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

1 Purpose

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

2 Liquid Chromatography – Mass Spectrometer Analysis Procedure

2.1 Retrieve the following reagents:

Cytochrome C (1pmol/ µl) at -80°C
PCM Standard
Phase A
Acetone
Acetonitrile
Isopropanol

2.2 If batch contains low concentration samples, make Low Conc resuspension mixture in a new 1.5 mL microcentrifuge tube. Work on ice or in a -20°C cold tube rack.

Low Conc. Resuspension Mixture – Enough for 9 Low Conc. Samples	
Reagent	**Volume**
Phase A	93 µl
Cytochrome C (1 pmol/µl)	0.94 µl
Total volume:	93.94 µl

2.3 Resuspend samples as follows:

2.3.1 **REGULAR samples**: add 93µl Phase A and 0.94 µl Cytochrome C (1 pmol/µl) to reconstitute peptides. **LIMS can do the math for total of samples**

2.3.2 **LOW Concentration Samples**: add 10 µl of Low Conc resuspension mixture to reconstitute peptides.

2.4 Vortex.

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

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

2.7 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

2.8 Open "Batch Template" excel sheet located on desktop and save as date (YYYYMMDD), followed by a letter identifying batch order (e.g., A, B, C, etc.), an underscore, followed initials in "Batches" folder located on desktop.

2.9 To build batch fill in all highlighted areas as follows.

2.9.1 Sample Name: follow "Guidelines for Molecular Serology Body Fluids Proteomics" protocol

2.9.2 AcqMethod: Choose from drop down options. See table below.

Sample Type	AcqMethod
PepCalMix, Phase A	2Col Run1grad2 2ulLoading PepCalMix_2021July0mm.dam 2Col Run2grad2 2ulLoading PepCalMix_2021July0mm.dam
Cleaning Sol	2Col Run1grad2 cleaning SE SA B CytoC DI HISv2 2021Apr.dam 2Col Run2grad2 cleaning SE SA B CytoC DI HISv2 2021Apr.dam
ENeg, Ext Pos controls, Unknowns	2Col Run1grad2 2ulLoadingSE SA B CytoC DI Scheduled IA_2021Apr.dam 2Col Run2grad2 2ulLoadingSE SA B CytoC DI Scheduled IA 2021Apr.dam
Reruns (High)	2Col Run1grad2 4ulLoadingSE SA B CytoC DI Scheduled IA_October2022.dam 2Col Run2grad2 4ulLoadingSE SA B CytoC DI Scheduled IA_October2022.dam

2.9.3 VialPos place samples, controls, and prepared cleaning solution in Eksigent refrigerated auto sampler and fill out position accordingly.

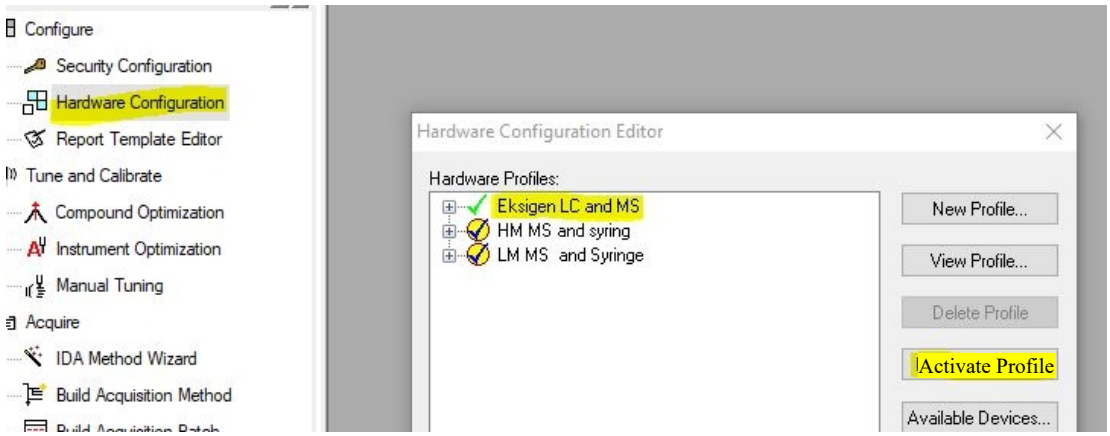
2.9.4 SetName: date (YYYYMMDD), followed by a letter identifying batch order (e.g., A, B, C, etc.), an underscore, followed initials

