FAIMS Pro Interface Coupled to Triple Quadrupole Mass Spectrometer for Quantification of Peptides in Complex Matrices

Michael Volný, Claudia P. B. Martins, Michael W. Belford, Mary L. Blackburn, Thermo Fisher Scientific, San Jose, CA, United States

ABSTRACT

Purpose: The Thermo Scientific™ FAIMS Pro™ interface provides the selectivity and ease-of-use for improved peptide quantifications in complex matrices and for lower abundance peptides.

Methods: Thermo Scientific™ EASY-nLC™ system was used with Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer

Results: Results show compatibility of FAIMS Pro interface with TSQ Altis Triple Quadrupole Mass Spectrometer and the applicability of this coupling for improvement of current peptide quantification workflows.

INTRODUCTION

The FAIMS Pro interface provides orthogonal precursor ion selectivity based on differential gas phase mobility. The Compensation Voltage (CV) setting determines which groups of ions are transmitted to the mass spectrometer for detection. A wide range of possible CV settings increases instrument performance for proteomics experiments. The increased selectivity and sensitivity enables researchers to improve quantification and to maximize efficiency and conserve sample. Many important peptides are biologically highly active and thus need to be monitored and quantified at ultra-low concentrations. The task of targeted peptide quantification is typically executed by LC-MS using triple quadrupoles in SRM/MRM mode. Such experiment can be disrupted by high background noise or interference from isobaric species. The application of differential mobility, enabled by the FAIMS Pro interface, offers an orthogonal mode of separation that enhances specificity and reduces the background interference resulting in improved LoQ levels.

MATERIALS AND METHODS

A TSQ Altis Triple Quadrupole Mass Spectrometer coupled with a Thermo Scientific™ EASY-nLC™ system was utilized for all measurements. Peptide standards and test matrices were obtained from Bachem, Thermo Fisher Scientific and Sigma-Aldrich. The FAIMS Pro interface attaches to the mass spectrometer with a flange and can be installed with Thermo Scientific NG sources.

Data Analysis

Thermo Scientific™ TraceFinder™ Software, Thermo Scientific™ FreeStyle™ Software and Skyline were used to process the results

FAIMS Pro software implementation

No Instrument configuration necessary, FAIMS Pro interface is recognized by the software when mounted and powered up

Initial DV Tune is available from Tune GUI

FAIMS parameters become part of Source and Scan tabs

CV optimization is available in Tune

Optimization files are automatically saved for further interrogation

CV value can be part of SRM or SIM table in the LC-MS method for each precursor ion

RESULTS

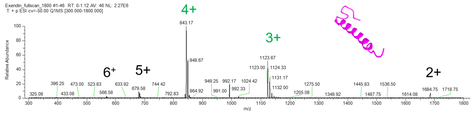

Figure 1. [9-39]Exendine (DLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS – NH2) direct infusion ESI-MS spectrum showing the charge state distribution

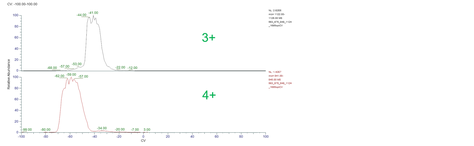

Figure 2. FAIMS Pro Compensation Voltage (CV) scan showing separation of Exendine $[M+3H]^{3+}$ and $[M+4H]^{4+}$ ions by FAIMS Pro

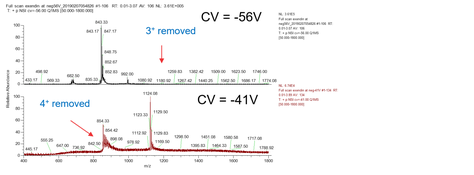

Figure 3. Setting CV to a specific value allows selective transmission of targeted charge state with removal or significant suppression of other charge states

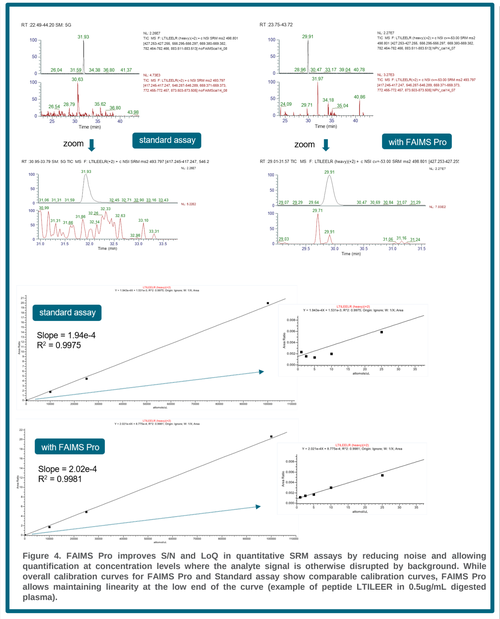

Figure 4. FAIMS Pro improves S/N and LoQ in quantitative SRM assays by reducing noise and allowing quantification at concentration levels where the analyte signal is otherwise disrupted by background. While overall calibration curves for FAIMS Pro and Standard assay show comparable calibration curves, FAIMS Pro allows maintaining linearity at the low end of the curve (example of peptide LTILEER in 0.5ug/mL digested plasma).

