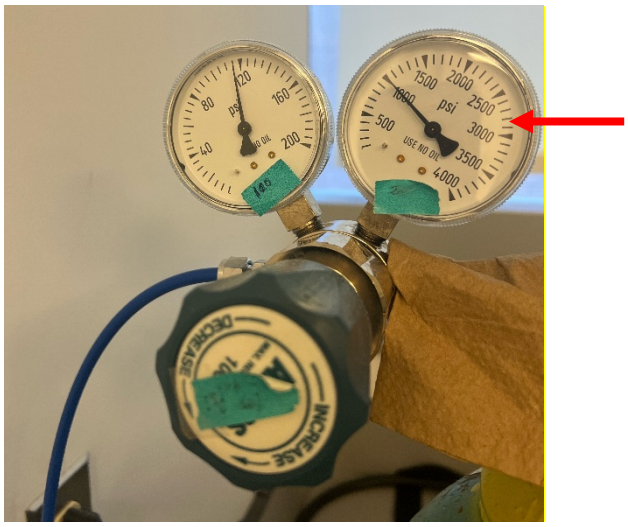
Body Fluid Identification by Proteomic Mass Spectrometry - Liquid Chromatography & Mass Spectrometer Processing

1 Purpose

1.1 Samples are run through liquid chromatography to separate peptide markers. Marker peptides are identified by mass spectrometry.

2 Check Before Starting

2.1 Check the Pure Air tank. If pressure on the large gauge (right gauge) is ≤ 300 psi, **do not proceed**. Contact the Proteomics Team to change the Pure Air tank if it is less than 300 psi.



2.2 Check the LC/MS instrument to see how many batches are in the queue. Ensure reagents are sufficient for all batches in queue. Assume all batches are full, i.e. consist of 82 runs.

2.2.1 To calculate how much reagent volumes you need to proceed, count the number of batches ahead of you, add your batch and add 1 (safety batch) to the total. For example, if there are three batches ahead of you add: 3 (batches ahead of you) + 1 (your batch) + 1 (safety batch) = 5.

2.2.2 From the table below you can calculate the volume of each reagent you need by multiplying the number of batches you calculated above (2.2.1) by the volumes of each reagent in the table. For example, above you would need 500 ml of 50% Methanol, 500 ml of Loading Solution A, 400 ml of Bottle A (HPLC water) and 300 ml of Bottle B (ACN).

Now, check the bottles. If you are unsure if there is enough volume of a reagent, contact the Proteomics Team.

LC Reagent	Volume Needed /1 Full Batch
50% Methanol	100 mL
Loading Solution Phase A	100 mL
Bottle A (HPLC water)	80 mL
Bottles B (ACN)	60 mL

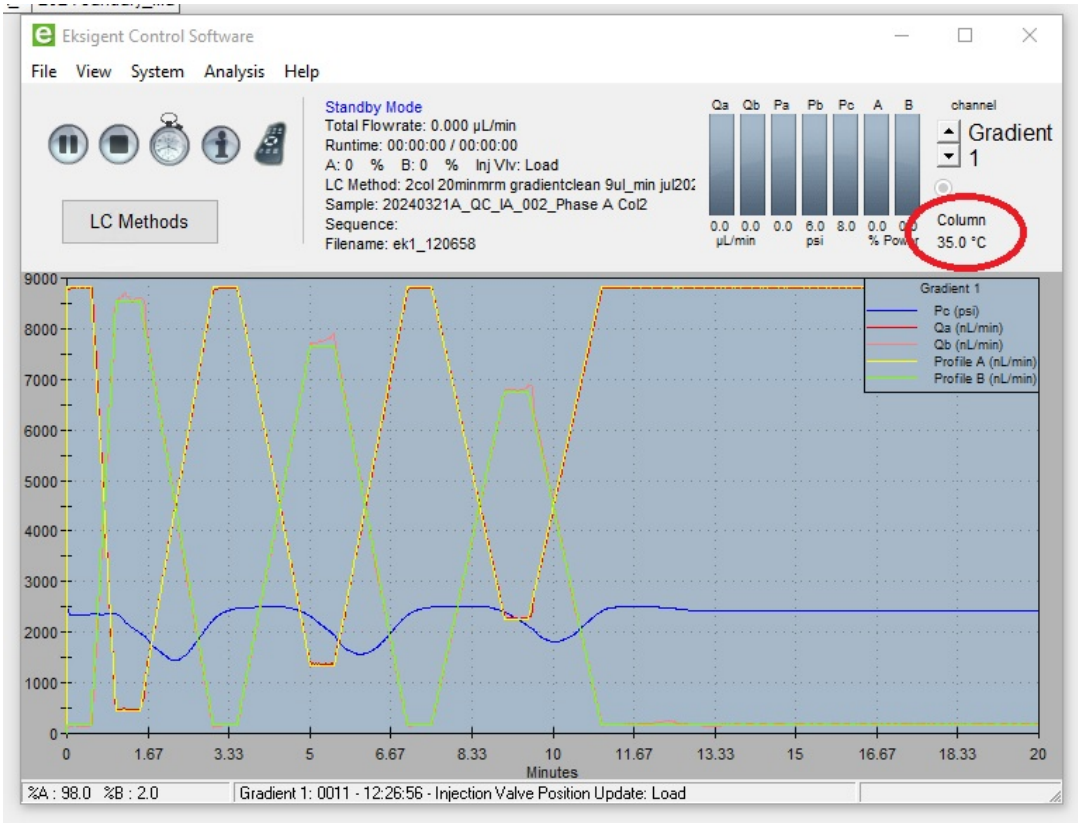
2.2.3 Check to see if there is a sufficient volume of PCM for your batch in the instrument tray.

