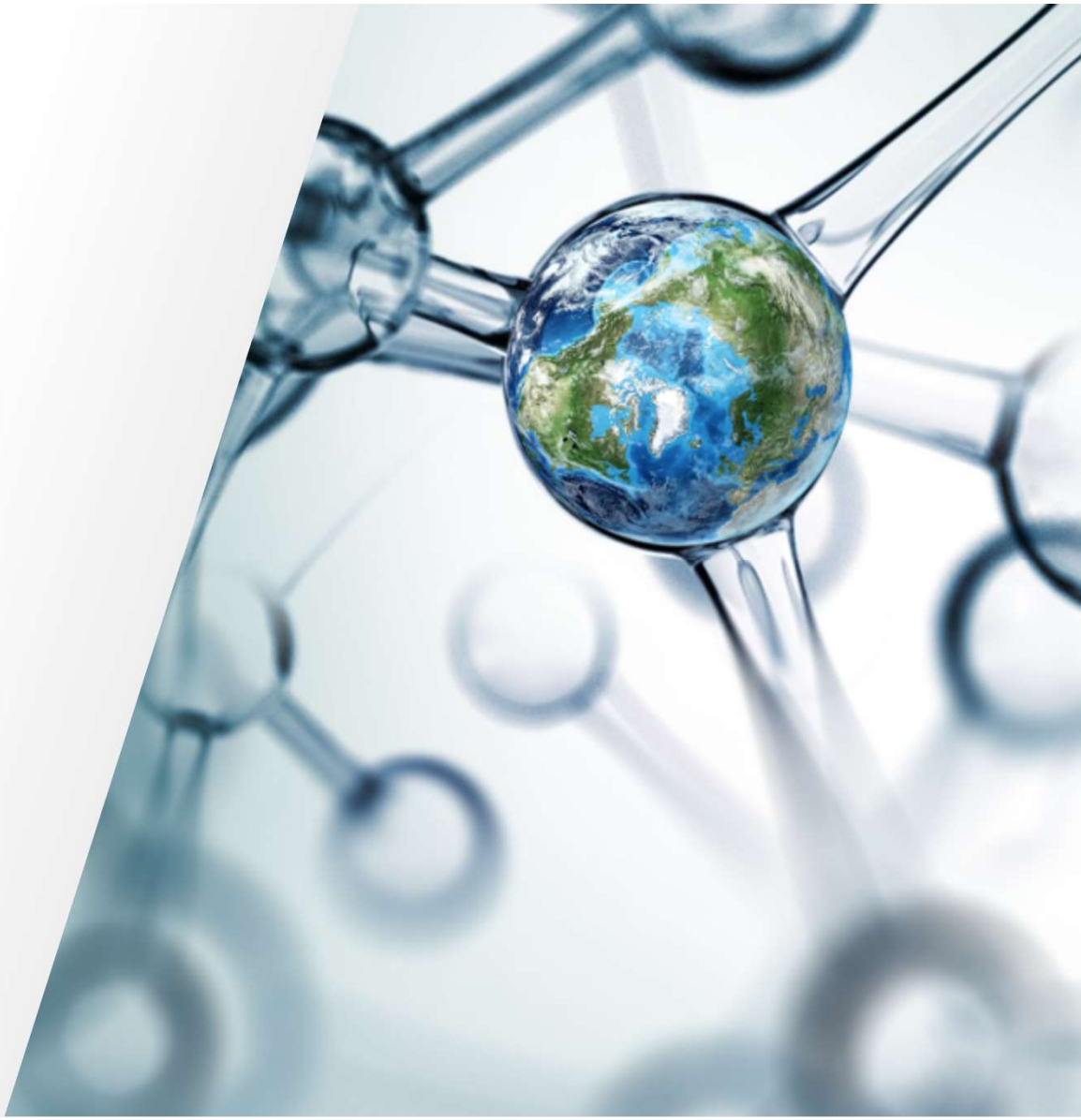
High-Flow LC/MS Analysis of Immunosuppressant Drugs in Human Blood Using FAIMS Technology

Kateryna Riedesel[1]; Cornelia L Boeser[1]; Mary Blackburn[1]

[1]Thermo Fisher Scientific, San Jose, CA



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Abstract

Introduction

Monitoring of immunosuppressant drugs (ISDs) in blood has high importance in ongoing clinical research to evaluate levels after organ transplantation surgeries. Complexity of blood matrix presents various issues such as ion signal suppression by endogenous compounds, fast contamination of ionization source and reduced throughput of analysis. In this study four commonly used ISDs: Sirolimus, Tacrolimus, Everolimus and Cyclosporin A were analyzed in blood by high-flow LC/MS by incorporating FAIMS technology. We were able to demonstrate improved signal-to-noise ratio for analysis of ISDs in blood when compared to setup without FAIMS technology, as well as longer instrument uptime and higher throughput.

