

Automating Optimization of Compensation Voltages for Differential Mobility Separation using Skyline

Using Skyline Software and the QTRAP® Systems with SelexION® Technology

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Peptide quantification in proteomics research typically involves complex matrices and therefore avoiding interferences in quantitative experiments is a key workflow consideration. The QTRAP® system technology offers multiple valuable options for higher selectivity quantitation in addition to MRM analysis, the MRM³ workflow and the differential mobility separation (DMS) workflow.

Differential mobility separation (DMS) on QTRAP® systems, known as SelexION® Technology, can be used to separate peptides based on different chemical properties and can be useful in the separation of isobaric or isomeric compounds². Use of DMS can improve the quantitation of peptides by reducing both specific confounding interferences as well as reducing the general background noise, simplifying peak integration. Each peptide of interest will have a unique compensation voltage (CoV) and therefore must be tuned like all compound dependent parameters on a mass spectrometer.

In this note, we demonstrate a new iterative tuning strategy for the semi-automated optimization of CoV values using Skyline Software (Figure 1).



Key Features of Compensation Voltage Optimization for QTRAP® Systems Using Skyline Software

- QTRAP® System with SelexION® Technology provides added specificity for peptide quantitation in complex matrices
- Semi-automated tuning strategy has been developed in Skyline software for the on-column optimization of compensation voltages (CoV) for large numbers of peptides
 - Scheduled MRM™ Algorithm for highest multiplexing of peptide MRMs and highest efficiency of tuning
 - ~100 peptides can be tuned in complex matrices without need for an internal standard in 3 LC-MS injections
 - Compatible with other compound dependent parameter optimization steps

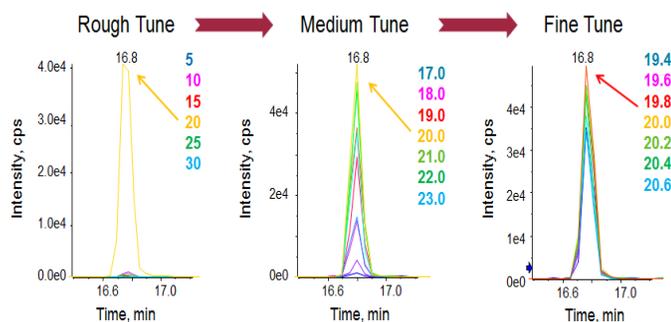


Figure 1. Strategy for Iterative Tuning of CoV. Three step on-column tuning strategy for large number of peptides simultaneously has been developed in Skyline Software. In the first injection, a rough tune is performed to determine the approximate CoV value for each peptide within the typical peptide CoV range. Using that value, a second injection is performed with CoV values in 1 V steps on either side of that first rough tune value, this provides a value to within 1 V from the optimum. A fine tune step is then performed with very small steps (0.2 V) to provide a final optimized CoV value for every peptide.

Workflow Steps for Optimizing the Compensation Voltages using Skyline Software

1. The first step in on-column optimization is to develop a good set of MRMs for each peptide of interest and determine the retention times for each peptide in matrix. This will allow *Scheduled MRM™* Algorithm to be used in the steps below, for the iterative optimization of CoV values (Figure 3 – Step 1).
2. In the first LC-MS DMS optimization run, a very rough tune of CoV is performed, using 5 V steps to cover the required CoV range (6 – 30 V). From that injection, the CoV that had the most signal for each MRM was found and then used as the center point for the next CoV ramp (Figure 3 – Step 2). The user can edit the CoV ramp parameters (Figure 2). Only the highest intensity MRM per peptide is chosen for optimization as CoV is a peptide level property, this provides greatest workflow efficiency.
3. In the second LC-MS optimization run, a medium tune was performed using CoV steps of 1 V with 3 steps on either side of the center point. Again, the CoV with the maximum signal was determined and this was used as the center point for the next fine tune.

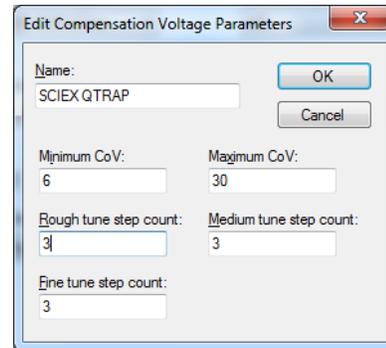


Figure 2. Default Settings for Compensation Voltage Tuning. The default settings that control the number and size of the sets can be adjusted in the Edit Compensation Voltage Parameter window found in Transition Settings on the prediction tab.

