**INTRODUCTION**

Therapeutic drug research is important for a variety of clinical research. Common tools for drug research include immunoassays and HPLC, both of which have drawbacks. Immunoassays may suffer from cross-reactivity, while high-throughput HPLC requires sample cleanup. By combining PaperSpray and FAIMS technology, background signal can be reduced, and signal-to-noise ratio (S/N) can be improved. FAIMS field ionization mass spectrometry (FAIMS-MS) is an emerging technique that is being explored for the analysis of whole blood and serum samples. The FAIMS device improved sensitivity of the PaperSpray method for Everolimus, Sirolimus and Tacrolimus, when analyzed by LC/MS. However, because of the lack of sample cleanup, PaperSpray samples need to be diluted in high throughputs and dried before analysis.

**MATERIALS AND METHODS**

**Sample Preparation**

Blood was collected in sodium heparin tubes. Blood samples were centrifuged at 1600 × g for 10 min to separate red blood cells and obtain serum. Serum samples were stored at −20°C until analysis.

**Method**

**PaperSpray**

The chronograms of the matrix blanks normalized to the maximal signal of the internal standard are shown in Figure 3. For everolimus and Sirolimus, the limits of detection (LODs) were less than 20 ng/mL and the limits of quantitation (LOQs) were above 30 ng/mL. For cyclosporin A, the LOD was less than 20 ng/mL and the LOQ was above 30 ng/mL. The sensitivity of the PaperSpray-MS/MS method was increased in comparison with the conventional method, with LODs and LOQs ranging from 0.8 to 21.0 ng/mL for Everolimus, Sirolimus and Tacrolimus, respectively.

**RESULTS**

**Background signal reduction with the FAIMS Pro interface**

Reduced background signal was observed throughout all standard levels for cyclones (80 ng/mL for Everolimus and Sirolimus, 50 ng/mL for Tacrolimus, and 2.5 ng/mL for Cyclosporin A). For Level B, there was a ~3x improvement for Everolimus, ~2x improvement for Sirolimus, and ~13x improvement for Tacrolimus when using the FAIMS Pro interface. The % CV of the response factor for Tacrolimus ranged from 0.987 to 1.2% for Levels A, B, and C (Table 2). The % CV of the response factor for cyclosporin A ranged from 1.2% to 14.2% for Levels A, B, and C (Table 2). The % CV of the response factor for cyclosporin A ranged from 1.2% to 14.2% for Levels A, B, and C (Table 2).

**Comparison of accuracy precisions and signal-to-noise ratio (S/N) with the FAIMS Pro interface**

The FAIMS Pro interface improved background signal relative to the internal standard by ~67% for Everolimus, ~74% for Sirolimus, and ~100% for Cyclosporin A, allowing for improved sensitivity of the PaperSpray method. Improved sensitivity and selectivity with the FAIMS Pro interface was used to fit the calibration curves and is shown in red on each plot. Average calculated concentrations, less than 20% coefficient of variance and being greater than or equal to 3x the LOD, were observed for all immunosuppressants. Reference samples with an installed Fisher Scientific™ TSQ Altis interface had a 0.992 signal-to-noise ratio (S/N), and compromise the LOQ by 0.992 without FAIMS and 0.987 with FAIMS.

**CONCLUSIONS**

The PaperSpray method was improved using the FAIMS Pro interface. Compared to the conventional method, the PaperSpray-MS/MS method improved in calibration curves with increased sensitivity for Everolimus (improved by 0.8 to 21.0 ng/mL), Sirolimus (improved by 0.8 to 21.0 ng/mL) and Tacrolimus (improved by 0.8 to 21.0 ng/mL).

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