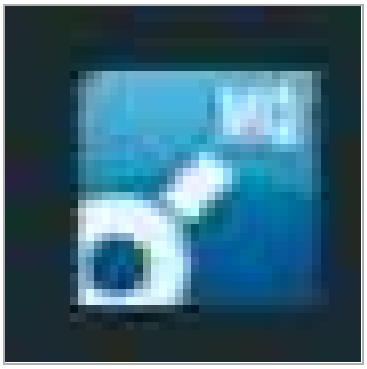

JUL 23, 2023

Using MultiQuant and Excel software to evaluate and report multi-analyte targeted LC-MS/MS data

Natalie ZM Homer[1]

[1]University of Edinburgh

Natalie ZM Homer
University of Edinburgh

ABSTRACT

This protocol describes how to evaluate and report targeted LC-MS/MS data that has been collected in Analyst software on AB Sciex mass spectrometers. It describes using a Processing Method built in MultiQuant to evaluate a targeted analysis data file (*.wiff) acquired in Analyst software. The LC-MS/MS data file must contain a calibration curve and unknowns analysed as a batch using the same Acquisition method in Analyst. The results from MultiQuant are then transferred to Microsoft Excel to filter and summarise the calculated amounts of the analytes of interest in the samples as a final result.

GUIDELINES

Use methods that have well defined calibration protocols, method details (masses, analyte retention times) and well defined calibration ranges and known units for the calibration ranges. MultiQuant is only successful if the calibration range is well defined and the calibration units are known. QCs used across multiple batches are best included in each batch, and give confidence to the results.

MATERIALS

- Analyst software package (AB Sciex)
- MultiQuant software license (AB Sciex)
- Excel software package (Microsoft)
- Details of the method used to acquire data in Analyst - including mass transitions of analytes and internal standards, retention times, names of analytes, calibration ranges and calibration units and volume (or mass) of sample extracted.

BEFORE START INSTRUCTIONS

Acquire a dataset in Analyst and use the .wiff file in MultiQuant

OPEN ACCESS

Protocol Citation: Natalie ZM Homer 2023. Using MultiQuant and Excel software to evaluate and report multi-analyte targeted LC-MS/MS data. **protocols.io** https://protocols.io/view/using-multiquant-and-excel-software-to-evaluate-an-cxmrxk56

License: This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: Jul 23, 2023

Last Modified: Jul 23, 2023

PROTOCOL integer ID: 85393

Create a processing method in MultiQuant using an acquire...

15m

1 Using a data file created in Analyst, with known analyte mass transitions and retention times, build a Processing method that integrates peaks with sufficient time window to integrate the peaks but not too wide that it chooses incorrect peaks. Save the processing file name in

MultiQuant and include details in the name that describe the column used and analytes measured as these are critical method details.

Using MultiQuant to evaluate Analyst acquired LC-MS/MS d... 30m

2 Create a result file in MultiQuant 3.0.3 by opening the software, selecting an LC-MS/MS data file acquired in Analyst software, that has been acquired following extraction using a protocol with carefully designed calibration curve and known calibration units. Select some or all of the samples in the batch of the file, selecting the processing method (as above) and allow the software to evaluate the data, before defining calibration ranges and assessing integration of peaks. To do this, follow these steps 1m

2.1 Select a data file (*.wiff) of a batch of data that includes calibration standards and unknowns, collected in Analyst and select all samples and standards or a selection. Click Next. 2m

2.2 Select the processing method created in MultiQuant that is appropriate to the data file (MultiQuant quantitation method file). Click Next. 1m

2.3 Save the file (a result file) with the file naming convention of the data file (yyymmdd_Exxxx_Analyte_initials) before beginning to process the data. 1m

2.4 In MultiQuant in the batch of samples define sample type - Blanks, Double Blanks, Standards, QCs and Unknowns (samples) and Solvents. Add known amounts to standards and QCs according to the Etraction protocol. 5m

2.5 Insert standard curve values and QC values in 'actual amount' column, according to the calibration table and QC table. 5m

2.6 Check all peaks have integrated and that there are peak areas for each analyte - reassign retention time if it is not quite picking the peak and use Metric plots to assess peak area and retention time to ensure correct peaks have been integrated 10m

Metric plot selected along with Calibration curve and identified peak.

2.7 Check calibration curves for accuracy (<20%) and exclude those points that are outwith. Ensure there are a minmum of 6 points in each calibration curve and that they have a regression coefficient R>0.99 — 5m

Transferring MultiQuant alphanumeric data into Excel to su... — 57m

3 Open Microsoft Excel and create a new spreadsheet and name it using the same file naming convention as used for the MultiQuant result file and name the first tab ‘Raw Data’ — 5m

4 Copy the alphanumeric data of all peak areas and calculated amounts from MultiQuant result file into Microsoft Excel — 2m

5 On the 'Raw Data' tab select 'filter' in the top menu of Excle and select the first analyte in the list (alphabetical). Copy this analyte excel data into the new tab and rename the tab with the analyte name. Repeat with all analytes until you have an excel spreadsheet with the following tabs - Raw Data; Analyte 1; Analyte 2, etc.... — 15m

6 In each of the separate tabs for the analytes - go into the tabs and rename the 'calculated amount' column on the Analyte tab to '[Analyte name] ng' and rename in the 2nd Analyte tab to '[2nd Analyte name] ng' (use the real analyte name!) — 10m

7 Create a new tab and name it 'Summary'. Copy the first three columns from the first Analyte tab, which contains sample details and calibration level details. Paste into the new tab. Then return to — 10m

the Analyte tab and copy the '[Analyte name] ng' column. Repeat this copying of the [Analyte name] ng column until all analytes in the MultiQaunt file and hence the excel file, have been copied. This results in a Summary table that is the major results of the analysis in terms of concentration

8 If the calibration curve units are ng and the sample extracted has been a volume, add a column ahead of all Analyte concentrations, include the volume extracted for each sample and then use this column to calculate the ng/mL amount by dividing the ‘calculated amount’ by the volume (uL) and multiply by 1000 to give the ng/mL amount of each analyte. 5m

8.1 If the sample analysed was urine, it is necessary to correct the amount of analyte detected by the creatinine concentration (measured elsewhere) OR if the 24 hour volume of urine is known this can be used to express the amount of analyte/day by calculating as a ratio of the extracted volume/day volume. 5m

9 If the calibration curve units are ng and the sample extracted has been a mass of tissue, add a column ahead of all Analyte concentrations, include the volume extracted for each sample and then use this column to calculate the ng/g. amount by dividing the ‘calculated amount’ by the mass in g to give the ng/g amount of each analyte 5m