4. An optional third LC-MS optimization run can be performed, with CoV steps of 0.2V with 3 steps on either side of the center point. The CoV with max signal is selected as the final optimized CoV value for each peptide.
5. A final method is created, with an optimized CoV value for every peptide, for use in quantitation experiments.

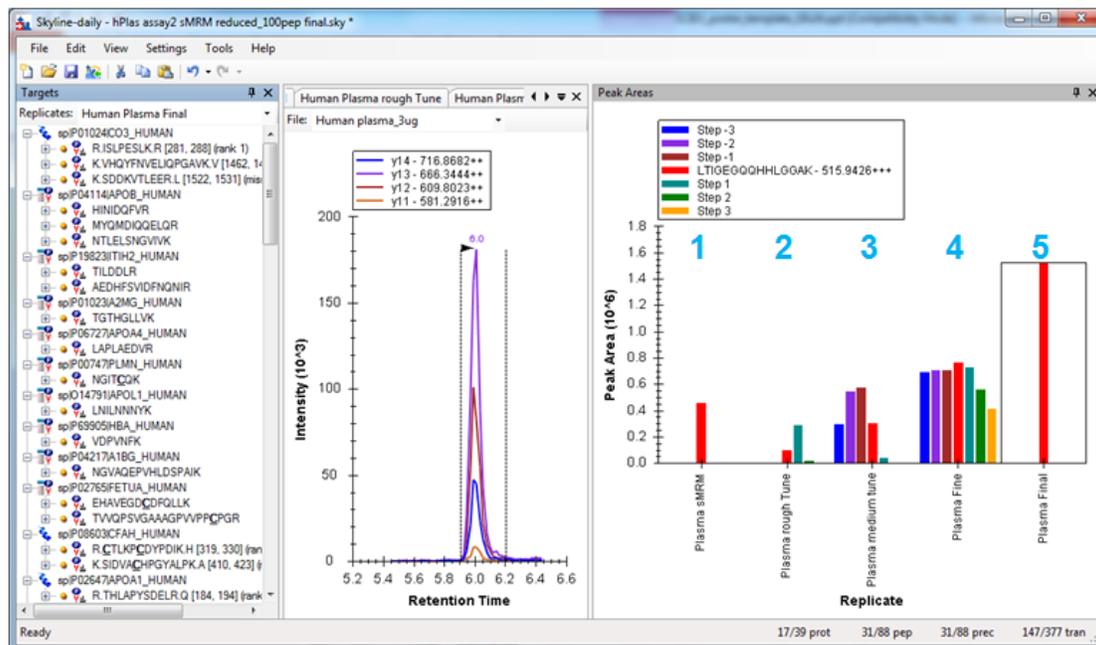
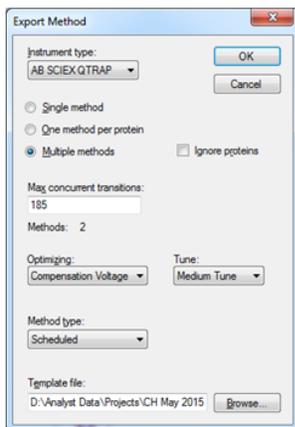


Figure 3. Overall Skyline Software Workflow with CoV Tuning. A screen capture of the Skyline workspace (left) highlights the multiple steps in performing the CoV optimization. This visual shows how the rough, medium and then fine tune really hones in on the CoV optimum in a short number of steps.

Exporting Acquisition Method from Skyline for Analyst® Software



Importing data from Analyst Software into Skyline

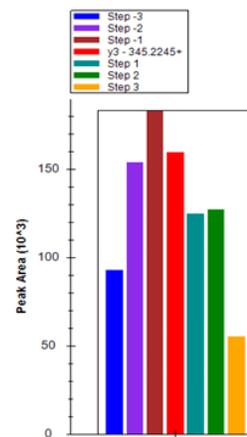
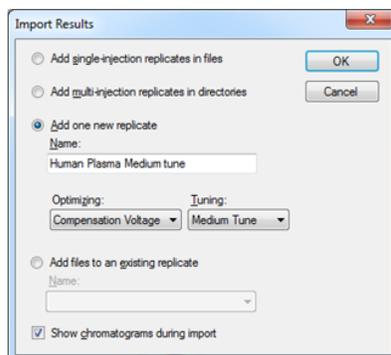


Figure 4. Exporting Methods and Importing Data. In Skyline, an acquisition method can be automatically created for the Analyst® software (left) by selecting Compensation Voltage (under Optimizing), and which tuning step they wish to perform (Rough, Medium or Fine Tune). After the data is collected, it is loaded into Skyline (middle) again specifying CoV Optimization and which tuning step. The CoV with the maximum signal is determined for each peptide (right) and is then used as the center point for the next optimization iteration. Three rounds of optimization produce a highly optimized CoV and can be performed on ~100 peptides per run (when using Scheduled MRM™ Algorithm).

