**Introduction**

LC-MS/MS is widely used to monitor immunosuppressive drug panels on a routine basis, for example for therapeutic drug monitoring of organ transplant patients. Multiplexing is a popular approach to speed the analysis time between individual samples. However, such techniques utilize a single electrospray probe and fast serial injections, typically requiring cumbersome wash cycles to ensure minimum carryover. This poster reports a novel approach to multiplexing the immunosuppressant analyses using a single ion source equipped with two electrospray probes. Previous reports demonstrated linearity and use of two ESI probes in a single source for analysis of Vitamin D. Here we demonstrate fast quantification of Sirolimus, Tacrolimus, Everolimus and Cyclosporin A using Ascomycin and Cyclosporin D as internal standards.

**Sample Preparation:**

The Tacrolimus, Sirolimus, Everolimus and Cyclosporin A stock standard solutions are liquid from Cerilliant Inc (Round Rock, Texas) and stored at -4°C. Whole blood spiked samples were cleaned up by mixing one volume of serum with two volumes 0.1 M ZnSO4 solution containing the internal standards: Cyclosporin D (1234/1217) and Ascomycin (809.6/756.6). Three level QCs were purchased from UTAK (Valencia, CA). The mixture was vortexed for one minute followed by centrifugation for 15 min. The supernatant was transferred to a clean vial for analysis.

**LC-MS/MS Conditions:**

The LC-MS/MS was performed using an IONICS 3Q 120 triple quadrupole mass spectrometer (Bolton, ON Canada) with a Shimadzu UFLC system. 20 µL of supernatant were loaded on a porous R1/20 pretreatment column (30x2.1mm) for on-line washing with water for 0.25 minutes at a liquid flow rate of 0.6 mL/min using Solvent A (water:methanol = 98:2, v/v, with 0.1% formic acid and 10mM ammonium acetate) and Solvent B (water:methanol = 2:98, v/v, with 0.1% formic acid and 10mM ammonium acetate). The LC-MS/MS was performed using an IONICS 3Q 120 triple quadrupole mass spectrometer (Bolton, ON Canada) with a Shimadzu UFLC system. 20 µL of supernatant were loaded on a porous R1/20 pretreatment column (30x2.1mm) for on-line washing with water for 0.25 minutes at a liquid flow rate of 0.6 mL/min using Solvent A (water:methanol = 98:2, v/v, with 0.1% formic acid and 10mM ammonium acetate) and Solvent B (water:methanol = 2:98, v/v, with 0.1% formic acid and 10mM ammonium acetate).

**Mass Spectrometry Conditions:**


**Results:**

This method covers a concentration range of three orders of magnitude from 0.2 to 200 ng/mL for Tacrolimus, Sirolimus, Everolimus and 2 to 2000 ng/mL for Cyclosporin A, while maintaining good linearity (R2 = 0.999) with 1/x weighting. The intraday and interday variability for three levels QCs were all <7% and <11%, respectively. No interference or cross contamination was observed.

**Conclusion**

A sensitive, reliable and accurate LC-MS/MS method was developed and validated for simultaneous quantification of Tacrolimus, Sirolimus, Everolimus and Cyclosporin A in whole blood. The use of an ESI/ESI novel dual source allows a sample analysis time of only 1.5 minutes, which doubles the throughput. This LC-MS/MS method requires simple sample preparation and is well-suited for routine therapeutic drug monitoring of immunosuppressive drugs.

**References:**


**Figure 1:** LC Cycle Time

**Figure 2:** The LC-MS/MS Setup Used During the Development of this Method.

**Figure 3:** Injection Sequence

**Figure 4:** EIC Chromatograms of Compounds for QC Level I in Whole Blood

**Figure 5A:** Calibration Curve for Sirolimus (0.2-200ng/mL)

**Figure 5B:** Calibration Curve for Tacrolimus (0.2-200ng/mL)

**Figure 5C:** Calibration Curve for Everolimus (0.2-200ng/mL)

**Figure 5D:** Calibration Curve for Cyclosporin (2-2000ng/mL)