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

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We use this protocol and it's working

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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.

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 of sample extracted.

BEFORE START INSTRUCTIONS

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

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

1 Create a new result file in MultiQuant 3.0.3 by opening the software, selecting a data file, selecting some or all of the samples in the batch of the file, selecting a processing method and allowing the software to evaluate the data, before defining calibration ranges and assessing

integration of peaks. To do this, do the following.

1.1 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.

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

1.3 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.

1.4 In MultiQuant in the batch of samples define sample type - Blanks, Double Blanks, Standards, Unknowns (samples) and Solvents.

1.5 Check all peaks have integrated for each analyte - use Metric plots to assess peak area and retention time to ensure correct peaks have been integrated

1.6 Insert standard curve values and QC valules in 'actual amount' column, according to the calibration table and QC table.

1.7 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

1.8 Copy the alphanumeric data of all peak areas and calculated amounts into Microsoft Excel and name the excel file using the same file naming convention (yyymmdd_Exxxx_Analyte_initials) and name the first tab 'RawData'

Transferring MultiQuant alphanumeric data into Excel to su...

2 In the Excel spreadsheet create a new tab. Go back to 'Raw Data' select 'filter' 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; APAP 1; AT7519 1

3 In the two tabs for the analytes - rename 'calculated amount' on the APAP tab to '[APAP] ng' and rename in the APAP-Sul tab to '[APAP-Sul] ng'

4 Create a new tab and name it 'Summary'. Copy the first three columns from the APAP tab, which contain sample details and calibration level details. Paste into the new tab. Then return to APAP tab and copy the '[APAP] ng' column.