STANDARD OPERATING PROCEDURE

Title: MRM mass spectrometry for the analysis of immuno-MRM assay samples using a 6500 QTRAP

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Purpose

The purpose of this document is to describe a multiple reaction monitoring (MRM) method for quantitative analysis of peptides from immunoaffinity enriched samples (immuno-MRM samples). The method employs retention time scheduling for enhanced quantification.

Scope

This procedure includes the setup of MRM methods on a 6500 QTRAP. Liquid chromatography (LC) parameters and methods are described in a separate SOP.

Responsibilities

It is the responsibility of person(s) performing this procedure to be familiar with laboratory safety procedures. The interpretation of results must be done by a person trained in the procedure and familiar with such interpretation.

Equipment

- Mass spectrometer: Sciex 6500 QTRAP
- Ion Source: Sciex NanoSpray III
- Emitter tip: TaperTip 20 μm ID (NewObjective)

Materials

- Water, Optima® LC/MS, suitable for UHPLC-UV (W6-4, Fisher Scientific)
- Acetic acid (ACS reagent, ≥99.7%, 242853, Sigma)
- Acetonitrile, Optima® LC/MS, suitable for UHPLC-UV (A955-4, Fisher Scientific)
- Formic acid (FA) (1.11670.1000, EMD Millipore)

Procedure

1. Setup MRM method parameters
    a. Source/Gas Parameters:
        i. Curtain Gas (CUR): 20
        ii. IonSpray Voltage (IS): 3.5 kV
        iii. Ion Source Gas 1 (GS1): 18
        iv. Ion Source Gas 2 (GS2): 0
        v. Interface Heater Temperature (IHT): 160 °C
        vi. Collision Gas (CAD): Medium
    b. Scheduled MRM Parameters
    Note: These parameters were used with the aim of obtaining at least 10 data points per peak.
        i. MRM detection window: 60 sec
        ii. Target Scan Time: 0.5 sec
    c. MS Parameters:
        i. Declustering Potential (DP): 100 V
        ii. Entrance Potential (EP): 10 V
        iii. Collision Energy (CE): From Skyline[1], based on optimized values from synthetic peptides.
        iv. Collision Cell Exit Potential Q1 (CXP): 10 V
    d. Advanced MS Parameters:
        i. Resolution Q1: Unit
        ii. Resolution Q3: Unit
        iii. Intensity Threshold (total count): 0
        iv. Settling time: 0 ms
        v. Pause between mass ranges: 5.007 ms
2. Test the system suitability with an appropriate standard once the column is conditioned.
Note: Pierce iRT standard (88320, Pierce) was injected on a conditioned column at least twice to be able to assess retention time reproducibility and whether peak shapes and intensities are acceptable based on historic data.
    a. Retention time shift of <0.5 minutes (a third injection might be needed if the first two runs' retention times shift by >0.5 min; the LC system needs to be troubleshot if the retention times still shift >0.5 min after a third run).
    b. Minimal tailing or fronting (check the LC connections if necessary).
    c. No drop-out of electrospray (sparge the LC solvents or replace the emitter tip if needed).
    d. If the peak intensities are unacceptable, troubleshoot the autosampler or clean the MS.
3. Identify scheduling times for target peptides/transitions
    a. Target LC-MRM method preparation
        i. Load the Skyline file containing peptides and transitions that will be monitored during the analysis.

ii. Export a scheduled transition list from Skyline using the "values from a single data set" and choosing the correct data file.
iii. Import the scheduled transition list into an MRM acquisition method on the 6500 with all parameters set as above (step 1)
iv. Set up the autosampler and LC methods as in the LC SOP.
v. Inject one immuno-MRM sample.

b. Instrument performance evaluation and adjustment of scheduled retention times
    i. Import the latest data file into the Skyline file.
    ii. Check the automatic integration of all peaks.
        1. Make sure integration start and stop is identical for all transitions of a precursor (go to "Settings", and check "Integrate All" to enable this feature automatically).
        2. Manually adjust the integration of peaks if necessary.
    iii. Check that peak shapes are acceptable.
        1. Minimal tailing or fronting.
        2. No drop-out of electrospray (for this, no smoothing should be applied in Skyline (View/Transform/None)).
        3. No missing transitions.
    iv. Export a new scheduled transition list from Skyline if needed (the retention times can shift based on whether peptides are tested in a buffer background or whether the matrix is more complex, such as in the eluted samples after an immunoaffinity capture from a plasma matrix).

4. Once the instrument is performing acceptably, run the remaining samples of the immuno-MRM experiments in the order given in the SOPs for response curves, validation samples, or unknown samples. The file naming convention follows:
    a. For the response curve experiments: AssayGroup_TypeOfExperiment_AnalyteAmountLevel_CaptureReplicate#_InjectionReplicate#
    b. For the repeatability (validation) experiments: AssayGroup_AnalyteLevel_Day#_CaptureReplicate#_InjectionReplicate#

References

1. MacLean, B., Tomazela, D. M., Shulman, N., Chambers, M., Finney, G. L., Frewen, B., Kern, R., Tabb, D. L., Liebler, D. C., and MacCoss, M. J. (2010) Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics* 26, 966-968.