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C458 quantification using LC-MS/MS

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Sergey Trushin[1], Thi Kim Oanh Nguyen[1], Andrea Stojakovic[1], Mark Ostroot[1], Jonathan T. Deason[1], Su-Youne Chang[1], Liang Zhang[1], Slobodan I. Macura[1], Toshihiko Nambara[1], Wenyan Lu[1], Takahisa Kanekiyo[1], Eugenia Trushina[1]

[1]Mayo Clinic

Mark Ostroot
Mayo Clinic

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Abstract

This protocol is for LC-MS/MS quantification of C458. This protocol has been used to detect tissue concentrations of C458.

1 Paclitaxel (Sigma, St. Louis, MO) was used as an internal standard (IS). Ultra-pure water was generated using a Barnstead nanopure diamond system (Thermo, Marietta, OH). Optima LC/MS-grade methanol (MeOH, Fisher Scientific, Waltham, MA), analytical grade formic acid (Fisher Scientific, Waltham, MA), 96 well protein crash plates and 1 ml 96 well polypropylene collection plates (Chromtech, Apple Valley, MN), Kunststoff-Kapillaren end-to-end K2EDTA coated plastic 30 µl capillary tubes (Fisher Scientific, Waltham, MA) and K2EDTA pre-sprayed microtainer collection tubes (500 µl, Becton, Dickinson and Company, Franklin Lakes, NJ) were utilized in this study. For controls, drug free mouse plasma was obtained from healthy CD-1 mice containing 0.1% K2EDTA that was purchased from Valley Biomedical and stored at -20 °C for later use. The LC-MS/MS consisted of a Waters Acquity H class ultra-performance liquid chromatography (UPLC) system containing a quaternary solvent manager and sample manager-FTN coupled to a Xevo TQ-S mass spectrometer equipped with an electrospray ionization (ESI) source. Data were acquired and analyzed using Waters MassLynx v4.1 software. Detection of C458 and paclitaxel (internal standard, IS) was accomplished by multiple reaction monitoring (MRM) using the mass spectrometer in positive ESI mode with capillary voltage, 2.5 kV; source temperature 150 °C; desolvation temperature 400 °C; cone gas flow 150 L/h; desolvation gas flow 800 L/h. The cone voltages and collision energies for C458 and paclitaxel were determined by MassLynx-Intellistart v4.1 software and were 12 and 18 V (cone) and 30 and 66 eV (collision), respectively. MRM precursor and product ions were monitored at m/z 339.26> 121.11 for C458 and 854.29 > 105.08 for paclitaxel. Data was collected from 2 - 5.5 for both C458 and paclitaxel. Separation of C458 and paclitaxel was achieved using an Agilent Poroshell 120 EC-C18 (2.7 µm, 2.1 × 100 mm) with an Agilent EC-C18 pre-column (2.7 µm, 2.1 × 5 mm) and a gradient elution program containing ultra-pure water and MeOH, both with 0.1% formic acid. The gradient begins with 70% aqueous, decreases to 10% aqueous over 3 min and holds there for 2 min, then returns to baseline over 0.1 min and holds to equilibrate for 2.9 min. The flow rate was 0.4 ml/min, total run time was 8 min, injection volume was 5 µl, and the column and autosampler temperatures were 40 ˚C and 20 ˚C, respectively. Stock solutions of C458 (5 mg/mL, dissolved in DMSO) and paclitaxel (200 ng/mL, dissolved in acetonitrile, ACN) were prepared in salinized glass vials and stored at -20 °C. 20X working stock solutions were prepared daily and diluted in 1:1 MeOH:H2O and stored at -20 °C. Plasma standards containing C458 (0.2 - 200 ng/mL) were prepared by adding (5 µl) aliquots of 20 X C458 to plasma (95 µl) in 1.5 mL slick microfuge tubes, 50 µl of this plasma dilution was transferred to a 96 well protein crash plate. Analytes were isolated using protein precipitation with 150 µl ACN containing IS (200 ng/ml). The plate was capped and shaken for 20 min at 1100 rpm. The sample was then vacuum filtered into a Chromtech (Apple Valley, MN) 1 mL 96 well polypropylene collection plate, evaporated to dryness under a gentle stream of nitrogen and reconstituted with 200 µl H2O/ACN (1:1). The collection plate was capped and shaken at 900 rpm for 20 min, and 5 µl aliquots were injected into the LC/MS/MS. Mass Spectra and ion chromatograms of C458 and IS were

processed using MassLynx v4.1 software with TargetLynx. Standard curves for C458 and paclitaxel were analyzed using the peak area ratio of C458 versus IS. C458 standard curve concentrations were 0.1, 0.2 0.5, 1, 5, 10, 20, 50,100 and 200 ng/ml. Concentration of C458 in the brain was detected using the same approach.