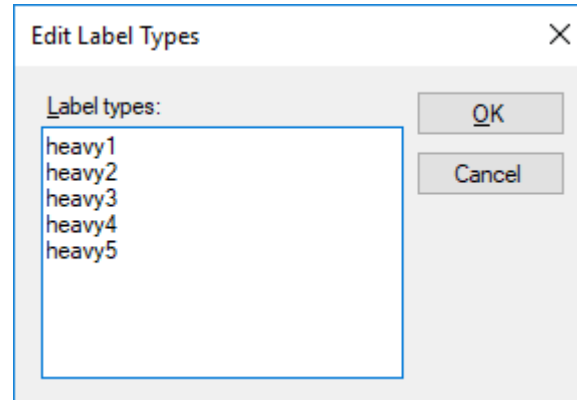
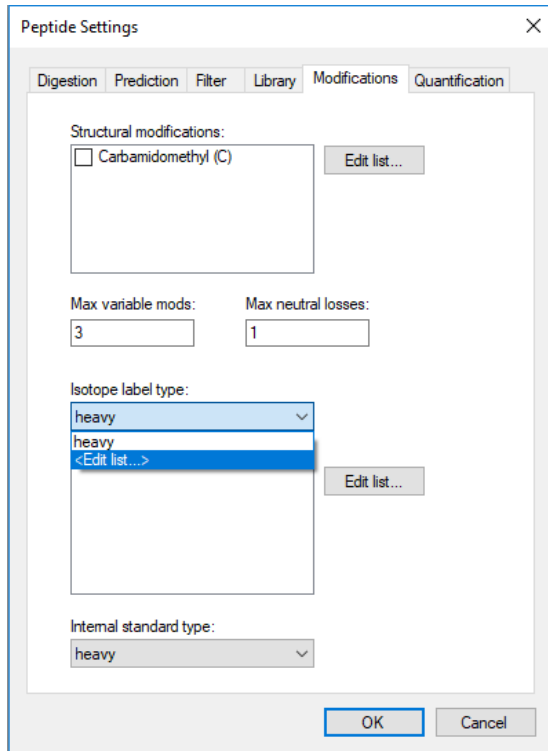


# Isotopolog Calibration Curve

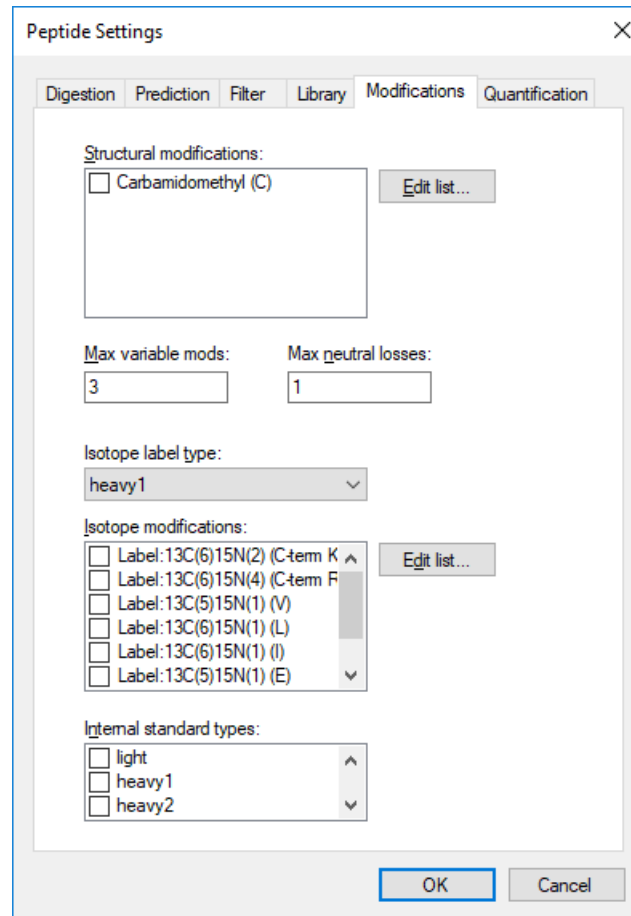
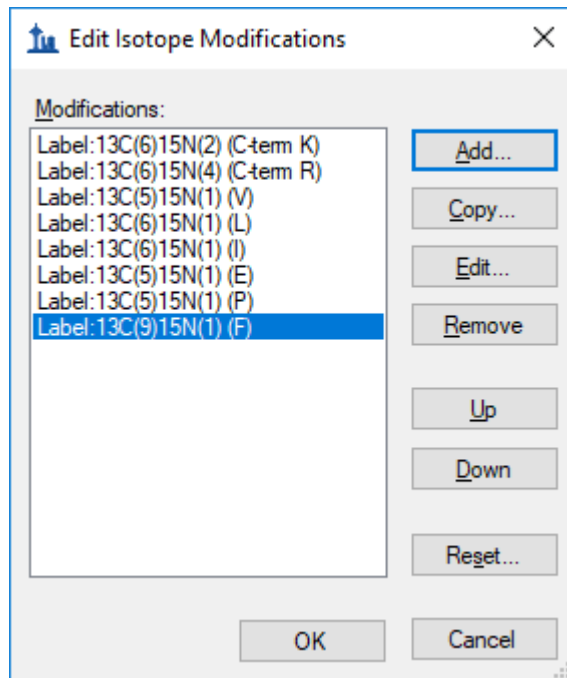
Nicholas Shulman