2.9.5 OutputFile: Copy and paste Sample name

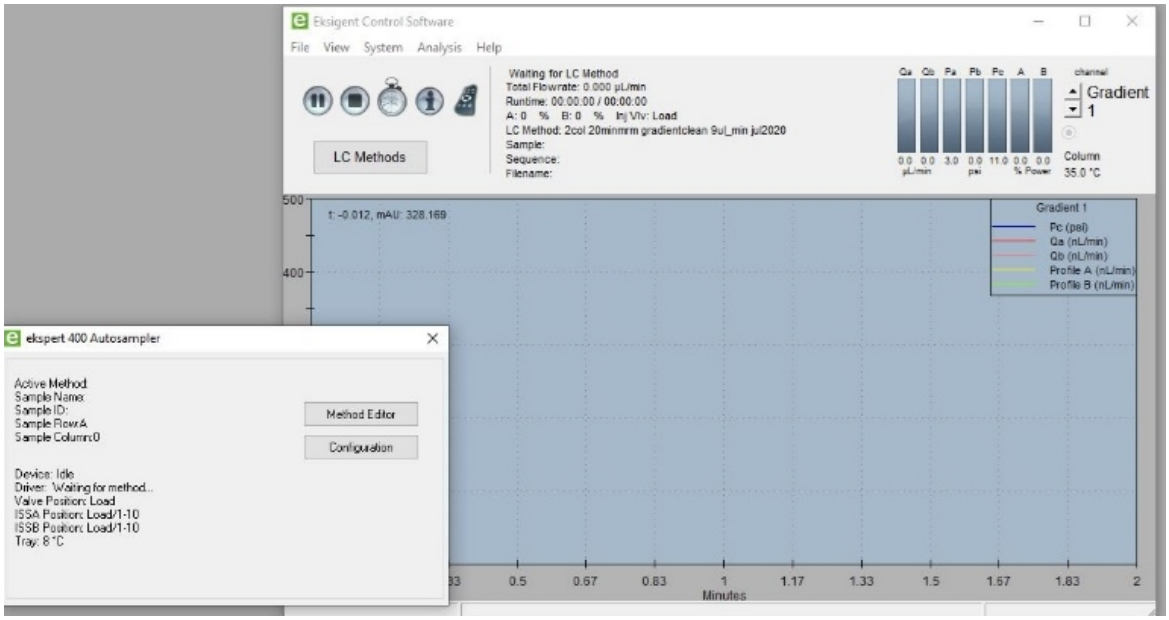
2.10 Save Excel Sheet as Text (tab delimited) file in "Batches" folder located on the desktop.

