**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. [1] Multiplexing is a popular approach to speeding 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. [2] 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 [3]. Here we demonstrate fast quantification of Sirolimus, Tacrolimus, Everolimus and Cyclosporin A using Ascomycin and Cyclosporin D as internal standards.

**Method**

Sample and Preparation: The Tacrolimus, Sirolimus, Everolimus and Cyclosporin A standard stock solutions were prepared in a liquid form from Cerilliant Inc (Round Rock, Texas) and stored at -8°C. Whole blood spiked samples were cleaned up by mixing one volume of serum with two volumes of 0.1 M ZnSO4 one minute followed by centrifugation for 15 min. The supernatant was transferred to a clean vial for quantitation.

LC-MS/MS Conditions: The LC-MS/MS was performed using an LCQ 120 triple quadrupole mass spectrometer (Bolton, ON, Canada) with a Shemyad UPLC system. All solvents used in this method are HPLC grade. Whole blood spiked samples were cleaned up by mixing one volume of serum with two volumes of 0.1 M ZnSO4 precipitation solution containing the internal standards: Cyclosporin D (1234/1217) and Ascomycin (809/676/6). 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 clear vial for quantification.

**Results**

A sensitive, reliable and accurate LC-MS/MS method was developed and validated for 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, 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**

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.

**Reference**


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**High Throughput Simultaneous Analysis of Immunosuppressants by ESI/ESI Dual Source Coaxial Flow Ion Source LC-MS/MS System**

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