Sept 10, 2019

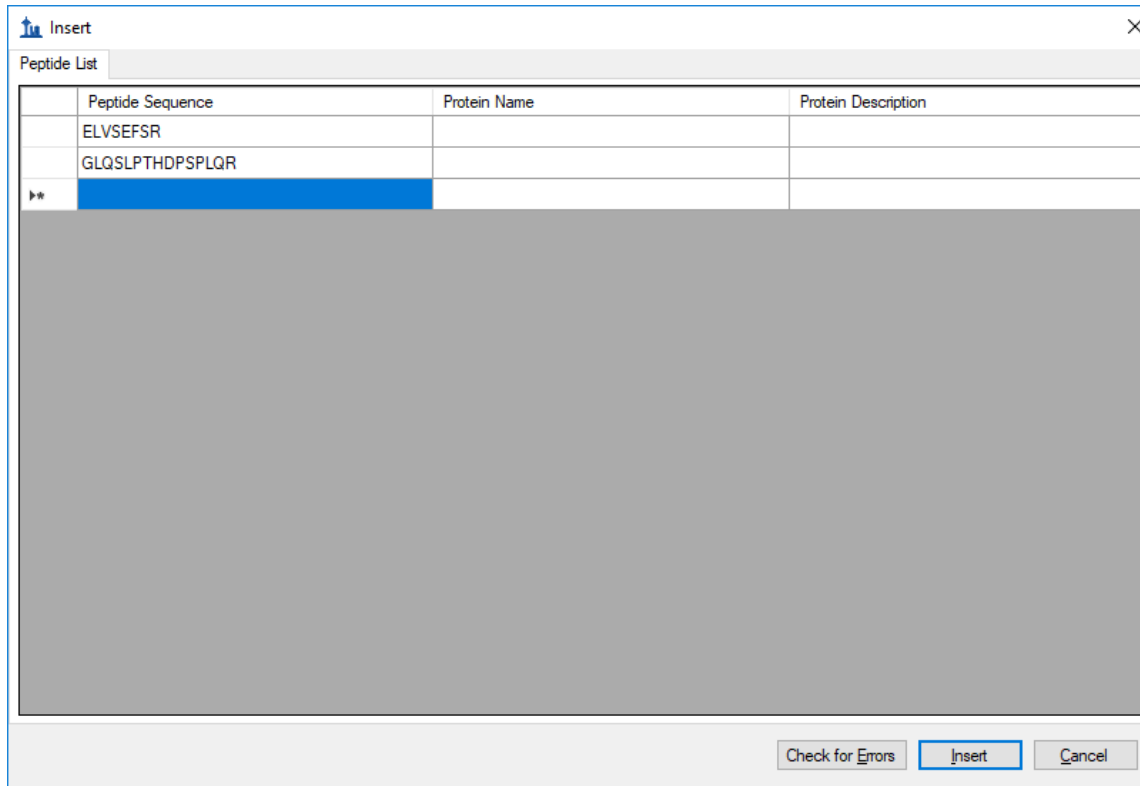
# Define 5 label types



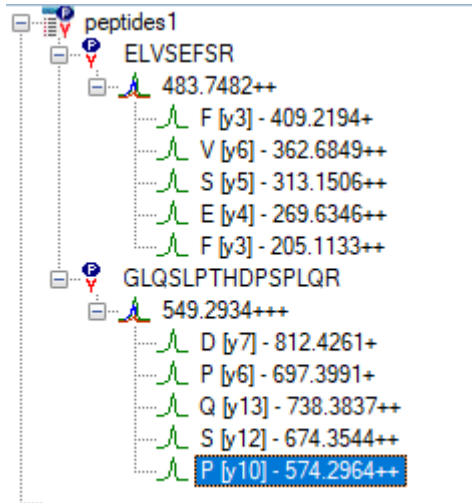
# Add necessary isotope modifications



# Edit > Insert Peptides



# Choose appropriate precursors and transitions



# Modify the peptides

**Edit Modifications** [Close]

Structural:		Isotope heavy1:	Isotope heavy2:	Isotope heavy3:	Isotope heavy4:	Isotope heavy5:
[Dropdown]	E	[Dropdown]	[Dropdown]	[Dropdown]	[Dropdown]	Label:13C(5)15N(1) [Dropdown]
[Dropdown]	L	[Dropdown]	[Dropdown]	Label:13C(6)15N(1) [Dropdown]	Label:13C(6)15N(1) [Dropdown]	Label:13C(6)15N(1) [Dropdown]
[Dropdown]	V	[Dropdown]	Label:13C(5)15N(1) [Dropdown]	Label:13C(5)15N(1) [Dropdown]	Label:13C(5)15N(1) [Dropdown]	Label:13C(5)15N(1) [Dropdown]
[Dropdown]	S	[Dropdown]	[Dropdown]	[Dropdown]	[Dropdown]	[Dropdown]
[Dropdown]	E	[Dropdown]	[Dropdown]	[Dropdown]	[Dropdown]	[Dropdown]
[Dropdown]	F	[Dropdown]	[Dropdown]	[Dropdown]	Label:13C(9)15N(1) [Dropdown]	Label:13C(9)15N(1) [Dropdown]
[Dropdown]	S	[Dropdown]	[Dropdown]	[Dropdown]	[Dropdown]	[Dropdown]
[Dropdown]	R	Label:13C(6)15N(4) [Dropdown]	Label:13C(6)15N(4) [Dropdown]	Label:13C(6)15N(4) [Dropdown]	Label:13C(6)15N(4) [Dropdown]	Label:13C(6)15N(4) [Dropdown]

[OK] [Cancel]  Create copy [Reset]

