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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Multiple Reaction Monitoring (MRM) using an 8040 triple quadrupole mass spectrometer** | | | **Version #: 1.1** | **Author: Hui Zhang Laboratory – Johns Hopkins University** | | **Date: 06/10/2016** |  | |

**Purpose**

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

**Scope**

This procedure describes the setup of the mass spectrometer and the MRM method parameters for the 8040 triple quadrupole mass spectrometer. 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: LCMS 8040 (Shimadzu Scientific)

**Procedure**

1. Setup MS method and tune file parameters
   1. ESI Source/Gas parameters
      1. Spray voltage: 4500 V
      2. Desolvation line temperature: 210 °C
      3. Nebulizing gas flow rate: 3 L/min
      4. Drying gas flow rate: 15 L/min
      5. Heat block temperature: 400 °C
   2. MS parameters
      1. Q1 & Q3 resolution: Unit
      2. Detection window: 180 sec
      3. Maximum loop time: 1.59 sec
      4. Maximum dwell time: 50 msec
2. Test system suitability with 500 fmol *E. coli* Beta galactosidase tryptic digest (QC sample)
3. Identify scheduling time for target peptides/transitions
   1. LC-MRM method preparation
      1. Load the Skyline file containing peptides and transitions that will be monitored
      2. In Lab Solutions, run the MRM Optimization Wizard to adjust precursor m/z, optimize voltage (select 5 transitions), and adjust product m/z of all heavy and light peptides
      3. Run mixture of all heavy and light peptides twice using LC-MRM method created from MRM Optimization Wizard results (set max dwell time to 10 ms)
      4. Import .lcd file into Skyline and refine transitions (3 transitions/precursor)
      5. Update Lab Solutions .lcm LC-MRM method based on refined transitions from Skyline
   2. Timing the peptide detection
      1. Set up the autosampler and LC methods as indicated in the Liquid Chromatography SOP
      2. Inject the QC sample three times
      3. Run mixture of all heavy and light peptides twice
      4. Import .lcd file into Skyline and check the automatic integration of all peaks
      5. Export the scheduled transition list using 180 sec scheduled windows
      6. Update Lab Solutions .lcm LC-MRM method based on scheduled windows determined by Skyline