Optimizing lower limits of quantification and detection by choosing transitions in Skyline

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Introduction

For a particular analyte, the lower limits of quantification and detection can be estimated by using a calibration curve. The amount of noise in the measurements is highly dependent on the set of transitions being monitored.
Skyline is an open source Windows application for analyzing mass spectrometry results.

An upcoming version of Skyline (23.2) will have features which automatically choose the set of transitions to quantify that result in the lowest limits of quantification and/or detection.

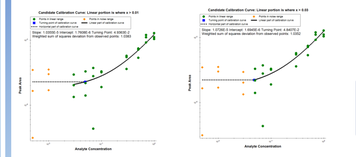
Methods

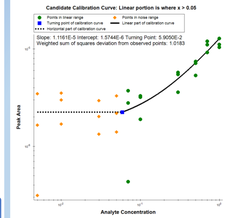
A matrix matched calibration curve was prepared by diluting human plasma into chicken plasma at human plasma concentrations ranging from 1 ug/uL to 0.005 ug/uL. For LC-MS/MS analysis, 1 ug of total protein was injected onto a Thermo Easy-nanoLC coupled to a Thermo Orbitrap Eclipse Tribrid Mass spectrometer. Peptides were quantified in the linear ion trap with parallel reaction monitoring using a scheduled acquisition method.

Results

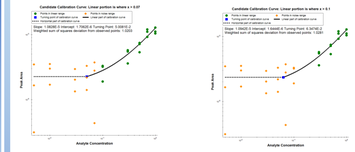
Fitting piecewise linear curves to the calibration points

For each calibration level, generate a candidate calibration curve by fitting a sloped straight line to the subset of points with concentrations above that level. Augment the curve with a horizontal line whose height is the mean plus one standard deviation of the y-values of the points at or below that calibration level. For any x-value, the y-value of the curve is the maximum of either the horizontal line or the sloped line.





Select the candidate calibration curve which minimizes the average weighted sum of squares difference between the observed points and the piecewise curve

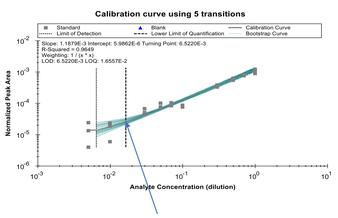


The reason that these do not look like straight lines is that they have been plotted using a logarithmic scale.
The logarithmic scale is being used so that it is easy to see the horizontal part of the calibration curves.
The points in the sloped part of the curve have a weight which is proportional to $1/x^2$.
The points in the horizontal (noise) part of the curve are weighted proportional to the x value of the highest calibration level in the noise portion.

Determining the Limit of Detection:
The limit of detection is declared to be the place where the horizontal (noise) line intersects with the linear portion of the calibration curve.

Determining the lower limit of quantification

One hundred calibration curves are generated using random

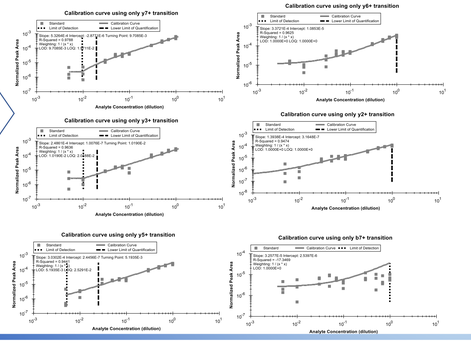


The lower limit of quantification is declared to be the point at which the coefficient of variation of the Y-values of these curves is less than 20%.

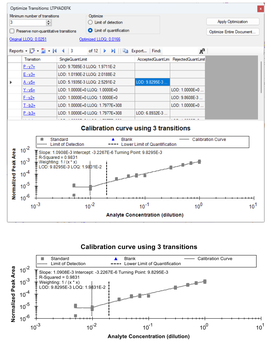
Finding the optimal set of transitions

Step 1: Generate calibration curves using results from only one transition at a time

Order those transitions by figure of merit



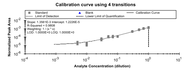
Step 2: Generate a calibration curve using only the best transitions from step 1



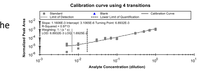
Step 3: Try adding the other transitions one at a time

Accept only those transitions that improve the calibration curve.