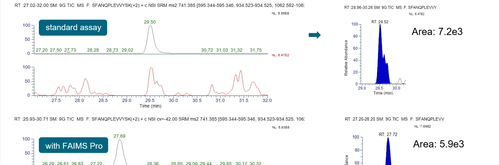

Figure 5. Example of peptide SFANQPLEVY in digested plasma matrix shows how FAIMS Pro (bottom panel) improves peak shape by removing a coeluting interference. As a result, automatic integration provides more accurate value without a necessity to reintegrate manually as would be required in the standard assay (top panel)

Thermo Scientific™ Pierce™ LC-MS/MS System Suitability Standard (7 x 5 Mix) with FAIMS Pro on TSQ Altis MS

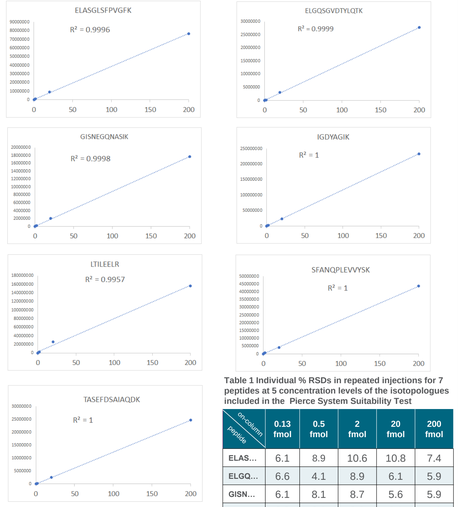

Table 1 Individual % RSDs in repeated injections for 7 peptides at 5 concentration levels of the isotopologues included in the Pierce System Suitability Test

Concentration / Peptide	0.13 fmol	0.5 fmol	2 fmol	20 fmol	200 fmol
ELAS…	6.1	8.9	10.6	10.8	7.4
ELGQ…	6.6	4.1	8.9	6.1	5.9
GISN…	6.1	8.1	8.7	5.6	5.9
IGDY…	2.9	2.1	2.9	3.1	1.9
LTIL…	8.2	8.8	12.6	4.3	11.7
SFAN…	9.6	8.4	11.8	15.1	15.1
TASE…	4.8	3.8	3.8	2.9	3.5

Figure 6 The Pierce LC-MS/MS System Suitability Standard (7 x 5 Mix) enables users to assess performance (sensitivity and dynamic range) of LC-MS/MS systems. The mixture contains 7 peptides, each having 5 isotopologue sequences present in a dilution series. Here the 7x5 Mix was used to evaluate performance of FAIMS Pro with TSQ Altis MS. The mixture was prepared according to Pierce protocol and 0.3ug/uL digested plasma was used as a matrix. The results - obtained with optimized CV values and with 4 SRM transitions for each isotopologue - show linearity for all peptides and low %RSDs for repeated injections.

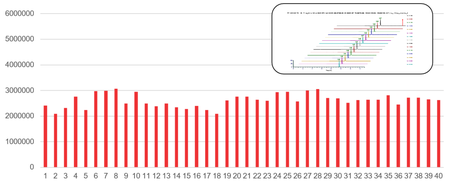

Fig 7 FAIMS Pro – TSQ Altis MS: 40 consecutive injections of Angiotensin in 0.5ug/uL digested plasma. The overall %RSD was <10% across this 2 day experiment (cleaning injections were inserted every 10 analytical injections). The inset show XIC of 15 consecutive injections.

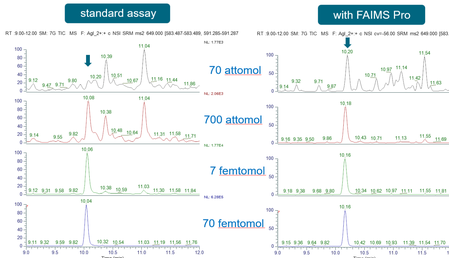

Fig 8 FAIMS Pro – TSQ Altis: Comparison of Angiotensin LC-MS assay with and without FAIMS Pro interface.

CONCLUSIONS

- FAIMS Pro interface coupled to TSQ Altis Triple Quadrupole Mass Spectrometer provides improvement of peptide quantification due to reduced noise and removal of co-eluting interference species.
- FAIMS Pro interface offers orthogonal precursor ion selectivity based on differential gas phase mobility. The Compensation Voltage (CV) setting determines which groups of ions pass the FAIMS Pro interface further to the mass spectrometer. In SRM workflows on triple stage quadrupoles, CV value can be used as another parameter in SRM table to set selective transmission of targeted precursor ion, while suppressing other ions.
- Utilization of FAIMS Pro interface is robust and the analyte signals are reproducible and stable in time as shown by performing consecutive injections over multiple days with low %RSD and by analyzing Pierce System Suitability Standard.

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TRADEMARKS/LICENSING

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