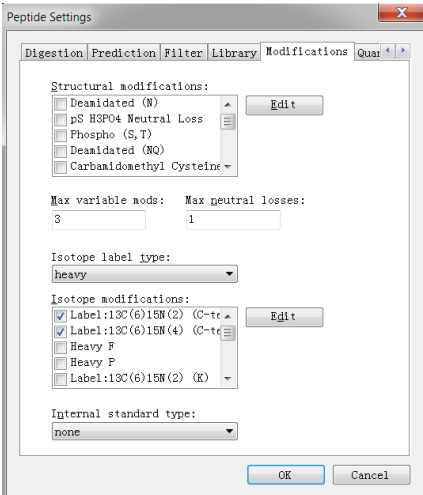
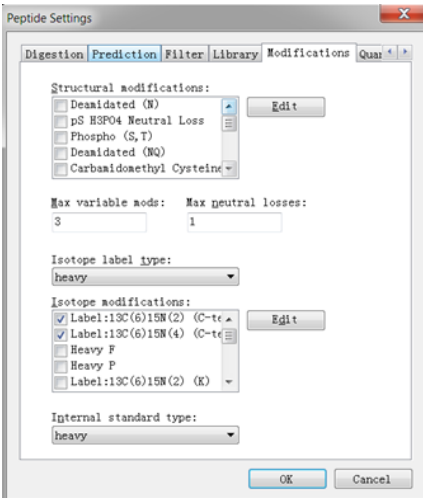
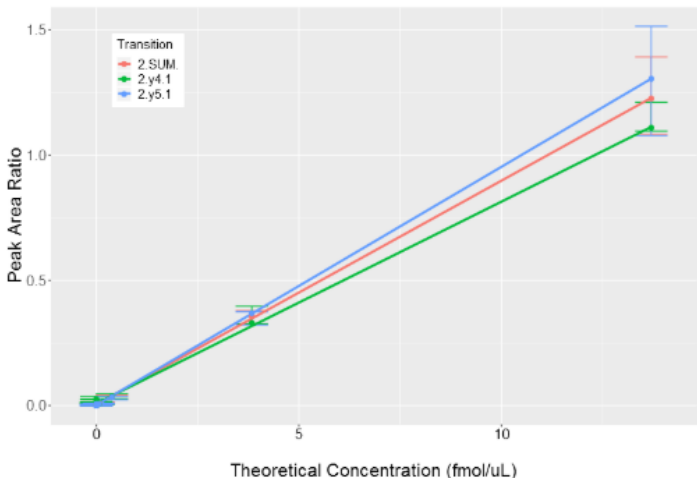


Response Curve (Experiment 1)

Error category	Error message	Example case	Solution to the issue
Internal standard	Internal standard is set to be none.	<p>The internal standard in the skyline file is set to be none. Errors happen for all the peptides.</p> 	<p>In Skyline, settings -> Peptide Settings -> Modifications -> Internal standard type, set Internal standard.</p>
Internal standard	Internal standard is incorrect according to the inferred internal standard.	<p>The internal standard in the skyline file is set to be heavy, while the inferred internal standard is light. Errors happen for all the peptides.</p> 	<p>In Skyline, settings -> Peptide Settings -> Modifications -> Internal standard type, set Internal standard to be the correct type.</p>