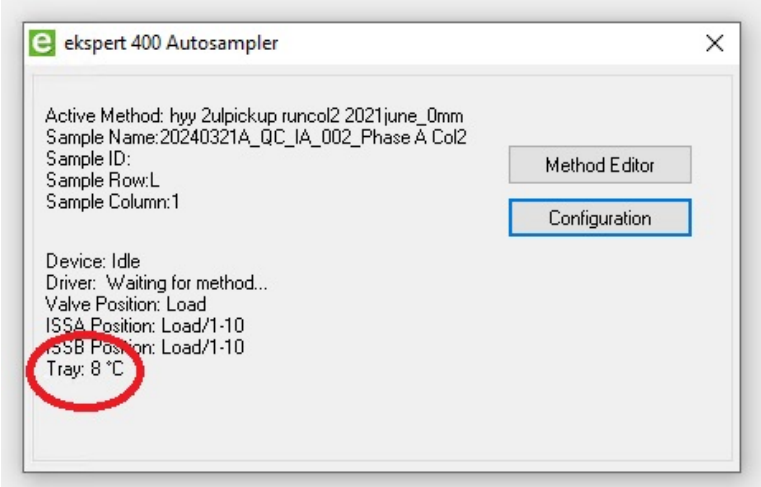
2.2.4 To calculate how much reagent volume you need to proceed, count the number of batches ahead of you and add your batch to the total. For example, if there are three batches ahead of you add: 3 (batches ahead of you) + 1 (your batch) = 4.

2.2.5 Multiply the number of batches calculated above (2.2.4) by 18 µl. For example, if you calculated four above, multiply by 18 µl = 72 µl.

2.2.6 To determine if there is enough PCM available, pipette the calculated volume using water into a new LC vial. Remove the PCM vial from the LC tray and compare it to the volume of water you pipetted into the new vial. If there is sufficient PCM in the PCM vial, replace the vial in the LC tray and proceed. If it is insufficient or you are unsure if there is enough PCM, contact the Proteomics Team.

2.3 Check Column and Tray temperatures as shown in figures below. Column temperature should be ~35°C and can be seen only on Gradient 1 window (shown below). Tray temperature should be ~8°C. If temperatures are not within ± 2oC of recommended temperature, contact the Proteomics Group.





2.4 For **samples that need to be rerun**, follow the instructions above (2.1 to 2.4 above) and then proceed to section 3.10.

3 Liquid Chromatography – Mass Spectrometer Analysis Procedure

3.1 Retrieve the following reagents:

Cytochrome C (8 pmol/ µl) at -80°C
PCM Standard
Phase A
Acetone
Acetonitrile
Isopropanol

3.2 Prepare a working solution of Cytochrome C Master Mix sufficient to resuspend all samples (see 3.3)Work on ice or in a -20°C cold tube rack.

3.3 Input into LIMS the number of regular quant samples + the number of low quant samples divided by eight. If the number is a fraction round up to the next integer. For example, if you have five regular quants and three low quant samples you have 5 + 3/8 = 5.38, therefore input 6 into LIMS.