Methods

The robustness study was performed using Vanquish LC coupled to Thermo Scientific™ Orbitrap™ Exploris™ 240 at a high flow rate of 0.8 mL/min. Compensation voltages (CVs) were optimized on-line by injection for each ISD. Ionization was performed using a HESI ion source with auxiliary gas, sheath gas and vaporizer temperature settings optimized for the high-flow analysis. Mass spectrometry analysis was performed by the orbitrap analyzer in full scan mode. Continuous injections of Sirolimus, Tacrolimus, Everolimus and Cyclosporin A spiked in blood were performed over 5 days.

Preliminary Data

In this study we demonstrate the performance of FAIMS technology coupled with high-flow LC/MS for analysis of ISDs in blood. Incorporation of FAIMS technology resulted in reduced matrix interference and enhanced signal-to-noise ratio for monitoring of ISDs. The analysis throughput was enhanced by improvements of instrument uptime and minimizing the frequency of front-end cleaning. For Research Use Only – Not for Patient or Diagnostic Use.

Novel Aspect

The study demonstrates numerous benefits of using FAIMS technology for analysis of ISDs in complex matrices by high-flow LC/MS.

Introduction

Analysis of immunosuppressant drugs (ISDs) after organ transplantation surgeries is very important because low doses result in therapeutic inefficiency or, in case of overdose, may cause toxicity. LC/MS is considered a common technique for selective, sensitive and accurate quantitation of ISDs in biological fluids. However, routine analysis of clinical samples by this technique is challenging due to fast contamination of mass spectrometer parts by the components of sample matrix. Acquisition queue often needs to be stopped to perform cleaning and necessary maintenance.

Incorporation of FAIMS Pro interface helps improving instrument uptime and throughput by removal of matrix contaminant ions. FAIMS operates by applying asymmetric waveform and generating high and low electric fields between inner and outer electrodes. Only ions with certain ion mobilities can be transmitted between the electrodes while others get neutralized. Applying compensation voltages (CVs) helps to promote ions of interest into the mass spectrometer. Optimization of CVs for each analyte is done empirically and is very quick and easy process. Mass-spectrometer can be operated continuously for at least a week without interruption for maintenance when using FAIMS Pro technology.

FAIMS Pro has been interfaced with various sample inlets such as low-flow LC or PaperSpray. This new technology showed low LOQs, improved signal-to-noise and robustness. In this study we demonstrate capabilities of FAIMS Pro upgraded to high-flow for LC/MS analysis of immunosuppressants in whole human blood. The interface is easy-to-use and improves mass spectrometer uptime and analysis throughput.

Materials and Methods

Sample preparation

- Cyclosporin A, Everolimus, Tacrolimus and Sirolimus were spiked into whole blood following precipitation with Zinc Sulphate. Ascomycin was used as Internal Standard (IS) for all ISDs. The mix was vortexed, let equilibrate and centrifuged. Supernatant was collected for subsequent LC-MS analysis.
- Calibrators that span therapeutic range of ISDs were purchased from Iris Technologies International GmbH and prepared in the similar way as samples.

LC-MS parameters

- LC separation was performed at 0.8 mL/min flow rate using Vanquish UHPLC Horizon. Total run time was 3 min. Injection volume was set to 15 µL.
- H-ESI source at 3000 V was used for ionization. Sheath, aux, sweep gas parameters were optimized, ion transfer tube temperature and vaporizer temperature were optimized.
- Compensation voltages (CVs) were optimized on-line by injection for each ISD when FAIMS Pro upgraded to high-flow was in use.
- Orbitrap Exploris (OE) 240 was used in full-scan mode to collect the date in m/z range 700 – 1400. Resolution was set to 60,000.

Data Analysis

- Data was collected during 5 consecutive days of sample injection for each of the study (without FAIMS and with FAIMS Pro) yielding 1800 injections per study and 360 injections per day.
- OE Calibration and cleaning of transfer tube was done only once at the beginning of each study.
- Data Analysis was performed in Thermo Scientific™ TraceFinder™ 5.1.SP2 Software.
- Quantitation was done using raw peak areas of each ISD and their response ratios to Ascomysin IS.