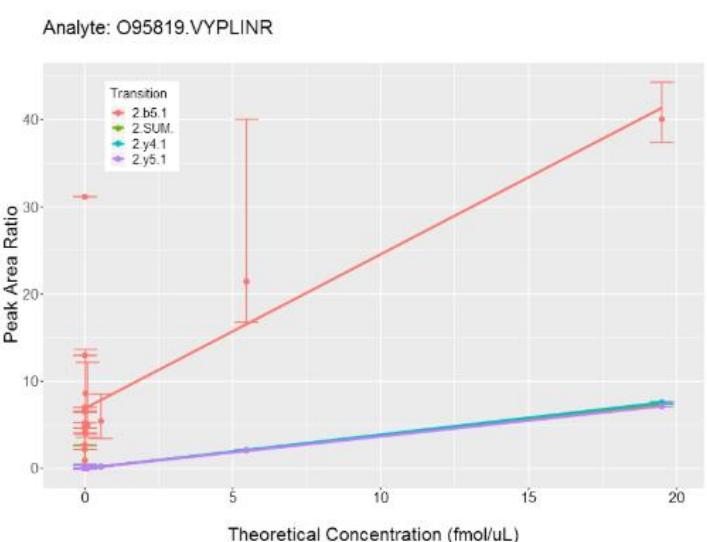
Attribute	Essential attributes have missing values.	Essential attribute(s) has(have) missing values, including ISSpike or PeptideConcentrationIS. Essential attribute(s) has(have) missing values, including ReplicateName; Replicate; SampleGroup.	1. In Skyline, for the old template, View -> Document Grid -> Views -> Replicates , check the corresponding attributes; for the new template, View -> Document Grid -> Views -> Assay Curve Replicates , check the corresponding attributes. 2. If the corresponding attributes are normal in step 1, in Skyline, View -> Targets -> By Name , in Targets panel, navigate to the peptide sequence, check the fragment ions from precursors of light and heavy isotopes, find the fragment ions with no data, and delete them.
Attribute	Essential attributes are annotated in unqualified data type.	None	In Skyline, for the old template, View -> Document Grid -> Views -> Replicates , check the data types of corresponding attributes; for the new template, View -> Document Grid -> Views -> Assay Curve Replicates , check the data types of corresponding attributes.
Internal standard peptide concentration	The annotation of the concentration of the internal standard peptide has issues.	The annotation of the concentration of the internal standard peptide has issues. Please check the annotation of IS Spike or PeptideConcentrationIS.	In Skyline, for the old template, View -> Document Grid -> Views -> Replicates , check the attribute of IS Spike, then View -> Document Grid -> Views -> Peptides , check the attribute of PeptideConcentrationIS; for the new template, View -> Document Grid -> Views -> Assay Peptides , check the attribute of Internal Standard Concentration.
Concentration	More than one concentration levels are needed.	None	In Skyline, for the old template, View -> Document Grid -> Views -> Replicates , check the attribute of Concentration; for the new template, View -> Document Grid -> Views -> Assay Curve Replicates , check the attribute of Analyte Concentration.
Area values of heavy or light Isotope	More than one area values of heavy or light isotope exist for the combination of protein, peptidemodifiedsequence, precursorcharge, productcharge, fragmentation, replicate, concentration,	Peptide sequence with errors: ETPAASEAPSSAAK Issue message: More than one area values of heavy or light isotope exist for the combination of protein, peptidemodifiedsequence, precursorcharge, productcharge, fragmentation, replicate, concentration, samplegroup, isspike and isotopelabel.	In Skyline, in the Targets panel, navigate to the peptide with errors, click each fragment ion and check the Peak Areas – Replicate Comparison panel, more than one Peak Area value could be observed in one Replicate . Re-import the data related to the peptide into the current Skyline document to make sure there is one area value of heavy or light isotope kept.

	samplegroup, isspike and isotopelabel.		
Fragment ion	In response curve, no fragment ion with both heavy and light isotopes exists.	None	In Skyline, in the Targets panel, navigate to the peptide with errors, click each fragment ion (heavy or light isotope) and check the Peak Area value in Peak Areas – Replicate Comparison panel. Re-import the data related to the peptide into the current Skyline document to make sure the heavy and light isotope appear only one time for the fragment ions of the peptide.

Warning category	Warning message	Example case	Solution to the issue
Fragment ion	In response curve, the number of fragment ions with both heavy and light isotopes is less than 3.	<p>Analyte: Q96B36.LNT[+80]SDFQK</p>  <p>The graph displays three linear data series for the analyte Q96B36.LNT[+80]SDFQK. The x-axis represents Theoretical Concentration in fmol/uL, ranging from 0 to 15. The y-axis represents the Peak Area Ratio, ranging from 0.0 to 1.5. The three transitions are: 2 SUM (red line), 2 y4.1 (green line), and 2 y5.1 (blue line). All three series show a positive linear correlation between concentration and peak area ratio. The 2 y5.1 transition has the highest peak area ratio for a given concentration, followed by 2 SUM, and then 2 y4.1. Error bars are shown for each data point, indicating variability in the measurements.</p>	Check the response curve graph to make further decisions.

