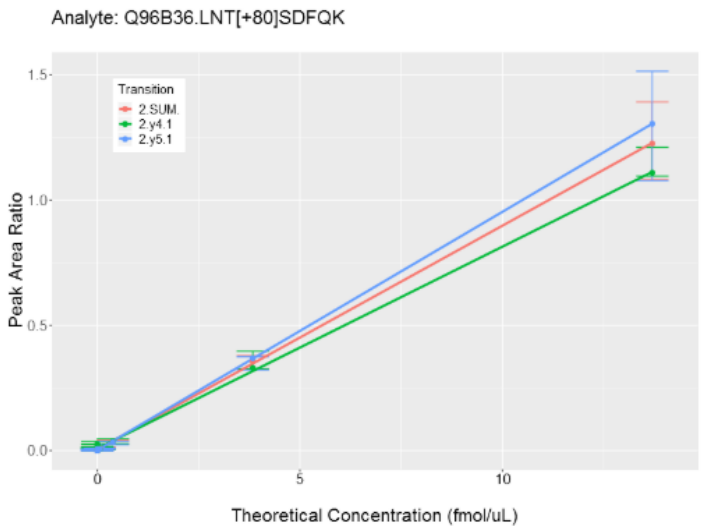
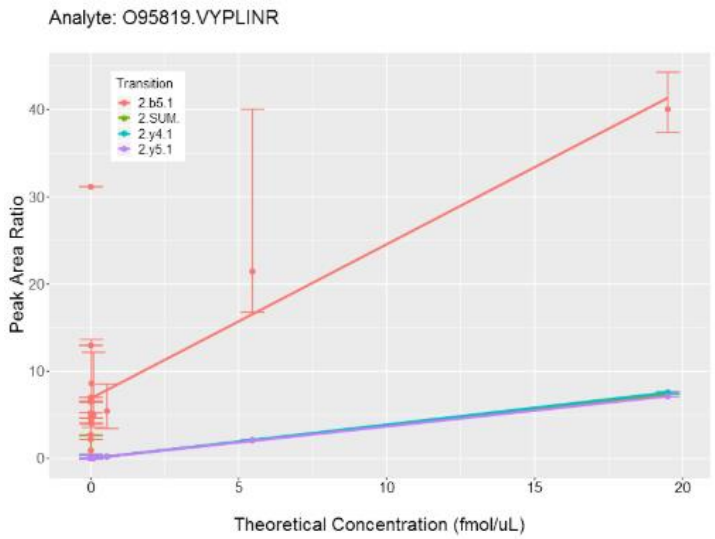
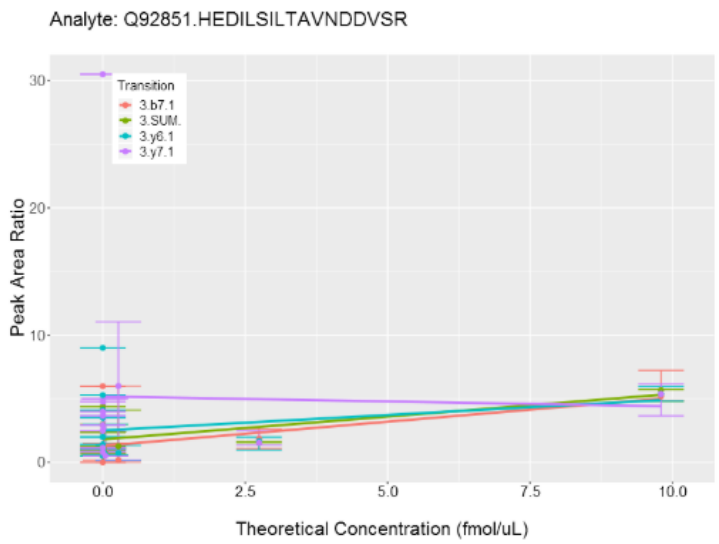
Response Curve (Experiment 1)

