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| STANDARD OPERATING PROCEDURE |
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| **Title: Liquid Chromatography, Waters nanoACQUITY UPLC system**  |
|  |  |
| **Version #: 3** | **Author: PNNL lab** |
| **Date: 07/01/2016** |  |

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# Purpose

The purpose of this document is to describe the Liquid Chromatography (LC) method for development of CPTAC MRM assays.

# Scope

This procedure is designed to help the setup of LC gradient and method parameters on Waters nanoACQUITY UPLC system.

# 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

nanoACQUITY UPLC (Waters, Milford, MA, Catalog # 176016000)

Milli-Q Integral Water Purification System (EMD Millipore, Darmstadt, Germany, Catalog # ZRXQ010TO)

# Materials

ACQUITY UPLC M-Class Peptide BEH C18 Column, 130Å, 1.7 µm, 100 µm X 100 mm (Waters, Milford, MA, Catalog # 186007485)

# Reagents

Acetonitrile, Optima LC/MS Grade (Fisher Scientific, Asheville, NC, Catalog # A955-4)

Acetonitrile/Formic Acid 0.1% Mobile Phase (EMD Millipore, Darmstadt, Germany, Catalog # FX0437P-1)

Formic Acid, LC-MS Grade (Thermo Fisher Scientific, Waltham, MA, Catalog # 28905)

# Solutions

Mobile phase A: 0.1% FA in H2O

Mobile phase B: 0.1% FA in ACN

Strong Needle Wash buffer: 100% ACN

Weak Needle Wash buffer: 0.1% FA in H2O

Seal Wash buffer: 10% ACN in H2O

# Procedure

**Instrument Configuration**

1. **Autosampler parameters:**

**Under General tab:**

‘Partial loop’ under ‘sample loop option’

Wash Solvent: Weak 600 ul, Strong 200 ul

Column Temperature: 42 ºC

Autosampler temperature: 4 ºC

Run time: 8 min

**Under Event tab:**

 Check ‘run events’ box:

|  |  |  |
| --- | --- | --- |
| Time (min) | Event | Action |
| 10.00 | Switch 1 | On |
| 10.01 | Switch 1 | Off |

1. LC gradient methods:

|  |  |  |  |
| --- | --- | --- | --- |
| Time (min) | %Mobile Phase A | %Mobile phase B | Flow rate (ul/min) |
| 0 | 99.5 | 0.5 | 0.5 |
| 11 | 99.5 | 0.5 | 0.4 |
| 11.5 | 99.5 | 0.5 | 0.4 |
| 13 | 99.5 | 0.5 | 0.4 |
| 13.5 | 90 | 10 | 0.4 |
| 17 | 85 | 15 | 0.4 |
| 38 | 75 | 25 | 0.4 |
| 49 | 61.5 | 38.5 | 0.4 |
| 50 | 5 | 95 | 0.4 |
| 54 | 5 | 95 | 0.4 |
| 55 | 5 | 95 | 0.5 |
| 56 | 5 | 95 | 0.75 |
| 58 | 5 | 95 | 0.75 |
| 59 | 90 | 10 | 0.5 |
| 60 | 5 | 95 | 0.5 |
| 62 | 5 | 95 | 0.5 |
| 64 | 99.5 | 0.5 | 0.5 |

# Referenced Documents

List any publications or documents referenced in the SOP.