**ELVSEFSR Precursors**

- 483.7482++
- 488.7523++ (heavy1)
- 491.7592++ (heavy2)
- 495.2678++ (heavy3)
- 500.2814++ (heavy4)
- 503.2883++ (heavy5)
- 322.8345+++
- 326.1706+++ (heavy1)
- 328.1752+++ (heavy2)
- 330.5143+++ (heavy3)
- 333.8567+++ (heavy4)
- 335.8613+++ (heavy5)

503.2883++ (heavyb)

GLQSLPTI

549.293

D

P

Q

S

P

- Cut
- Copy
- Paste
- Delete
- Pick Children
- Set Standard Type
- Modify...

Edit Modifications

Structural:		Isotope heavy1:	Isotope heavy2:	Isotope heavy3:	Isotope heavy4:	Isotope heavy5:
	G					
	L					Label:13C(6)15N(1)
	Q					
	S					
	L				Label:13C(6)15N(1)	Label:13C(6)15N(1)
	P					
	T					
	H					
	D					
	P			Label:13C(5)15N(1)	Label:13C(5)15N(1)	Label:13C(5)15N(1)
	S					
	P					
	L		Label:13C(6)15N(1)	Label:13C(6)15N(1)	Label:13C(6)15N(1)	Label:13C(6)15N(1)
	Q					
	R	Label:13C(6)15N(4)	Label:13C(6)15N(4)	Label:13C(6)15N(4)	Label:13C(6)15N(4)	Label:13C(6)15N(4)

OK

Cancel

Create copy

Reset

GLQSLPTHDPSPQLQR Precursors

- 823.4365++
- 828.4406++ (heavy1)
- 831.9492++ (heavy2)
- 834.9561++ (heavy3)
- 838.4647++ (heavy4)
- 841.9732++ (heavy5)
- 549.2934+++
- 552.6295+++ (heavy1)
- 554.9685+++ (heavy2)
- 556.9731+++ (heavy3)
- 559.3122+++ (heavy4)
- 561.6513+++ (heavy5)

