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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Liquid Chromatography, Waters nanoACQUITY UPLC system** | | |  |  | | **Version #: PRISM** | **Author: PNNL lab** | | **Date: 07/20/2016** |  | |

# Purpose

The purpose of this document is to describe the 2nd dimention LC-SRM Liquid Chromatography (LC) method for development of CPTAC PRISM-SRM 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

UPLC: nanoACQUITY (Waters, Part Number 176016000)

# Materials

Injection loop: 5 uL peeksil

Column: 100 μm x 100 mm, BEH C18, 1.7 μm 130 Å (Waters, Part Number 186007485)

Water, HPLC grade (H2O)

Acetonitrile, HPLC grade (ACN) (Fisher Scientific, A955-4)

Formic Acid (0.1%)/Acetonitrile (EMD, FX0437P-1)

Formic Acid (FA) (Agilent Technologies, G2453-85060)

# Reagents

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

**LC-SRM Instrument Configuration**

1. nanoACQUITY autosampler parameters:

Under General tab:

‘Partial loop’ under ‘sample loop option’

Wash Solvent: Weak 600 ul, Strong 200 ul

Autosampler temperature: 4 ºC

1. Injection volume: 3 uL
2. LC-SRM gradient methods:

*Column Temperature: 42 ºC*

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

# Referenced Documents

List any publications or documents referenced in the SOP.