Error category	Error message	Example case	Solution to the issue
Internal standard	Internal standard is set to be none.	<table><tr><th>Skyline File Name</th><th>Internal Standard Type</th><th>Quality</th></tr><tr><td>MousePlasmaV2Exp1B3_refined.sky</td><td>none</td><td>The internal standard in the skyline file is set to be none. Errors happen for all the peptides.</td></tr></table>	In Skyline, **settings** -> **Peptide Settings** -> **Modifications** -> **Internal standard type**, set Internal standard.
Internal standard	Internal standard is incorrect according to the inferred internal standard.	<table><tr><th>Skyline File Name</th><th>Internal Standard Type</th><th>Quality</th></tr><tr><td>MousePlasmaV2Exp1B3_refined.sky</td><td>heavy</td><td>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.</td></tr></table>	In Skyline, **settings** -> **Peptide Settings** -> **Modifications** -> **Internal standard type**, set Internal standard to be the correct type.
Attribute	Essential attributes have missing values.	<table><tr><th>Skyline File Name</th><th>Peptide sequence with errors</th><th>Issue reason</th></tr><tr><td>DDRpanel_052316_curves_05_QCTEST.sky</td><td>DLSHIGDAVVISC[+57]AK[+114]DGVK</td><td>• Essential attribute(s) has (have) missing values, including ReplicateName; Replicate; SampleGroup. • Essential attribute(s) has (have) missing values, including ISSpike or PeptideConcentrationIS.</td></tr></table>	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.	<table><tr><th>Skyline File Name</th><th>Peptide sequence with errors</th><th>Issue reason</th></tr><tr><td>MousePlasmaV2Exp1B3_refined.sky</td><td>LPLPALFK</td><td>• The annotation of the concentration of the internal standard peptide has issues. Please check the annotation of IS Spike or PeptideConcentrationIS.</td></tr></table>	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	None	In Skyline, for the old template, **View** -> **Document Grid -> Views -> Replicates**, check the attribute of Concentration; for the new

<table>
<tr><td></td><td>needed.</td><td></td><td>template, View -> Document Grid -> Views -> Assay Curve Replicates, check the attribute of Analyte Concentration.</td></tr>
<tr><td>Light and Medium isotope</td><td>Both light and medium isotope labels are found in the peptide.</td><td>None</td><td>In Skyline, in the Targets panel, navigate to the peptide with errors, click each fragment ion to check its isotope label type.</td></tr>
<tr><td>Area values of heavy or light Isotope</td><td>More than one area values of heavy or light isotope exist for the combination of protein, peptidemodifiedsequence, precursorcharge, productcharge, fragmention, replicate, concentration, samplegroup, isspike and isotopelabel.</td><td><table><tr><th>Skyline File Name</th><th>Peptide sequence with errors</th><th>Issue reason</th></tr><tr><td>20160720_MousePlasmaExp1Batch5_refined.sky</td><td>ETPAASEAPSSAAK</td><td>• More than one area values of heavy or light isotope exist for the combination of protein, peptidemodifiedsequence, precursorcharge, productcharge, fragmention, replicate, concentration, samplegroup, isspike and isotopelabel.</td></tr></table></td><td>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.</td></tr>
<tr><td>Fragment ion</td><td>In response curve, no fragment ion with both heavy and light isotopes exists.</td><td>None</td><td>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.</td></tr>
</table>

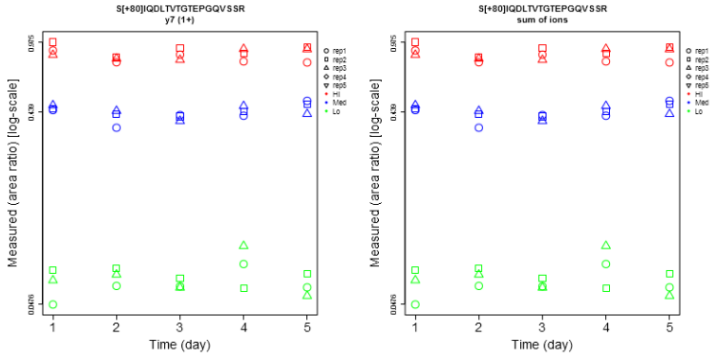
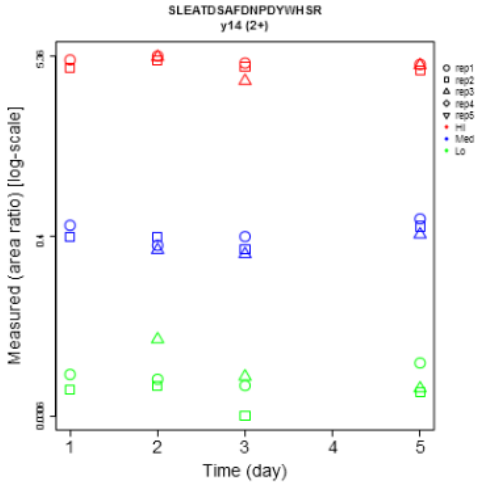
Warning category	Warning message	Example case	Solution to the issue
Fragment ion	In response curve, the number of fragment ions with both heavy and light isotopes is less than 3.	In response curve, only two fragment ions 2.y4.1, 2.y5.1 (with both heavy and light isotopes) exist. 	Check the response curve 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.

