STANDARD OPERATING PROCEDURE

Title: MRM mass spectrometry, 5500 QTRAP

Version #: 1   **Author: Paulovich lab**

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Purpose

The purpose of this document is to describe the mass spectrometry (MS) method for quantitative analysis of peptides using multiple reaction monitoring (MRM).

Scope

This procedure encompasses the setup of the MS and method parameters. LC parameters are contained in a separate document.

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: ABSciex 5500 QTRAP
- Source: ADVANCE CaptiveSpray Source for ABSciex (Michrom Bioresources/Bruker)
- Emitter tip: CaptiveSpray tapered tip 20um ID (Michrom Bioresources, SS9/25000/20)
- LC-to-source connection: PEEKsil, 1/32" x 25um x 20cm (Upchurch, 32520).

Procedure

1. Setup MS method parameters
   a. Source/Gas Parameters:
      i. Curtain Gas (CUR): 10

      ii.   IonSpray Voltage (IS): 1200

      iii. Ion Source Gas 1 (GS1): 0

      iv. Ion Source Gas 2 (GS2): 0

      v.   Interface Heater Temperature (IHT): 110

b. Scheduled MRM Parameters:

      i.   MRM detection window (sec): 200

      ii. Target Scan Time (sec): 1.5

c. MS Parameters:

      i.   Declustering Potential (DP): 90 (or from Skyline)

      ii. Entrance Potential (EP): 10

      iii. Collision Energy (CE): From Skyline (PO4 peptide 5500 regression)

      iv. Collision Cell Exit Potential Q1 (CXP): 10

d. Advanced MS Parameters:

      i.   Resolution Q1: Unit

      ii. Resolution Q3: Unit

      iii. Intensity threshold (total count): 0

      iv. Settling time (ms): 0

      v.   Pause between mass ranges (ms): 3

2. Test system suitability with appropriate standard once column is conditioned.

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 QC analysis.

      ii. In the Skyline file under Settings/Transition Settings/Predictions, select 'ABI 5500 QTrap PO4' under 'Collision energy:' and 'Static' under 'Declustering potential'.

      iii. Export the unscheduled transition list as 'Multiple methods' (6), ignoring proteins, with a dwell time of 10ms.

      iv. Import the unscheduled transition lists into MRM acquisition method on the 5500 with all other parameters set as above (step 1).

  b. Timing the peptide detection

      i.   Set up the autosampler and LC methods as in the accompanying LC SOP.

      ii. Inject the QC sample 6x.

      iii. Import the data files into the Skyline file.

      iv. Check the automatic integration of all peaks.

      v.   Export the scheduled transition list using above scheduled parameters.

  c. QC sample analysis

      i.   Import the scheduled transition list into an MRM acquisition method on the 5500 with all parameters set as above.

      ii. Set up the autosampler and LC methods as in the accompanying LC SOP.

      iii. Inject the QC sample 5 times.

  d. Instrument performance evaluation

      i.   Import the data files into the Skyline file.

      ii. Check the automatic integration of all peaks.

         1. Manually adjust integration of peaks, if necessary.

      2. 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).

   iii. Check that peak shape is acceptable.
      1. No tailing or fronting.
      2. No drop-out of electrospray.
      3. No missing transitions.
      4. If the peaks are unacceptable, troubleshoot the LC system and re-run the column conditioning procedure.

   iv. Check that peaks pass reproducibility criteria.
      1. Retention time shift is less than 0.5 minutes.
      2. Peak area ratio CV is less than 20% for all peaks.

3. Analysis of response curve for characterization of assays.
   a. LC-MRM method preparation
     i. Open Skyline file containing targeted peptides, transitions, and retention times.
     ii. In the Skyline file under Settings/Transition Settings/Predictions, select 'ABI 5500 QTrap DQ' under 'Collision energy:' and 'Static' under 'Declustering potential'.
     iii. Export the scheduled transition list.
   b. Import the scheduled transition list into an MRM acquisition method on the 5500 with all parameters set as above (step 1).
   c. Set up the autosampler and LC methods as described in SOP LC-03 (Liquid chromatography trap elute).

Referenced Documents

SOP LC-03 Liquid chromatography trap elute.pdf