Cytochrome C Master Mix Reagent Ratios (multiply volumes by the number of samples to be resuspended)	
Reagent	**Volume**
Phase A	93 µl
Cytochrome C (8 pmol/µl)	1.45 µl
Total volume:	94.45 µl

3.4 Resuspend samples as follows:

3.5 **REGULAR Samples**: add 94.45µl of Cytochrome C Master Mix (8 pmol/µl) to reconstitute peptides.

3.6 **LOW Concentration Samples**: add 10 µl of Cytochrome C Master Mix solution to reconstitute peptides.

3.7 Vortex.

3.8 Place in refrigerated centrifuge at 4°C and spin at 18,000 g (RCF) for 30 minutes. Record instrument and temperature in LIMS.

3.9 Pipette supernatant into LC Vial (avoid pellet if present) and store at 4°C.

3.10 Prepare the LC cleaning solution to be run alongside samples and controls:

LC Cleaning Solution	
Reagent	**Volume**
Acetone	2 µl
Acetonitrile	9 µl
Isopropanol	9 µl
Total Volume:	**20 µl /sample**

3.11 Place samples, controls, and prepared cleaning solution in Eksigent refrigerated auto sampler and fill out location of each vial in the input load plate on LIMS.

3.12 Fill in LIMS output data entry sheet and select the appropriate Acquistion Method for the sample type.

Sample Type	AcqMethod
PepCalMix, Phase A	Col1 PepCalMix Col2 PepCalMix
Cleaning Sol	Col1 Cleaning Col2 Cleaning
ENeg, Ext Pos controls, Unknowns	Col1 Unknown Col2 Unknown
Reruns (High)	Col1 Unknown Hi Col2 Unknown Hi

3.12.1 Your batch should not end on a “col 1” method. If your batch does end in a “col 1” method, add an additional phase A line at the end of the batch with a “col 2” method.

3.13 Output Plate Name should be the date (YYYYMMDD), followed by a letter identifying batch order (e.g. A, B, C, etc.) an underscore, followed by analyst initials.

3.14 Ensure the output samples are in the desired injection order before loading plate (in its default - vertical mode)

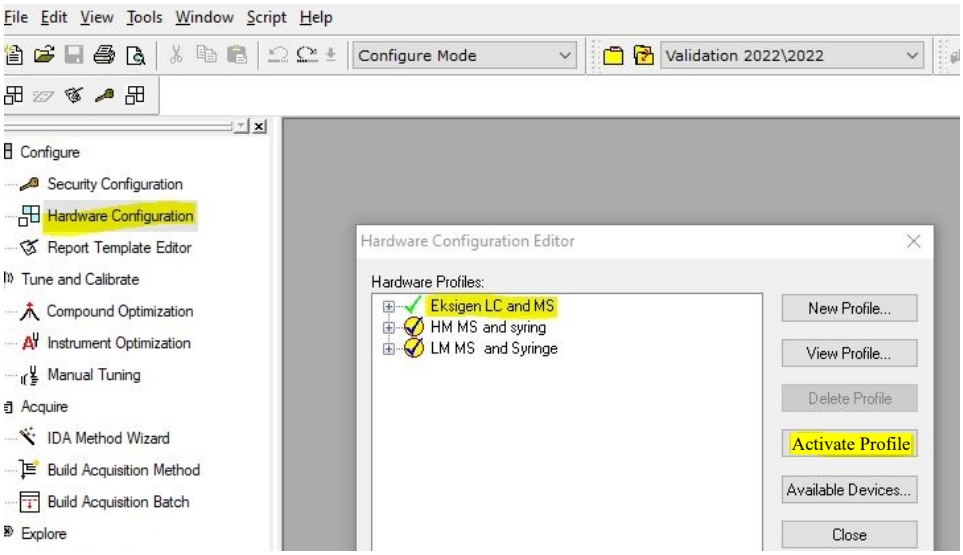
3.14.1 Positive controls should be run after unknown samples when creating batch.