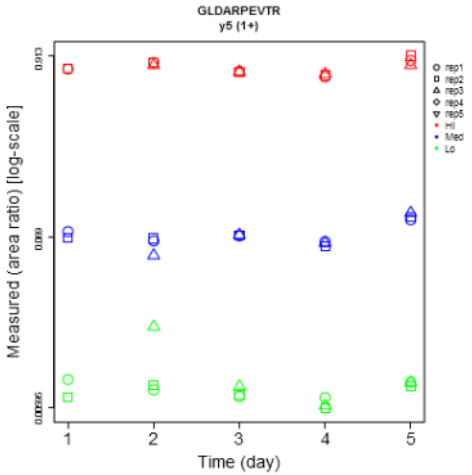
Bad linear regression fitting	The fit of the linear regression model is poor due to $R^2 < 0.5$ or $p > 0.05$ in the significance test for linear regression.		Check the response curve graph to make further decisions.

Repeatability (Experiment 2)

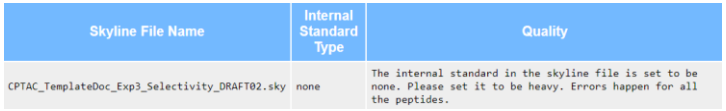
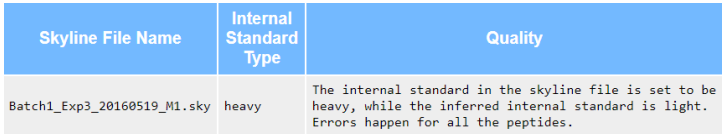
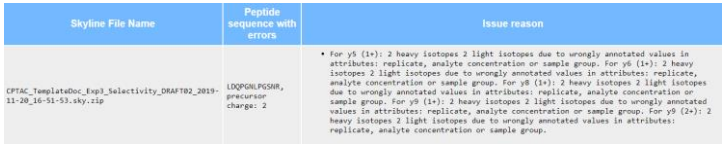
Error category	Error message	Example case	Solution to the issue
Internal standard	Internal standard is set to be none.	None	In Skyline, **settings** -> **Peptide Settings** -> **Modifications** -> **Internal standard type**, set Internal standard.
Internal standard	Internal standard is incorrect according to the inferred internal standard.	<table><tr><th>Skyline File Name</th><th>Internal Standard Type</th><th>Quality</th></tr><tr><td>TCGA glyco 26 peps_repeatability.sky</td><td>light</td><td>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.</td></tr></table>	In Skyline, **settings** -> **Peptide Settings** -> **Modifications** -> **Internal standard type**, set Internal standard to be the correct type.
Attribute	Essential attributes have missing values.	<table><tr><th>Skyline File Name</th><th>Peptide sequence with errors</th><th>Issue reason</th></tr><tr><td>DDRpanel_052316_repeatability_03_QCTEST.sky</td><td>LSLEFPSGYPYNAPTVK, precursor charge: 2</td><td>• Essential attribute(s) has (have) missing values, including Replicate; Concentration; SampleGroup.</td></tr></table>	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.

<table>
<tr>
<td>Area values of heavy or light Isotope</td>
<td>The number of light isotopes or heavy isotopes is not one due to wrongly annotated values in attributes.</td>
<td>
<table>
<tr><th>Skyline File Name</th><th>Peptide sequence with errors</th><th>Issue reason</th></tr>
<tr><td>MousePlasmaExp2B3_Refined.sky</td><td>DFALQNPGVVPR, precursor charge: 2</td><td>• For b2 (1+): 2 heavy isotopes 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 | Day4_Std3_R03). For b3 (1+): 2 heavy isotopes 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 | Day4_Std3_R03). For y2 (1+): 2 heavy isotopes 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 | Day4_Std3_R03). For y3 (1+): 2 heavy isotopes 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 | Day4_Std3_R03). For y6 (1+): 2 heavy isotopes 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 | Day4_Std3_R03).</td></tr>
</table>
</td>
<td>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. 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 the new template, View -> Document Grid -> Views -> Assay Repeatability Replicates, check the values of the attribute of Replicate Number for the problematic replicate names.</td>
</tr>
</table>

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.	In the repeatability graph below, the number of fragment ions is 1 < 3; the only fragment ion is: y7 (1+). 	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 are no points 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	None	Check the repeatability graph to make further decisions.

