.750 mg/mL. For the preparation of QC samples, a second set of stock solutions were prepared at a concentration of 1.50 mg/mL each. Equal volumes of the solutions were mixed to a final concentration of 0.750 mg/mL. Further dilutions were done in a 50/50 (v/v) mixture of acetonitrile and DMSO. For CAL and QC samples, eight and three different concentrations of all analytes spiked into human urine were used, respectively. The concentrations were calculated under consideration of purity and salt factor respectively. For preparation of the CAL and QC samples, working solutions in a 50/50 (v/v) mixture of acetonitrile and DMSO were added to human urine using a ratio of 2/98 (v/v). The stock solutions, CALs and QCs were stored at -25°C±5°C. For the preparation of the ISTD solution, Nimodipine was dissolved in MeOH to a concentration of 1.00 mg/mL and was diluted in ACN to 2000 ng/mL. The concentration was calculated under consideration of purity. Human urine samples were vortexed and centrifuged for 20 minutes at approximately 4000 rpm (3362 g) and approximately 8°C after unassisted thawing at room
temperature. To an aliquot of 20 μL human urine, 80 μl human blank K-EDTA plasma and 200 μl of acetonitrile containing a concentration of 2000 ng/ml of the ISTD were added. After protein precipitation at room temperature and short mixing, urine samples were centrifuged for 20 minutes at approximately 4000 rpm (2770 g) and approximately 8°C. An aliquot of 120 μL of the supernatant was diluted with 200 μL water. An aliquot of 10 μL was injected onto the HPLC column. The samples were stored in an autosampler tray at approximately 8°C±5°C. The analysis was done by separation with an injection volume of 10 μL (10 μL in a 20 μL sample pool) in reversed phase chromatography (column temperature of 50 °C) followed by detection with triple-stage quadrupole with a negative ion mode in the selected reaction monitoring mode. The concentration of the analyte was calculated by the internal standardization method. A linear regression model was used. The area ratio of analytes to internal standards against the concentration of calibration samples was used for quantification. A weighting factor, 1/x was used for quantification. Calculation of results was done using Xcalibur 1.4 and 2.0 and LCquan 2.0 and 2.5 Software. For BIA 9-1103 and BIA 9-1106, the imprecision was ≤8.5% and ≤7.8% and the accuracy ranged from 98.4% to 102.7% and from 99.3% to 100.0%, respectively.