# Set the Precursor Concentrations

Customize Report

Report Name: PrecursorConcentrations

Columns Filter

- Proteins
  - Peptides
    - Precursors
      - Transitions
      - Precursor Results
        - Precursor Results Summary
        - Precursor Charge
        - Isotope Label Type
        - Precursor Neutral Mass
        - Transition Count
        - Precursor Ion Formula
        - Precursor Neutral Formula
        - Precursor Mz
        - Collision Energy
        - Declustering Potential
      - Modified Sequence
      - Explicit Compensation Voltage
      - Explicit Ion Mobility
      - Explicit Ion Mobility Units
      - Explicit Collisional Cross Section
      - Precursor Concentration**
      - Precursor Note
      - Library Name
      - Library Type
      - Library Score1
      - Library Score2
      - Library Score3
      - Is Decoy
      - Decoy Mz Shift
      - Auto Select Transitions
      - Precursor Locator
    - Peptide Results
    - Peptide Sequences

Pivot Replicate Name  Pivot Isotope Label

OK Cancel

Document Grid: PrecursorConcentrations

Reports 12 of 12 Export... Actions

	Precursor	Peptide	Isotope Label Type	Precursor Concentration
	<a href="#">483.7482++</a>	<a href="#">ELVSEFSR</a>	light	
	<a href="#">488.7523++ (he...</a>	<a href="#">ELVSEFSR</a>	heavy1	200
	<a href="#">491.7592++ (he...</a>	<a href="#">ELVSEFSR</a>	heavy2	100
	<a href="#">495.2678++ (he...</a>	<a href="#">ELVSEFSR</a>	heavy3	20
	<a href="#">500.2814++ (he...</a>	<a href="#">ELVSEFSR</a>	heavy4	10
	<a href="#">503.2883++ (he...</a>	<a href="#">ELVSEFSR</a>	heavy5	2
	<a href="#">549.2934+++</a>	<a href="#">GLQSLPTHDP...</a>	light	
	<a href="#">552.6295+++ (h...</a>	<a href="#">GLQSLPTHDP...</a>	heavy1	200
	<a href="#">554.9685+++ (h...</a>	<a href="#">GLQSLPTHDP...</a>	heavy2	100
	<a href="#">556.9731+++ (h...</a>	<a href="#">GLQSLPTHDP...</a>	heavy3	20
	<a href="#">559.3122+++ (h...</a>	<a href="#">GLQSLPTHDP...</a>	heavy4	10
	<a href="#">561.6513+++ (h...</a>	<a href="#">GLQSLPTHDP...</a>	heavy5	2