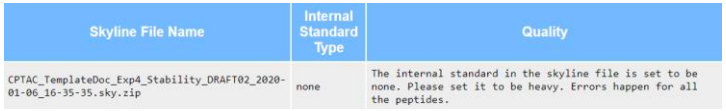
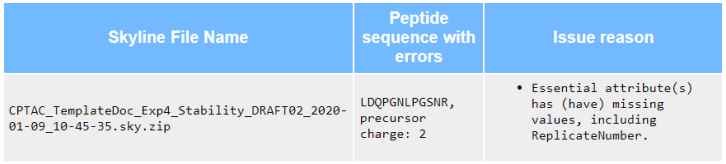
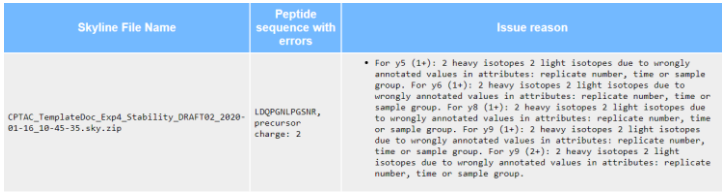
	day.		
Missing points	In repeatability graph, fragment ion has less than 3 replicates for a specific concentration on the specific day.	For fragment ion y5 (1+), there are less than 3 replicates in concentration(s) Hi, Lo, Med on day 1. 	Check the repeatability graph to make further decisions.
Bad distribution of points	In repeatability graph, for the fragment ion, the intra-assay, inter-assay or total coefficient of variance is larger than the threshold of 0.2.	None	Check the repeatability graph to make further decisions.

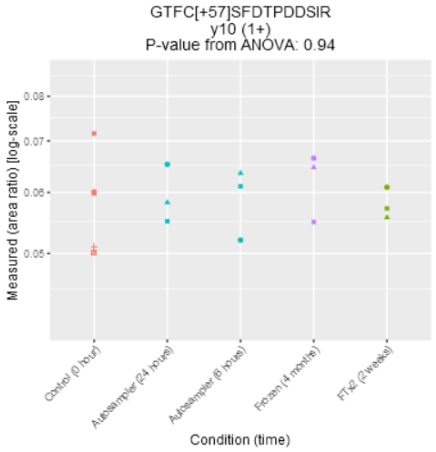
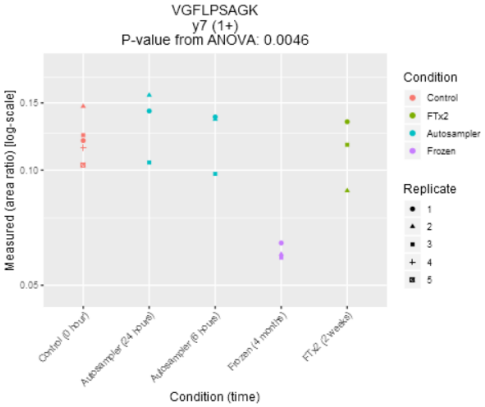
Selectivity (Experiment 3)

Error category	Error message	Example case	Solution to the issue
Internal standard	Internal standard is set to be none.		In Skyline, **settings** -> **Peptide Settings** -> **Modifications** -> **Internal standard type**, set Internal standard.
Internal standard	Internal standard is incorrect.		In Skyline, **settings** -> **Peptide Settings** -> **Modifications** -> **Internal standard type**, set Internal standard to be the correct type.
Attribute	Essential attributes have missing values.	None	In Skyline, **View** -> **Document Grid -> Views -> Assay Selectivity Replicates**, check the corresponding attributes.
Attribute	Essential attributes are annotated in unqualified data type.	None	In Skyline, **View** -> **Document Grid -> Views -> Assay Selectivity Replicates**, check the data types of 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.
Concentration	More than one concentration levels are needed.	None	In Skyline, **View** -> **Document Grid -> Views -> Assay Selectivity Replicates**, check the attribute of Analyte Concentration.
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.		In Skyline, **View** -> **Document Grid -> Views -> Assay Selectivity Replicates**, check the values of the attribute of Replicate Number, Analyte Concentration and Exp3 SampleGroup.