3.15 Export sample batch from the output plate in LIMS.

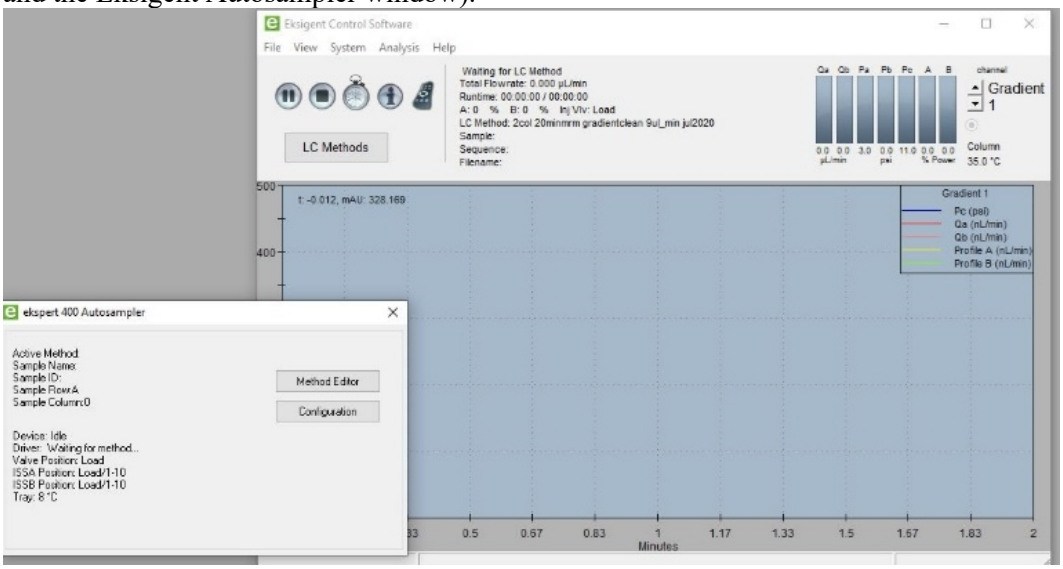
3.16 Open LIMS created batch excel sheet in the LIMS LCMS folder and confirm it is as desired and save document as .txt format.

3.17 Open analyst software.

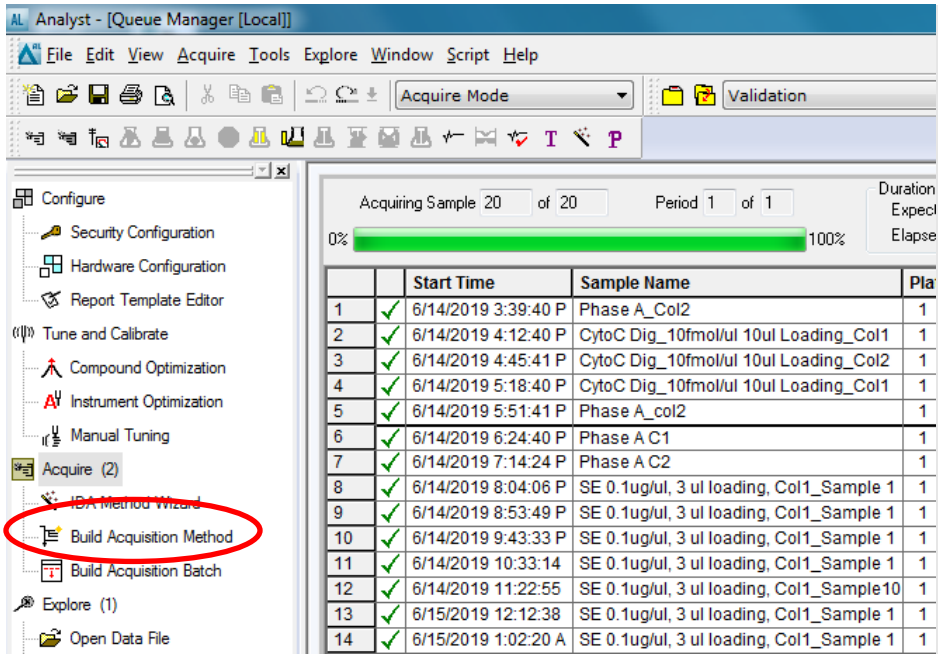
3.18 Double click Hardware Configuration → Eksigent LC and MS → Activate Profile