# Set Quantification Regression fit

Peptide Settings

Digestion Prediction Filter Library Modifications Quantification

Regression fit:  
Linear

Normalization method:  
None

Regression weighting:  
None

MS level  
All

Units

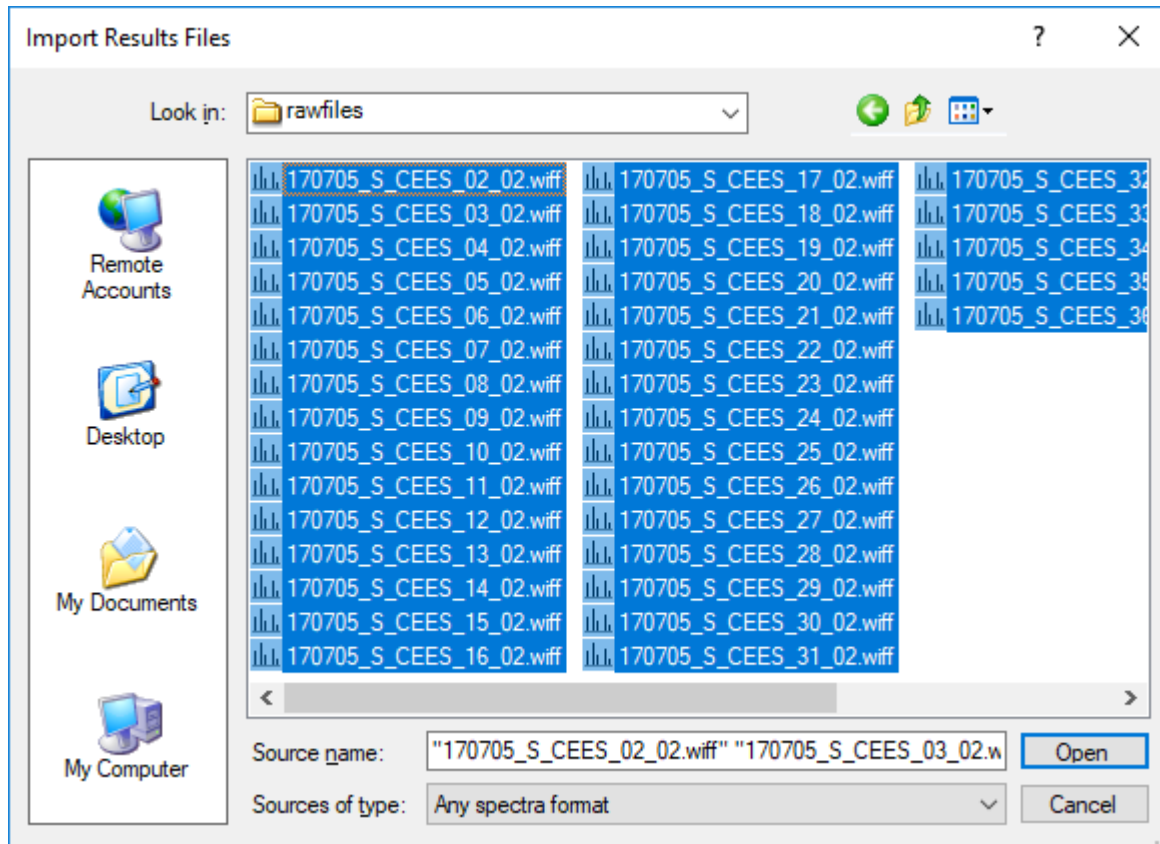
Figures of merit

Max LOQ bias:  %      Max LOQ CV:  %

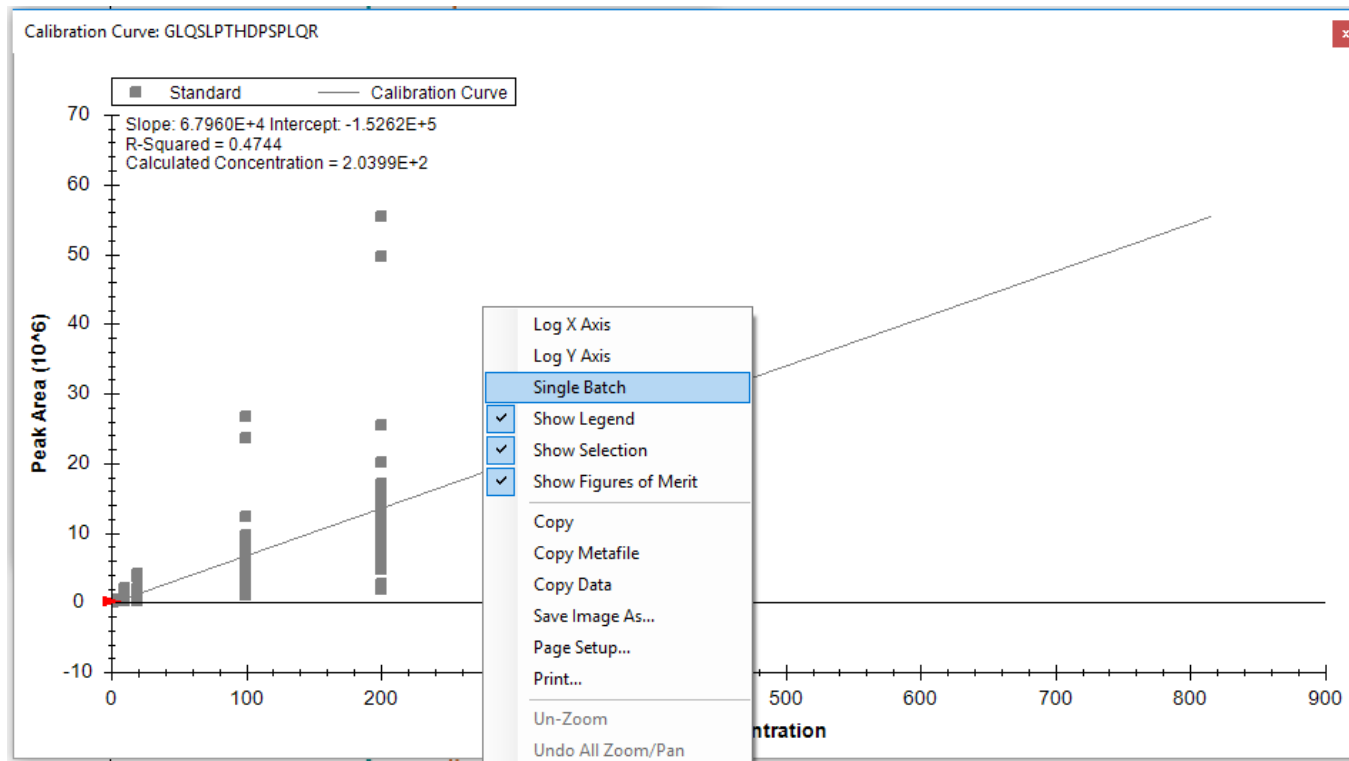
Calculate LOD by:  
None

OK Cancel

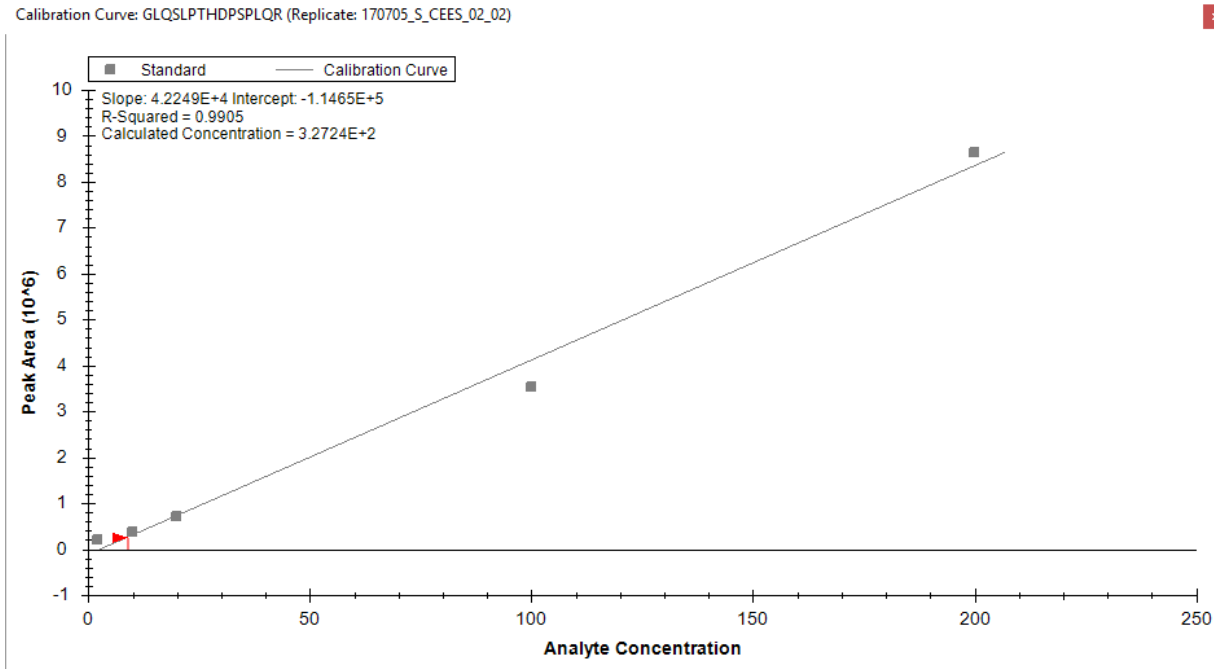
# Import Results



# View Calibration Curve, and choose “Single Batch”



# See calibration curve



Things that need to be fixed:

1. Any precursors for which we have not specified a concentration for should be drawn on the graph as “Unknown” instead of “Standard”.
2. The Calculated Concentration should reflect the currently selected precursor in the Targets tree. (Currently it shows a concentration calculated from the nonsensical sum of all of the transition areas across all precursors.)