2.11 Open analyst software.

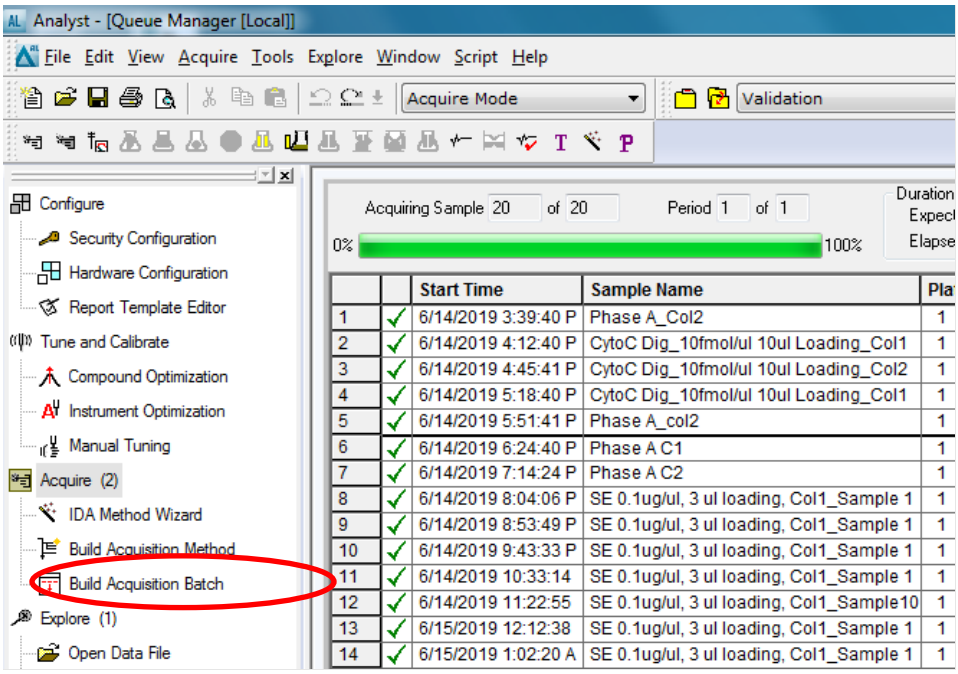
2.12 Double click Hardware Configuration → Eksigent LC and MS → Activate Profile



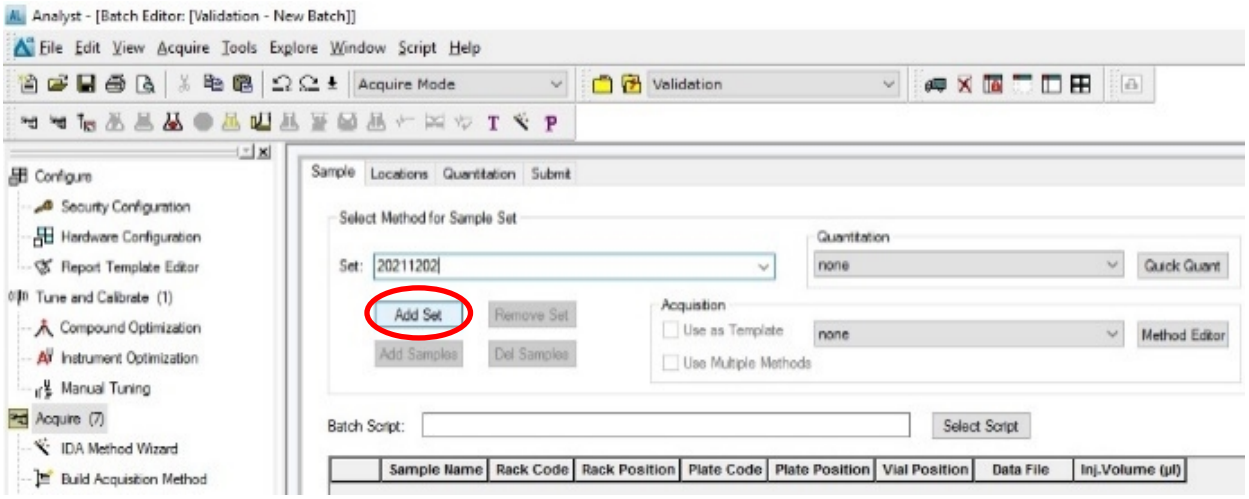
2.13 Ensure that Eksigent windows is open simultaneously (both Eksigent Control Software window and the Eksigent Autosampler window).