Results

Analyte Peak Area Change Across Five Days of Robustness Study

Samples were injected continuously during five days for both setups: with FAIMS Pro and without FAIMS Pro interface. Total of 1800 of injections were performed per study and 360 injections per day. Mass spectrometer was calibrated only once at the beginning of each study and the analysis was not interrupted for any type of maintenance, such as replacement of transfer tube or calibration. Optimization of compensation voltages (CVs) was performed for each immunosuppressant when using FAIMS Pro and is shown on Figure 1.

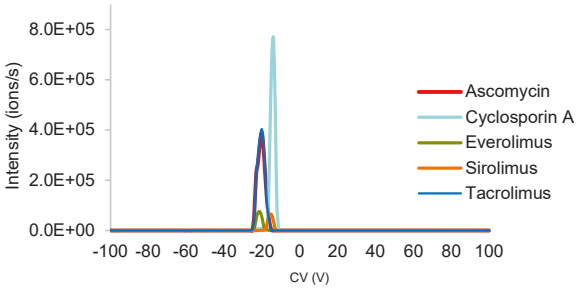

Figure 1. Optimization of CVs for immunosuppressants.

Cyclosporin A peak area decline of more than 20% is observed after three days of injections without FAIMS Pro (Figure 2). However, peak areas remain steady across all five days of continuous analysis when using FAIMS Pro (Figure 3). The signal has lower intensity than without FAIMS, which is expected, and is the result of transmission losses on electrodes. Similar trend is observed for other immunosuppressants Tacrolimus, Sirolimus and Everolimus (Figure 4 and Figure 5). This demonstrates increased up-time of the mass-spectrometer and analysis throughput when FAIMS Pro interface is in use.

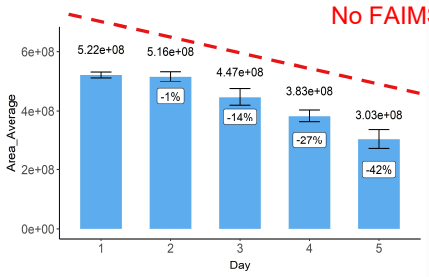

Figure 2. Cyclosporin A peak area change in human whole blood across five days of robustness study without FAIMS Pro.

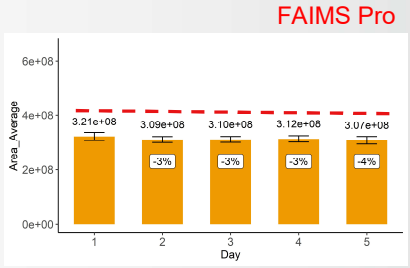

Figure 3. Cyclosporin A peak area change in human whole blood across five days of robustness study with FAIMS Pro.

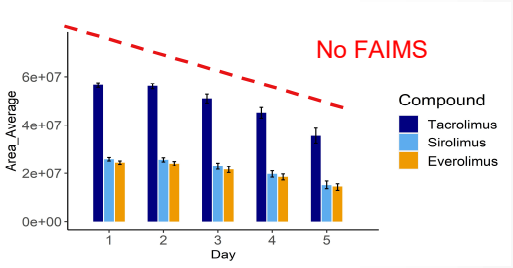

Figure 4. Tacrolimus, Sirolimus and Everolimus peak area change in human whole blood across five days of robustness study without FAIMS Pro.

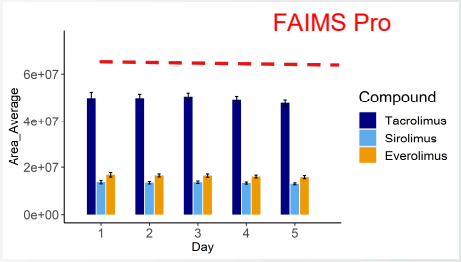

Figure 5. Tacrolimus, Sirolimus and Everolimus peak area change in human whole blood across five days of robustness study with FAIMS Pro.

Results

Comparison of fore-vacuum pressure and precision with and without FAIMS Pro

The signal decline without use of FAIMS Pro correlates with fore-vacuum pressure drop (Figure 6) and is indicative of transfer tube clogging by the whole blood matrix components. Incorporation of FAIMS Pro leads to stable fore-vacuum pressure and consistency of results. Optimization of CVs helps transmitting the ions of interest between the inner and outer electrodes of FAIMS into the mass spectrometer, while other contaminate ions drift away and get neutralized. The system can operate continuously for at least five days without stopping the queue to perform cleaning.

