Simultaneous Quantitation of BACE1 Inhibitor AZD3293 and Metabolite AZ13569724 in Several Human Matrices by LC-MS/MS

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Purpose
AZD3293 is a brain-permeable inhibitor of human beta-site amyloid precursor protein-cleaving enzyme1 (BACE1) in Phase 3 clinical development for Alzheimer’s disease (AD). Sensitive and robust bioanalytical methods were required to quantitate AZD3293 and its metabolite AZ13569724 in human biological matrices (plasma, plasma ultrafiltrate, cerebrospinal fluid [CSF], and urine), in part, to support clinical dose and dose regimen selection.

Methods
Sensitive LC-MS/MS assays using Shimadzu LC-20AD coupled with API 5000 triple quadrupole mass spectrometer were developed and validated in human plasma, plasma ultrafiltrate, CSF, and urine for AZD3293 and AZ13569724. Stable-isotope labeled AZD3293 and AZ13569724 were used as internal standards. Plasma samples were extracted by protein precipitation. Plasma ultrafiltrate was prepared using a Centrifree® centrifugal ultrafiltration device with a 30 KDa molecular weight cut-off. CHAPS (0.2M, 5 μL) was added to 250 μL plasma in each vial prior to transferring samples to the ultrafiltration device. Human CSF was collected by aspiration during the lumbar puncture procedure. Due to the potential for non-specific binding of the analytes in non-plasma matrices, CHAPS (0.2 M) was added to the CSF sample aliquots and urine sample aliquots to a final concentration of 1% (v/v) in the clinic prior to freezing. Non-specific binding of analytes to CSF collection tubing was evaluated by incubating spiked CSF samples in the tubing for 30 minutes. Recovery was calculated to estimate the non-specific binding in clinical usage.

Results
Calibration curves were validated for AZD3293 and AZ13569724 in human plasma (0.500-500 ng/mL), plasma ultrafiltrate (0.400-400 ng/mL), CSF (0.100-100 ng/mL), and urine (10.0-10,000 ng/mL). The dilution range was evaluated up to 10-fold in urine and up to 100-fold in plasma. The accuracy and precision of the methods were assessed in multiple analytical batches using multiple replicates of QCs and the overall performance met preset criteria. The methods demonstrated acceptable selectivity from matrices for the quantitation of AZD3293 and metabolite AZ13569724 in human plasma, plasma ultrafiltrate, CSF, and urine and minimal matrix effects were observed with high recovery of both analytes and internal standards. Furthermore, the presence of hemolyzed red blood cells or elevated lipid (fat) levels in human plasma did not affect the quantitation of AZD3293 and AZ13569724 in the plasma method. The stability of the AZD3293 and metabolite AZ13569724 was investigated in a variety of matrices and under a variety of conditions. Both analytes demonstrated acceptable stability under controlled conditions. Potential for non-specific binding of the analytes was observed in non-plasma matrices. In CSF, there was a 27% decrease in AZD3293 concentrations following incubation in the collection tubing, presumably due to non-specific binding.

Conclusion
The LC-MS/MS methods developed in human biological matrices (i.e., plasma, plasma ultrafiltrate, CSF, and urine) for AZD3293 and AZ13569724 have been successfully validated to current EMA and FDA regulatory agency guidelines. These methods have been used for the accurate and robust determination of AZD3293 and AZ13569724 concentrations in a number of clinical studies and the plasma and CSF methods are currently used to support on-going Phase 3 trials in patients with Alzheimer’s disease.