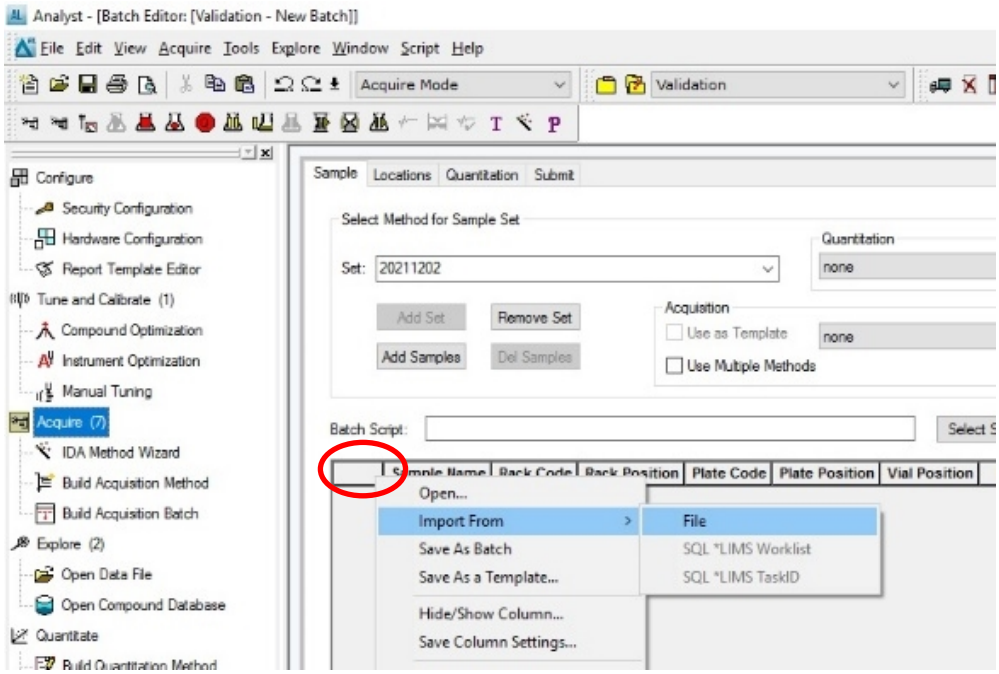
2.14 Double click Build Acquisition Batch



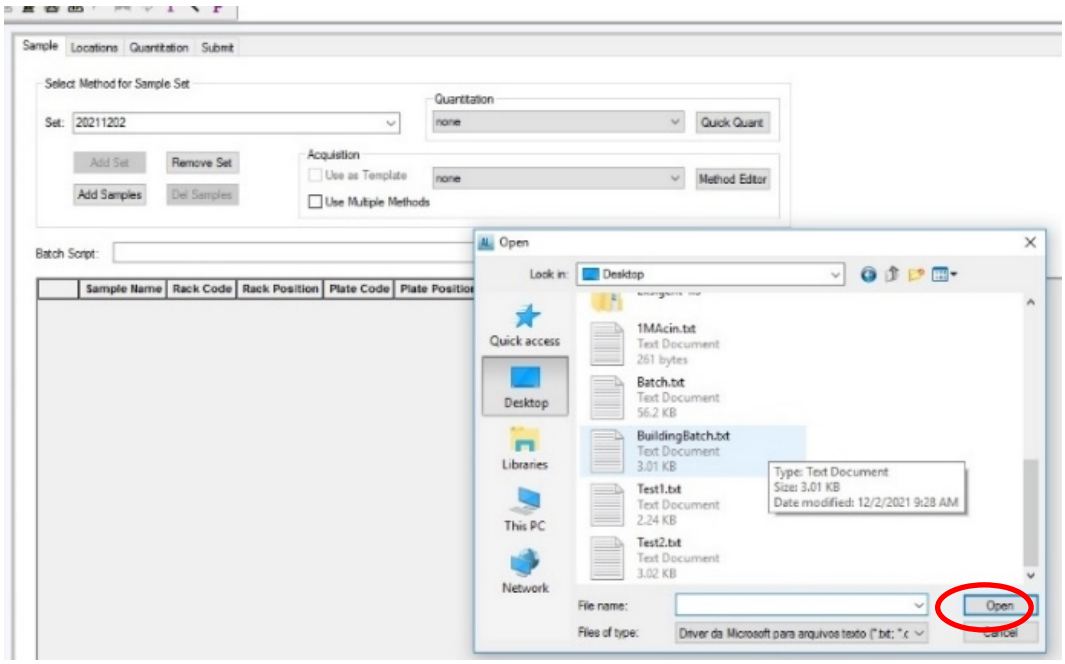
2.15 Click Add Set.