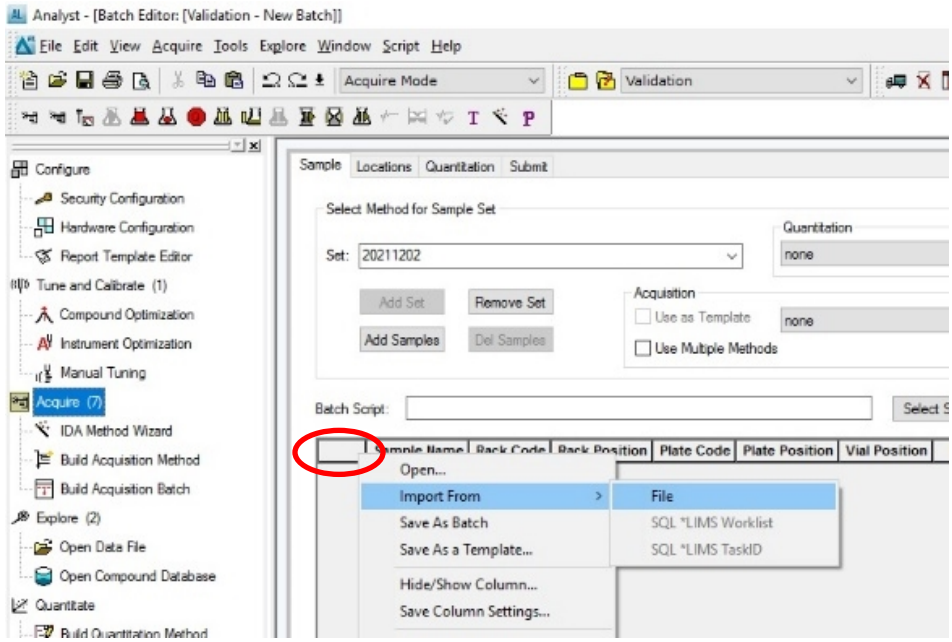
3.19 Ensure that Eksigent windows is open simultaneously (both Eksigent Control Software window and the Eksigent Autosampler window).



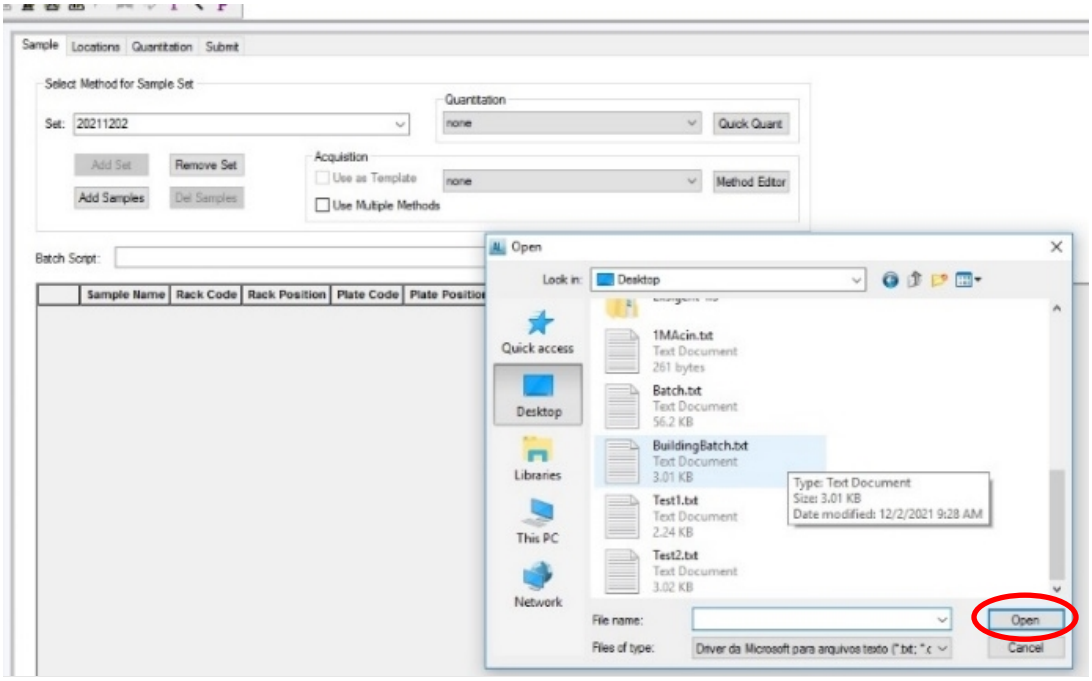
3.20 Double click Build Acquisition Batch