Bad linear regression fit

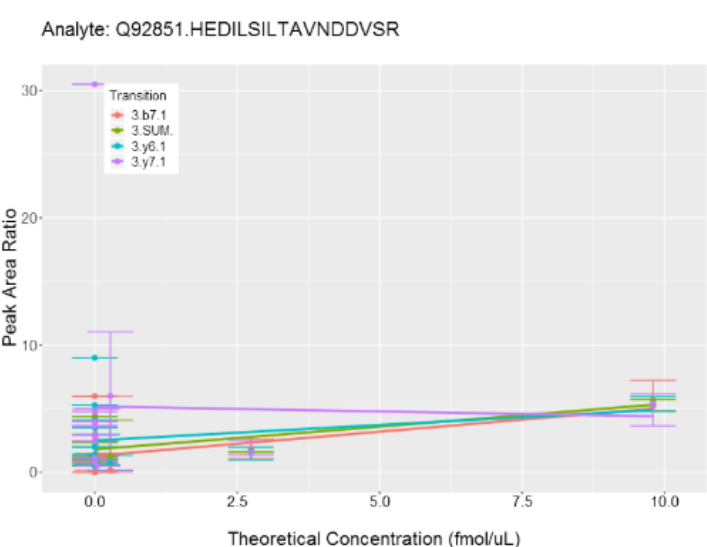
The coefficient of variance of slopes of the fragment ions is larger than 0.5.



Check the slopes of the fragment ions in the response curve graph to make further decisions.

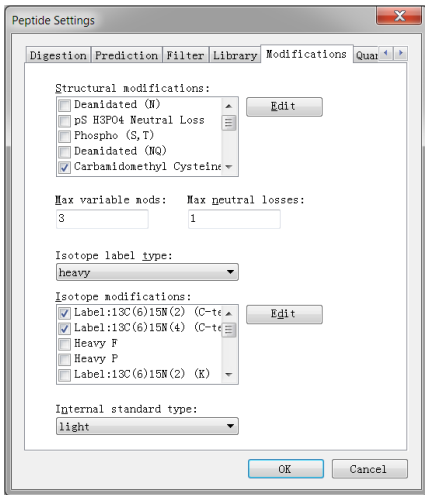
Bad linear regression fitting

The fit of the linear regression model is poor due to $R^2 < 0.5$ or $p > 0.05$ in the significance test for linear regression.