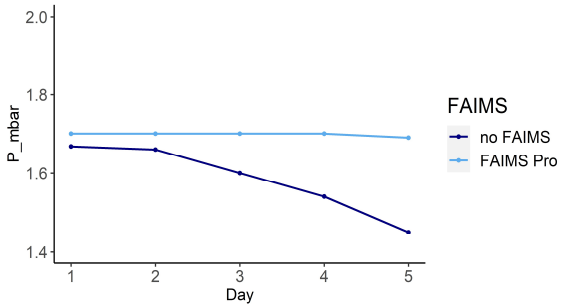

Figure 6. Fore-vacuum pressure change across all days of immunosuppressants analysis without FAIMS Pro and with FAIMS Pro.

Table 1. %RSD of immunosuppressants with FAIMS Pro Interface and without FAIMS Pro interface.

Compound	% RSD, no FAIMS	% RSD, FAIMS On
Cyclosporin A	20.9	4.3
Ascomycin	17.9	3.8
Everolimus	20.7	4.6
Sirolimus	20.6	4.3
Tacrolimus	17.6	3.9

%RSD values of raw peak areas for immunosuppressants across all five days of robustness studies were compared between the interfaces without FAIMS Pro and with FAIMS Pro (Table 1). Variability in peak areas was > 20% without FAIMS Pro and does not pass acceptance criteria for clinical protocols. Improved precision was observed for all analytes when using FAIMS Pro.

Results

Response ratios for immunosuppressants during five days of robustness study without and with FAIMS Pro interface

Response ratios (ratio of analyte peak area to internal standard Ascomycin) are represented on Figures 7-10. The ratio values for both setups are not equal due to the differences in transmission between the analyte and IS with and without FAIMS Pro. However, area ratios are consistent across all five days for each analyte for both types of robustness studies and demonstrate the importance of using internal standard to compensate any variations for accurate and precise quantification.

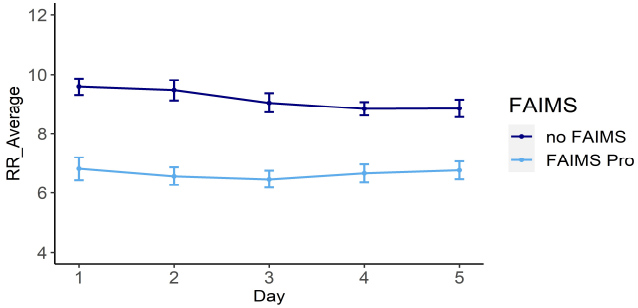

Figure 7. Cyclosporin A response ratio change in human whole blood across five days of robustness study without and with FAIMS Pro interface using Ascomycin as Internal Standard at 23.1 ng/mL.

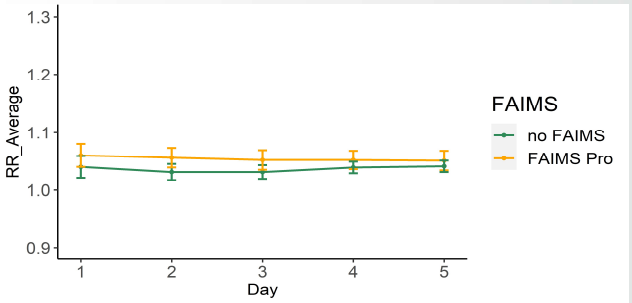

Figure 8. Tacrolimus response ratio change in human whole blood across five days of robustness study without and with FAIMS Pro interface using Ascomycin as Internal Standard at 23.1 ng/mL.

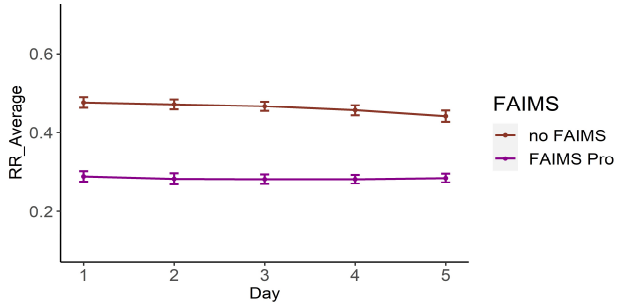

Figure 9. Sirolimus response ratio change in human whole blood across five days of robustness study without and with FAIMS Pro interface using Ascomycin as Internal Standard at 23.1 ng/mL.