Warning category	Warning message	Example case	Solution to the issue
Fragment ion	The number of fragment ions is less than 3.	None	Check the selectivity graph to make further decisions.
Sample group	The number of sample group is less than 6.	None	Check the selectivity graph to make further decisions.
Bad linear regression fit	The coefficient of variance of slopes of the fragment ion is larger than 0.5.	None	Check the selectivity graph to make further decisions.
Bad linear regression fitting	The fit of the linear regression model is poor due to $R^2 < 0.5$ for some fragment ions in the significance test for linear regression.	None	Check the selectivity graph to make further decisions.

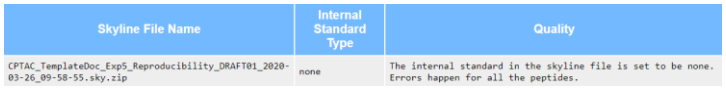
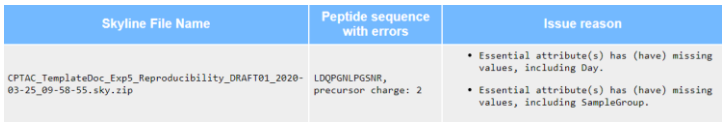
Stability (Experiment 4)

Error category	Error message	Example case	Solution to the issue
Internal standard	Internal standard is set to be none.		In Skyline, **settings** -> **Peptide Settings** -> **Modifications** -> **Internal standard type**, set Internal standard.
Attribute	Essential attributes have missing values.		1. In Skyline, **View** -> **Document Grid -> Views -> Assay Stability Replicates**, check the corresponding attributes.
Attribute	Essential attributes are annotated in unqualified data type.	None	In Skyline, **View** -> **Document Grid -> Views -> Assay Stability Replicates**, check the data types of corresponding attributes.
Attribute	Incorrect annotations in the attribute of Exp4 Sample Group or Freeze Thaw Cycles.	None	In Skyline, **View** -> **Document Grid -> Views -> Assay Stability Replicates**, check 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.		In Skyline, **View** -> **Document Grid -> Views -> Assay Stability Replicates**, check the values of the attribute of Replicate Number, Time, Time Units and Exp4 SampleGroup.

Warning category	Warning message	Example case	Solution to the issue
Attribute	Attributes of Time or Time Units have missing values.	None	In Skyline, **View** -> **Document Grid** -> **Views** -> **Assay Stability Replicates**, check the corresponding attributes.
Sample storage conditions and times	The number of sample storage conditions and times is less than 6.		Check the stability graph to make further decisions.
Replicate Number	The number of replicate is less than 2.	None	Check the stability graph to make further decisions.
Significant changes observed	The ANOVA p value is less than 0.05.		Check the stability graph to make further decisions.
Bad distribution of points	In stability table, for the fragment ion under the	None	Check the stability table to make further decisions.

	storage condition, the intra-assay of variance is larger than the threshold of 0.2.		
Bad distribution of points	In stability table, for the fragment ion, according to time zero analysis, the RMSE(s) is larger than the threshold of 0.2.	None	Check the stability table to make further decisions.

Endogenous (Experiment 5)

Error category	Error message	Example case	Solution to the issue
Internal standard	Internal standard is set to be none.		In Skyline, **settings** -> **Peptide Settings** -> **Modifications** -> **Internal standard type**, set Internal standard.
Attribute	Essential attributes have missing values.		In Skyline, **View** -> **Document Grid** -> **Views** -> **Assay Endogenous Replicates**, check the corresponding attributes.
Attribute	Essential attributes are annotated in unqualified data type.	None	In Skyline, **View** -> **Document Grid** -> **Views** -> **Assay Endogenous Replicates**, check the data types of 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.	None	In Skyline, **View** -> **Document Grid** -> **Views** -> **Assay Endogenous Replicates**, check the values of the attribute of Replicate Number, Day and SampleGroup.

Warning category	Warning message	Example case	Solution to the issue
Fragment ion	In reproducible detection of endogenous analyte graph, the number of fragment ions with both heavy and light isotopes is less than 3.	None	Check the reproducible detection of endogenous analyte graph to make further decisions.
Missing points	In reproducible detection of endogenous analyte graph, for the fragment ion, there is no point on at least one day.	None	Check the reproducible detection of endogenous analyte graph to make further decisions.
Missing points	In reproducible detection of endogenous analyte graph, fragment ion has less than 3 replicates on the specific day.	None	Check the reproducible detection of endogenous analyte graph to make further decisions.