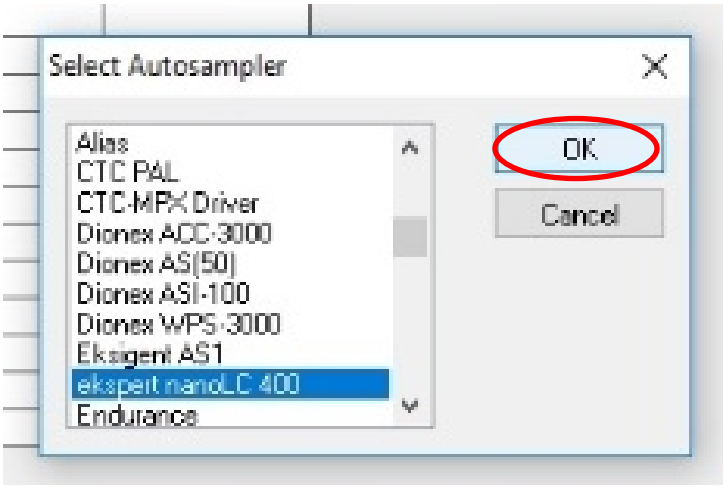
3.21 Right click to import acquisition batch.



3.22 Select .txt batch list exported from LIMS. Click open.

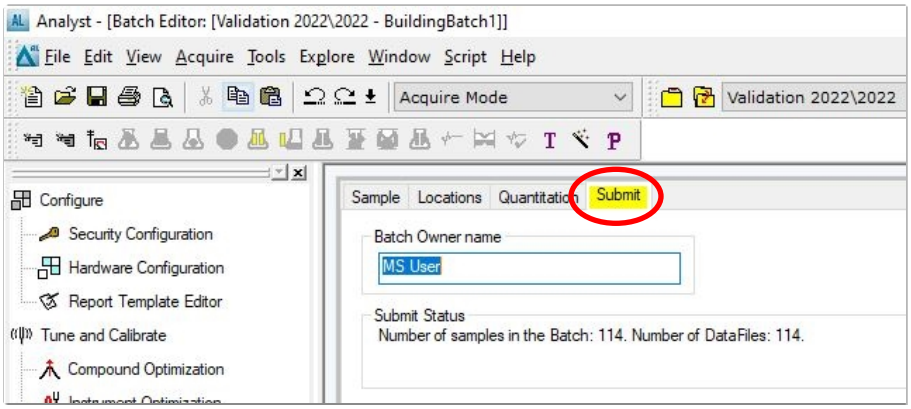


3.23 Select autosampler (**ekspert nanoLC 400).** Click OK.

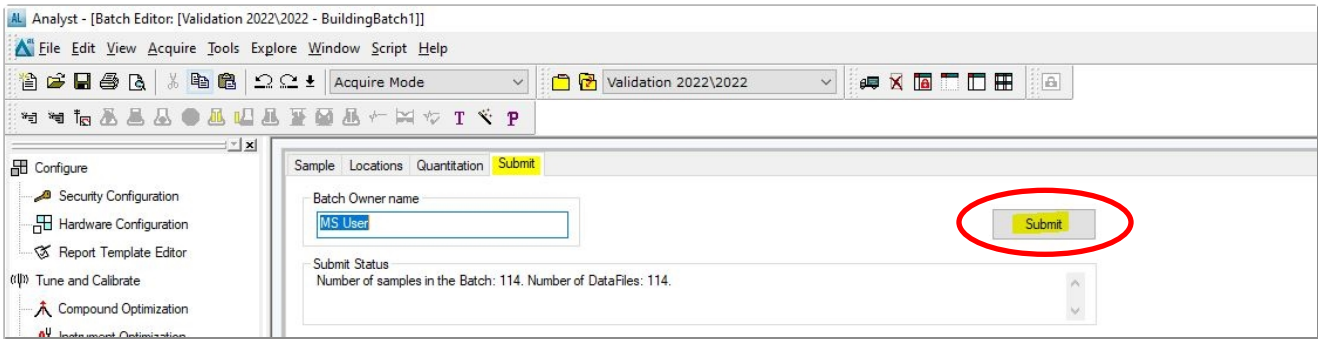


3.24 **Label and Position WITNESS**: Have a witness verify the selected autosampler, batch sample names, methods, and tube positions in autosampler match that in Vial Position column.