Exporting Methods and Importing Data

At each step of the iterative process, a specific MRM acquisition method is built using the standard method export process. When QTRAP system is selected and the *Optimizing* drop down menu is viewed, Compensation Voltage can be chosen (Figure 4, left). This selection creates a secondary selection option to choose which step in the iterative process is to be run (Rough tune, Medium tune or Fine tune). Select a template file that has been built with the appropriate source settings and the DMS device activated. This builds an MRM method using the most intense MRM per peptide and builds a series of MRM transitions with different CoV values. These steps can be controlled by adjusting the defaults (Figure 2). The method is then run in Analyst® Software on the QTRAP system with the DMS on.

Once the data is collected, it is imported back into Skyline software through the standard *Import Results* process (Figure 4, middle). Again, under *Optimizing*, the appropriate tuning step is chosen. As the data is imported, the MRM transition with the most signal intensity for each peptide is determined and the CoV value for that transition is selected as the optimum for that step.

This process is repeated up to three times to obtain a highly optimized CoV value for each peptide. This is a highly efficient process when using *Scheduled MRM™* Algorithm as large numbers of peptides can be tuned simultaneously in just 3 LC-MS runs.

After the tuning process is completed, all optimized values can be added to the Skyline Optimization Library (Figure 5). A user can create a new or add to an existing Optimization library, by selecting *Optimization Library* on the *Prediction* tab, under *Settings* → *Transition Settings* (Figure 5).

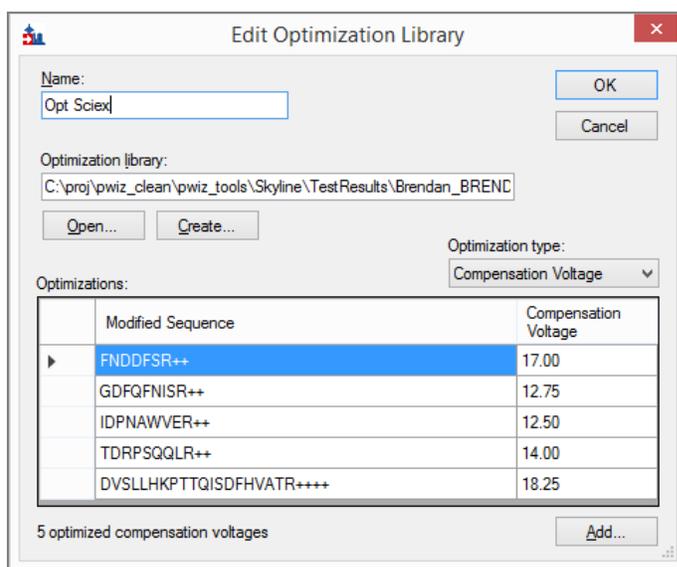


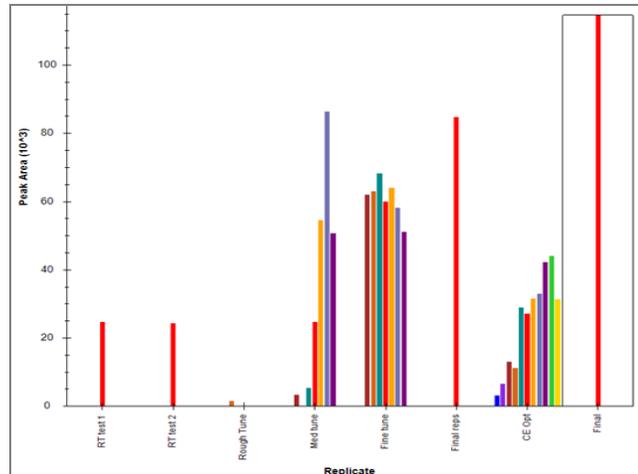
Figure 5. Storing CoV values in Skyline Optimization Library. After optimization, all optimized values including CoV can be added to the optimization library.