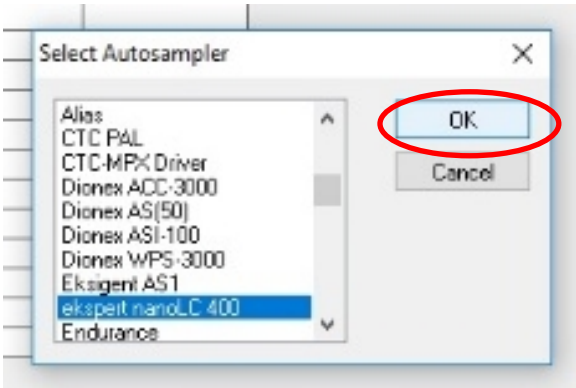
2.16 Right click to import acquisition batch.



2.17 Select batch list. Click open.

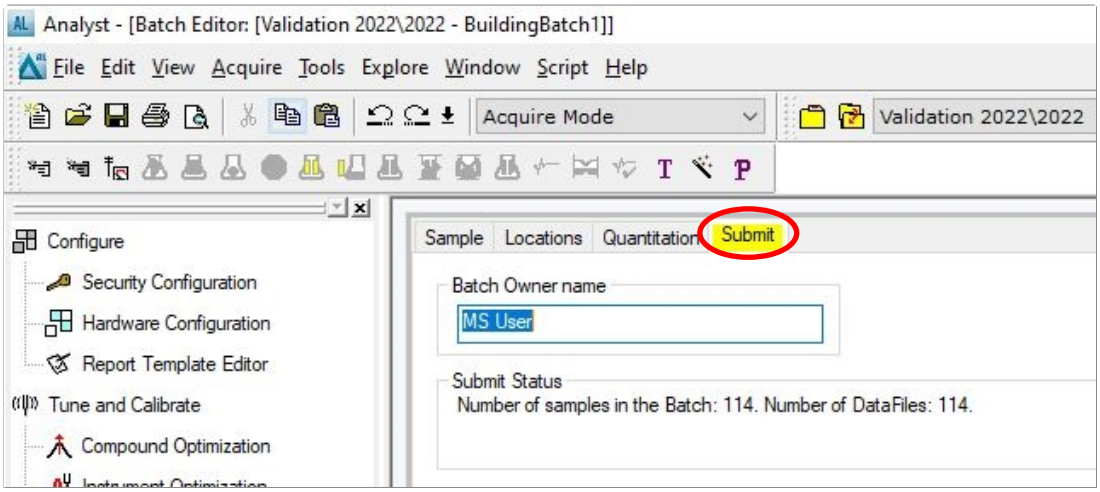


2.18 Select autosampler (**ekspert nanoLC 400).** Click OK.

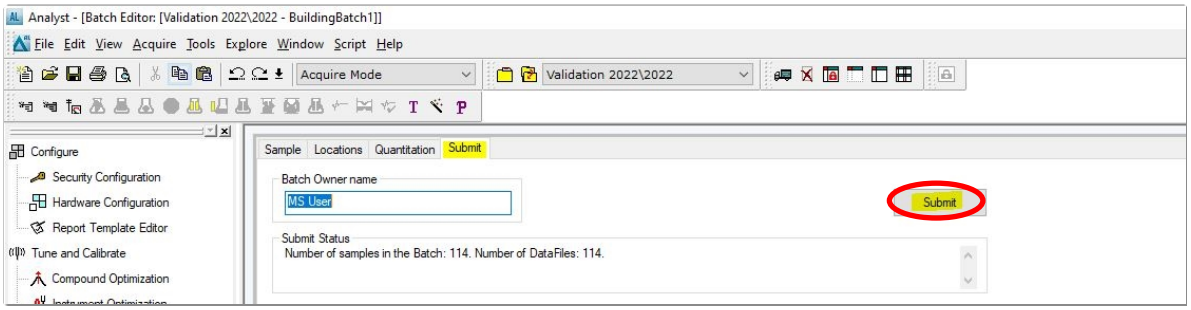


2.19 **Label and Position WITNESS**: Have a witness verify the selected autosampler, batch sample names, methods, and tube positions in autosampler match that in Vial Position column.