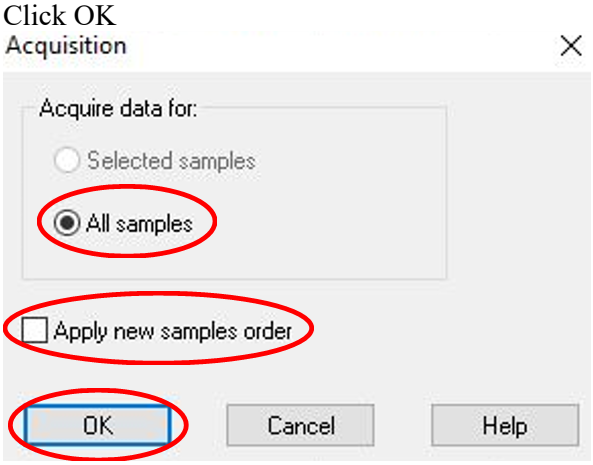
3.25 Click on Submit tab when sample list is ready.



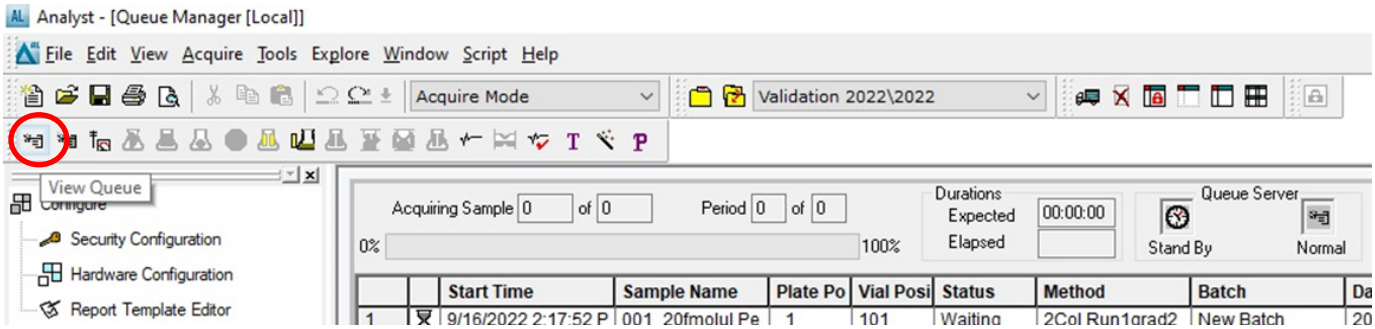
3.26 Click Submit button.



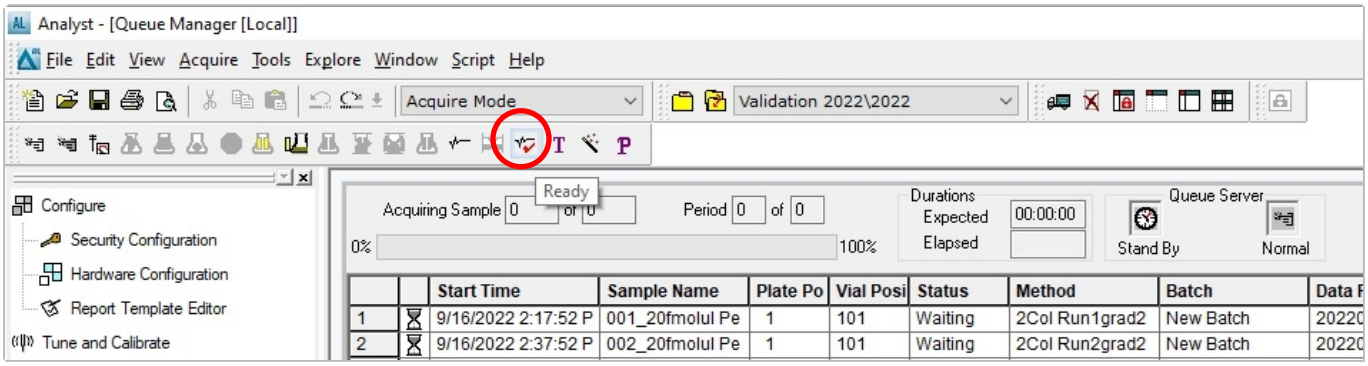
3.27 Ensure "All samples" is selected and that the box for "apply new samples order" is unchecked. Click OK



3.28 Click Queue button and double check all samples were submitted.



3.29 Click Ready button.



3.30 Click Start sample button. The Eksigent LC and 6500 MS will process all samples.