Adding the y6+ transition makes the LLOQ worse



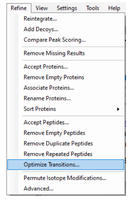
Adding the b3+ transition makes the LLOQ better



Step 4: If the number of accepted transitions is still less than the minimum number of transitions requested

Add initially rejected transitions so long as their effect on the quant limit is not catastrophic

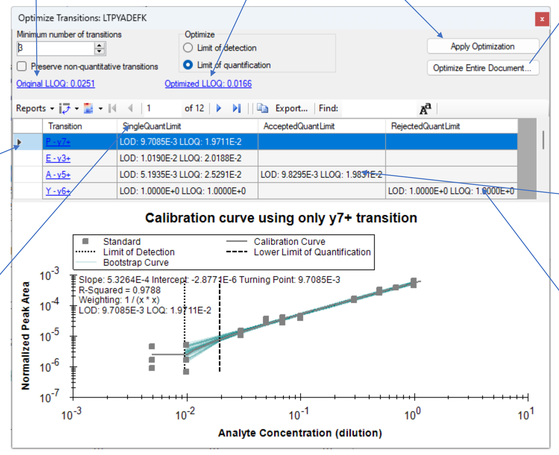
User Interface Overview



"Original LLOQ" shows what the lower limit of quantification was using all available transitions

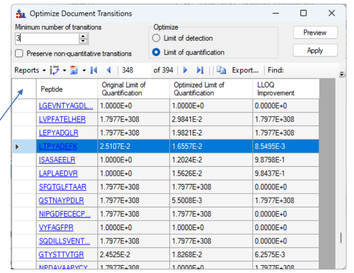
"Optimized LLOQ" link shows how the LLOQ could be improved by using only a subset of the transitions. Clicking the link selects the optimal subset of transitions in the grid.

"Apply Optimization" button modifies Skyline document so that the non-optimal transitions are marked non-quantitative



The selected rows in the grid control the set of transitions whose peak areas are included in the displayed calibration curve

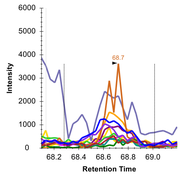
"SingleQuantLimit" column shows figures of merit of calibration curve using only one transition's peak area.



"AcceptedQuantLimit" column shows the figures of merit using the combined peak areas of the transition in this row as well as all of the accepted transitions above this row.
Since the requested minimum transitions were 3, Skyline included the three transitions with the best individual LLOQ values before Skyline started looking for other transitions which could be added and result in an improved LLOQ.

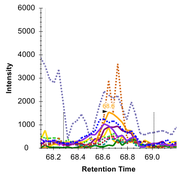
Values in the "RejectedQuantLimit" appear when adding in a particular transition resulted in a worse LLOQ. Transitions with text in the RejectedQuantLimit column were not included during Step 3 of the optimization process, but may have been included in Step 4 if the minimum number of transitions was unsatisfied.

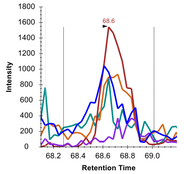
Chromatograms before and after



Before optimization, many of the transition chromatograms have interference

Skyline marks some of the transitions as non-quantitative (dotted lines) to improve the lower limit of quantification

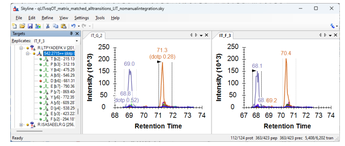




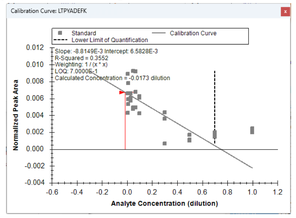
When the non-quantitative chromatograms are hidden the peak is less noisy

Future Directions

Skyline picks the wrong peak when the sample concentration is below the limit of detection



Unless manual adjustment to peak boundaries is performed, the peak areas for lower concentration samples are meaningless



Acknowledgements

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Conflict of Interest Statement

Philip M. Remes is an employee of Thermo Fisher Scientific, the manufacturer of the instrument used to generate the data shown on this poster. The MacCoss Lab at the University of Washington has a sponsored research agreement with Thermo Fisher Scientific. Michael J. MacCoss is a paid consultant for Thermo Fisher Scientific. All six major mass spectrometry vendors (Agilent, Bruker, Sciex, Shimadzu, ThermoFisher and Waters) provide financial support for the development and maintenance of Skyline.

References

Heil, Lilian R., Philip M. Remes, and Michael J. MacCoss. "Comparison of unit resolution versus high-resolution accurate mass for parallel reaction monitoring." *Journal of Proteome Research* 20.9 (2021): 4435-4442.

Pino, Lindsay K., et al. "Matrix-matched calibration curves for assessing analytical figures of merit in quantitative proteomics." *Journal of proteome research* 19.3 (2020): 1147-1153.