LC-MS/MS Analysis of Immunosuppressant Drugs in Whole Blood using the Xevo TQ Absolute with Capitainer B qDBS Devices and Volumetric Absorptive Microsampling for Clinical Research

Peter Harrsch, Waters Corporation

Introduction:
Traditional laboratory analysis of the immunosuppressant drugs cyclosporine, everolimus, sirolimus and tacrolimus is well-established in clinical research. However there remains a need for individuals to undergo an invasive, time-consuming and disruptive process under the supervision of trained staff in order to collect a sufficient volume of whole blood for laboratory analysis.

A reliable, remote sampling method may find utility in a clinical research setting. Here we describe the use of Capitainer™ B qDBS Devices to obtain analytically sensitive, precise and accurate data for cyclosporine, everolimus, sirolimus and tacrolimus analysis using small sample volumes.

The Waters ACQUITY™ UPLC™ I-Class System with Xevo™ TQ Absolute Mass Spectrometer was used to analyze these samples.

Methods:
An in-house laboratory developed LC-MS/MS method to analyze all four immunosuppressants in a single run was developed. Waters MassTrak™ Immunosuppressant Calibrator and Control Sets (IVD) and whole blood External Quality Assurance samples (LGC, Bury, UK) were used in conjunction with Capitainer B qDBS Devices to assess the performance of the method.

Samples (10µL) were collected using Capitainer® B qDBS devices, and the sample extracted using solvent containing internal standards. A water/methanol/ammonium acetate gradient was used with a Waters C18 HSS SB column on a Waters ACQUITY™ UPLC™ I-Class and Xevo TQ Absolute Mass Spectrometer operating in positive electrospray ionization mode with run time of less than 2 minutes.

Results:
Analytical sensitivity of the lowest calibrator at 1 ng/mL for everolimus, sirolimus and tacrolimus and 25 ng/mL for cyclosporine was demonstrated with S/N (PtP) > 10 across five analytical runs. We successfully demonstrated linearity of cyclosporine from 25-1500 ng/mL

and everolimus, sirolimus and tacrolimus from 1-30 ng/mL, with r2>0.995 over five analytical runs. Total precision and repeatability across the four immunosuppressants (2, 8 and 22 ng/mL for everolimus, tacrolimus and tacrolimus; 150, 400 and 900 ng/mL for cyclosporine) with five replicates over five analytical runs (n = 25) was ≤15% CV. External quality assurance samples for all drugs met the scheme acceptance criteria, with mean bias ≤15% CV.

Conclusions:
Using Capitainer B qDBS devices and sample small volumes (10 µL) of whole blood, an in-house laboratory method was used to meet validation goals for analytical sensitivity, linearity, precision and accuracy for cyclosporine, everolimus, sirolimus and tacrolimus. Furthermore, the advantages conferred by volumetric absorptive microsampling, notably removing the requirement for travel and a venous blood draw and facilitating home sampling, render this technique applicable to clinical research.

For Research Use Only. Not for Use in Diagnostic Procedures.

Waters™