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| STANDARD OPERATING PROCEDURE |
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| **Title: MRM Mass Spectrometry, TSQ Vantage** |
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| **Version #: 2** | **Author: PNNL Lab** |
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# Purpose

The purpose of this document is to describe the Mass Spectrometry (MS) method for developing peptide multiple reaction monitoring (MRM) assays.

# Scope

This procedure encompasses the setup of the MS and method parameters on Thermo TSQ Vantage.

# 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

Source: in-house built nano-sprary source

Emitter tip: In-house made emitter (20um ID, 360um OD)

Mass spectrometer: Thermo TSQ Vantage

# Materials

# Reagents

# Procedure

1. Setup MS method parameters:
2. Source Parameters:
3. Ion Spray Voltage: 2400 V
4. Capillary Temperature: 325 ºC
5. Scheduled MRM Parameters:
6. MRM detection window (sec): 300
7. Dwell time: 10 ms
8. MS parameters:
9. Use tuned S-lens value
10. Collision gas pressure: 1.5 mTorr
11. Q1 and Q3 unit resolution
12. Collision energy optimized by LC ramping for each transition
13. Intensity threshold: 500
14. Test system suitability with appropriate QC standard once column is conditioned.
15. Optimize collision energy
16. Load the Skyline file containing target peptides and transitions that will be monitored in MRM experiment
17. In the Skyline file under Settings/Transition Settings/Predictions, select ‘Thermo TSQ Vantage’ under ‘collision energy’
18. Export the transition list with ‘collision energy’ under ‘Optimizing’
19. Import the unscheduled transition list into MRM acquisition method on the TSQ with all other parameters set as above (step 1)
20. Import TSQ raw file into Skyline and obtain optimal collision energy for each transition
21. Identify scheduling times for target peptides/transitions
22. Set up the autosampler and LC methods as in the accompanying LC SOP file
23. Check the overall status by injecting QC sample
24. Export the transition list from Skyline with optimal collision energy
25. Inject target peptide mixture into TSQ Vantage
26. Import the data files into Skyline and manual check the retention time of each peptide
27. Export the scheduled transition list and optimal collision energy
28. Method performance evaluation
29. Spike the target peptide mixture into real complex matrix (cell lysate, tissue digest, plasma etc)
30. Inject the mixed sample
31. Import the data file into Skyline
32. Check the LC and transition condition:
33. Make sure scheduling is ok, no missing peaks
34. Make sure peak shape is acceptable, no tailing or fronting
35. Make sure each transition is ok, no obvious interference

# Referenced Documents

List any publications or documents referenced in the SOP.