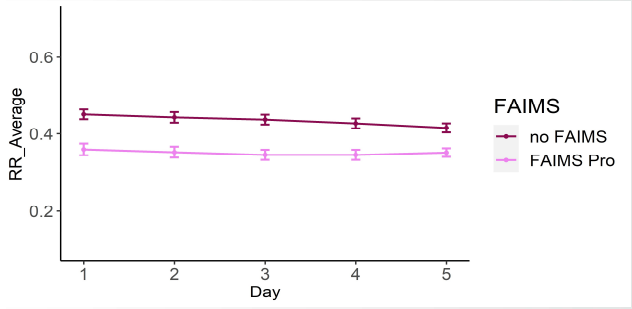

Figure 10. Everolimus response ratio change in human whole blood across five days of robustness study without and with FAIMS Pro interface using Ascomycin as Internal Standard at 23.1 ng/mL.

Results

Linearity for immunosuppressants during five days of robustness study without and with FAIMS Pro interface

Calibration curves for each immunosuppressant were run every day at the beginning of the queue. Linearity of $R^2 = 0.99$ was observed for all of analytes across 5 days when referenced to Ascomycin at 23.5 ng/mL as the internal standard. Figures 11 – 14 show calibration curves for Cyclosporin A with and without FAIMS Pro interface on Days 1 and 5.

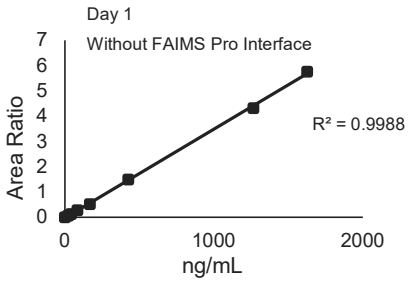

Figure 11. Cyclosporin A calibration curve without FAIMS Pro Interface on Day 1 of Robustness Study.

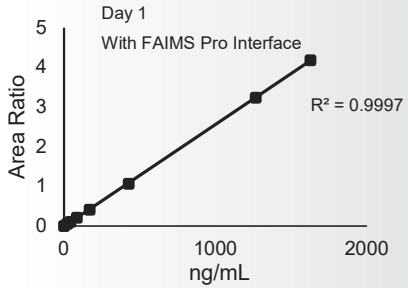

Figure 12. Cyclosporin A Calibration Curve with FAIMS Pro Interface on Day 1 of Robustness Study.

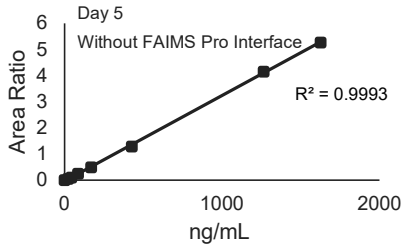

Figure 13. Cyclosporin A Calibration Curve without FAIMS Pro Interface on Day 5 of Robustness Study.

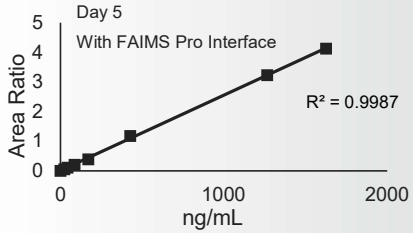

Figure 14. Cyclosporin A Calibration Curve with FAIMS Pro Interface on Day 5 of Robustness Study.

Conclusions

- We demonstrated that incorporating FAIMS Pro upgraded to high-flow increases throughput and efficiency for LC/MS analysis of immunosuppressant drugs in human whole blood. 360 injections per day were performed each day and total of 1800 injections per robustness study.
- The entire LC/MS system can be in use for five consecutive days of continuous sample injections without any maintenance performed on the system.
- Raw peak areas of immunosuppressants, response area ratios and fore-vacuum pressure remained consistent throughout the robustness study when using FAIMS Pro upgraded to high flow.
- Precision was below 5% for peak areas for immunosuppressants across five days with FAIMS Pro and is significant improvement when compared to 20% variability observed without FAIMS Pro.
- Signal-to-noise ratios were improved due to ability of FAIMS Pro interface to minimize interference from matrix components, which is crucial for sensitive and accurate analysis quantification of components in clinical studies.
- Overall, FAIMS Pro upgraded to high-flow posses significant benefits for busy clinical labs for routine analysis of samples without any interruptions.

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