Testing in Complex Matrices

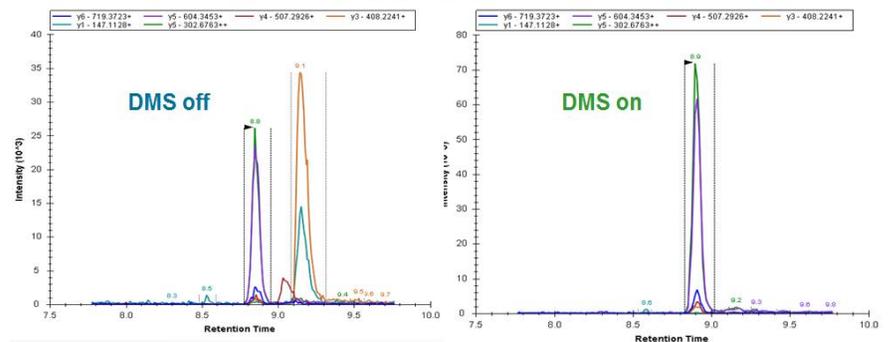
One key aspect of this on-column workflow is that this can be done in matrix without the need for tuning standards (synthetic peptides). A number of peptides per protein can be optimized and tested for performance before final selection and then heavy labeled synthetic peptides could then be obtained.

In this example, 88 peptides from 39 proteins were selected and taken through the entire process (Figure 6, top). This tuning process is fully compatible with other compound dependent parameter tuning steps that might be desired for assay optimization, such as collision energy optimization. The CE can be optimized either before or after CoV tuning.

After performing all tuning steps, the final optimized assay was exported and run on digested plasma, both with DMS on and with DMS in transparent mode. As expected, many peptides provided clean MRM transitions, highlighting the selectivity of MRM based assays. However, several peptides, that were critical for this assay, had interferences close in retention time to the correct peak (Figure 6, bottom left). These could often be removed by the DMS, as shown in Figure 6 (bottom right).



Peptide - VDPVNFK



Peptide - LAPLAEDVR

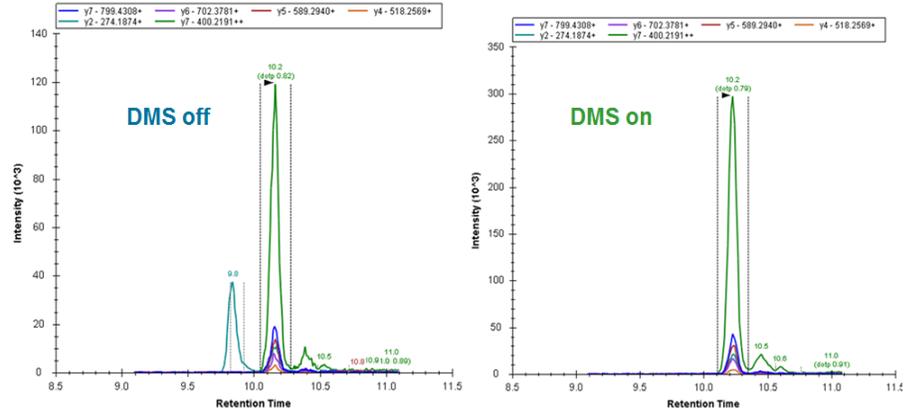


Figure 6. Removing Interferences from Peptides in Complex Plasma using DMS. In complex matrix, some peptides can have interferences in some of the MRM transitions. (Left) Shown here are the interferences for two peptides when the DMS cell is turned off (transparent mode). After CoV optimization, the LC-MS run was repeated and a significant reduction in interferences was observed (Right). This highlights the power of DMS for quantifying peptides in complex matrices, especially with fast gradients, like shown here with microflow chromatography.

Conclusions

Differential Mobility Separation (DMS) using SelexION® Technology on QTRAP® Systems provides an orthogonal level of selectivity by separating components based on their chemical properties and mobility.

- The iterative approach to tuning the compound dependent CoV value has been implemented in Skyline software, allowing for the easy, rapid optimizing of many peptide CoVs in a small number of LCMS runs. The tuning of 100 – 150 peptides in a single optimization round has been demonstrated to date.
- CoV tuning can be used in combination with other Skyline optimization features.
- Optimization can be performed on any type of peptide supported in Skyline, including post-translationally modified peptides².
- Using optimized CoV values, interferences could be significantly reduced.
- Finally, optimized CoV values can be stored in an optimization library for future use, along with all other optimized parameters for the peptide.

References

1. Multiple Mass Spectrometric Strategies for High Selectivity Quantification of Proteins and Peptides. SCIEX Technical Note RUO-MKT-02-3249-A.
2. Using Differential Mobility Spectrometry to Separate and Localize Sites of Post-Translational Modifications on Peptides - Using SelexION® Differential Mobility Technology on QTRAP® 5500 LC-MS/MS System, SCIEX Technical Note RUO-MKT-02-2750-A.

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