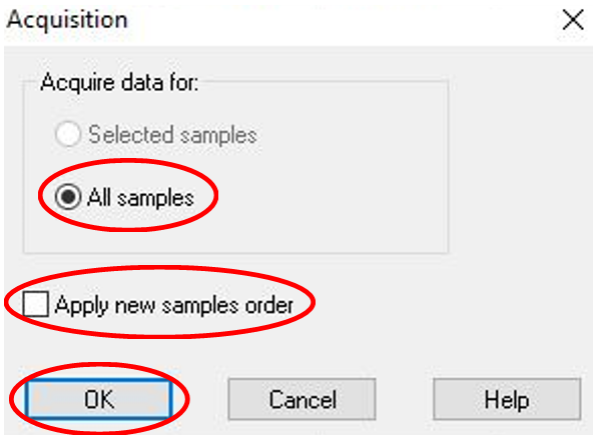
2.20 Click on Submit tab when sample list is ready.



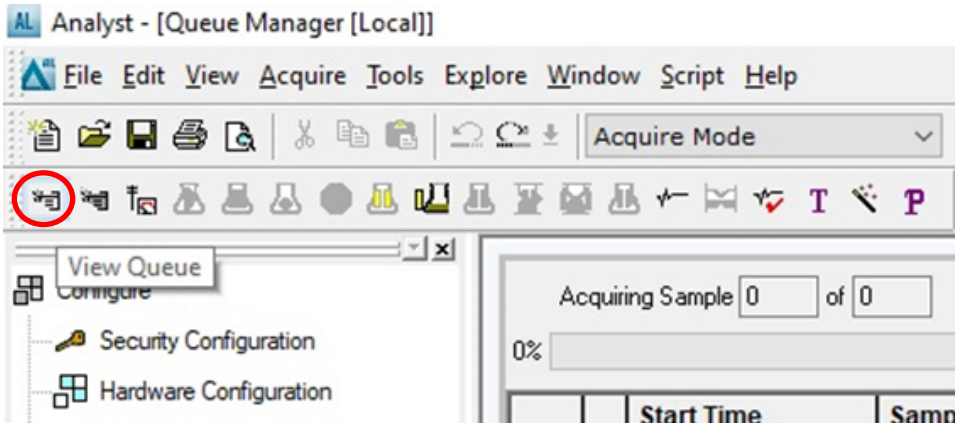
2.21 Click Submit button.



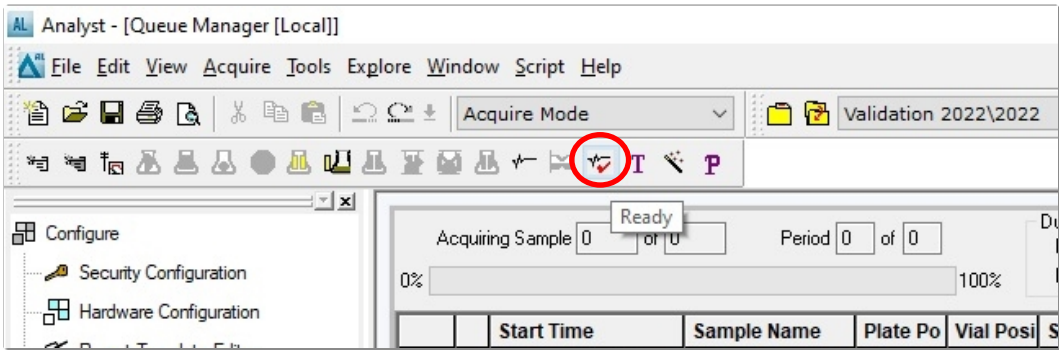
2.22 Ensure "All samples" is selected and that box is unchecked. Click OK.



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



2.24 Click Ready button.



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