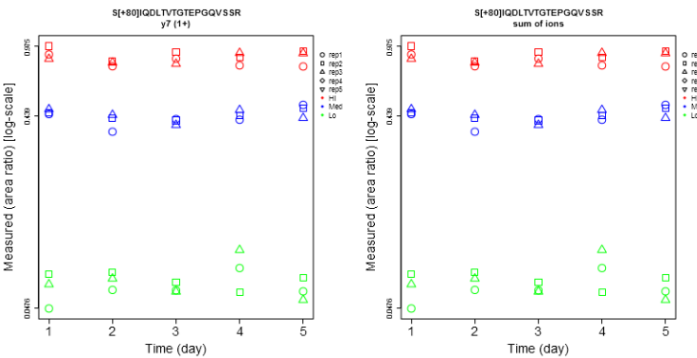


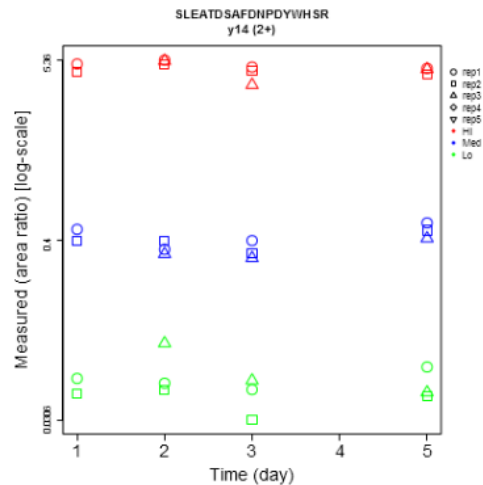
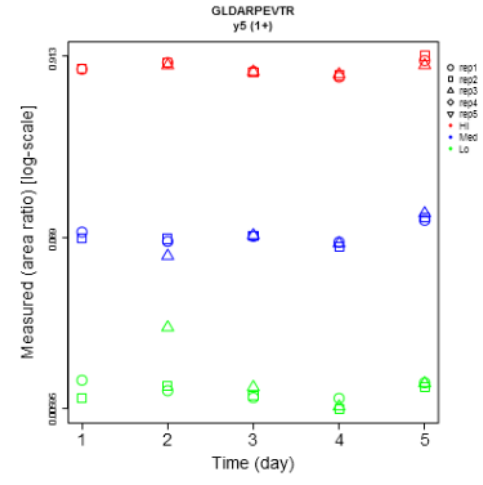
Check the response curve graph to make further decisions.

Repeatability (Experiment 2)

Error category	Error message	Example case	Solution to the issue
Internal standard	Internal standard is set to be none.	None	In Skyline, settings -> Peptide Settings -> Modifications -> Internal standard type , set Internal standard.
Internal standard	Internal standard is incorrect according to the inferred internal standard.	<p>The internal standard in the skyline file is set to be light, while the inferred internal standard is heavy. Errors happen for all the peptides.</p> 	In Skyline, settings -> Peptide Settings -> Modifications -> Internal standard type , set Internal standard to be the correct type.
Attribute	Essential attributes have missing values.	Essential attribute(s) has(have) missing values, including Replicate; Concentration; SampleGroup.	In Skyline, for the old template, View -> Document Grid -> Views -> Replicates , check the corresponding attributes; for the new template, View -> Document Grid -> Views -> Assay Repeatability Replicates , check the corresponding attributes.
Attribute	Essential attributes are using uncontrolled terms.	None	In Skyline, for the old template, View -> Document Grid -> Views -> Replicates , check the corresponding attributes; for the new template, View -> Document Grid -> Views -> Assay Repeatability Replicates , check the corresponding attributes.
Attribute	Essential attributes are annotated in unqualified data type.	None	In Skyline, for the old template, View -> Document Grid -> Views -> Replicates , check the data type of the corresponding attributes; for the new template, View -> Document Grid -> Views -> Assay Repeatability Replicates , check the data type of

			the corresponding attributes.
Light and Medium isotope	Both light and medium isotope labels are found in the peptide with a specific charge.	None	In Skyline, in the Targets panel, navigate to the peptide with errors, click each fragment ion to check its isotope label type.
Area values of heavy or light Isotope	The number of light isotopes or heavy isotopes is not one due to wrongly annotated values in attributes.	Peptide sequence with errors: DFALQNPGVVPR Error Message: For b2 (1+): 2 heavy isotopes 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 Day4_Std3_R03).	In Skyline, for the old template, View -> Document Grid -> Views -> Replicates , check the values of the attribute of Replicate for the problematic replicate names, for example, correct the values of the attribute of Replicate for the replicate name of Day4_Std3_R01 and Day4_Std3_R03; for the new template, View -> Document Grid -> Views -> Assay Repeatability Replicates , check the values of the attribute of Replicate Number for the problematic replicate names.

Warning category	Warning message	Example case	Solution to the issue
Fragment ion	In repeatability graph, the number of fragment ions with both heavy and light isotopes is less than 3.	<p>In repeatability graph, the number of fragment ions is 1 < 3, the fragment ions is(are): y7 (1+).</p> 	Check the repeatability graph to make further decisions.

Missing points	In repeatability graph, for the fragment ion, there is no point on at least one day.	For fragment ion y14 (2+), there is no point on day 4. 	Check the repeatability graph to make further decisions.
Missing points	In repeatability graph, fragment ion doesn't have three concentrations: Hi, Med, and Low on a specific day.	None	Check the repeatability graph to make further decisions.
Missing points	In repeatability graph, fragment ion has less than 3 replicates for a specific concentration on the specific day.	For fragment ion y5 (1+), there are less than 3 replicates in concentration(s) Hi, Lo, Med on day 1. 	Check the repeatability graph to make further decisions.