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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Liquid Chromatography using Dionex UltiMate 3000 RSLCnano LC system for PRM assays** | | | **Version #: 1.1** | **Author: Hui Zhang Laboratory – Johns Hopkins University** | | **Date: 02/18/2016** |  | |

# Purpose

The purpose of this document is to describe the liquid chromatography (LC) method for quantitative mass spectrometry-based analyses.

# Scope

This procedure describes the setup of the LC and the method parameters. It is specific to the operation of the Dionex UltiMate 3000 RSLCnano LC 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

* HPLC: Dionex UltiMate 3000 RSLCnano LC system (Thermo Fisher Scientific; cat. # NCS-3500RS)

# Materials

* Injection loop: 20 µL Dionex nanoViper sample loop (Thermo Fisher Scientific; cat. # 6826.2420)
* Column compartment: UltiMate 3000 Binary Rapid Separation Nano Flow Pump with Ternary Loading Pump and Column Compartment (Thermo Fisher Scientific; cat. # NCS-3500RS)
* Autosampler: UltiMate 3000 Thermostatted Rapid Separation Pulled Loop Wellplate Sampler (Thermo Fisher Scientific; cat. # WPS-3000)
* Solvent Degasser: UltiMate 3000 Integrated Solvent and Degasser Rack, 4 Channels (Thermo Fisher Scientific; cat. # SRD-3400)
* Trap Column: 300 µm I.D. x 5 mm packed with Acclaim PepMap 100 5 µm, 100 Å C18 (Thermo Fisher Scientific; cat. # 160454)
* Analytical Column: 75 µm I.D. x 50 cm EASY-Spray column packed with Acclaim PepMap RSLC C18, 2 µm (Thermo Fisher Scientific; cat. # ES803)
* Autosampler vials: 9 mm assembled amber autosampler vial kit (Thermo Fisher Scientific; cat. # C5000-196W)
* Autosampler vial inserts: Polyspring, glass conical insert (Thermo Fisher Scientific; cat. # C4010-630)

# Reagents

* Water: Optima LC/MS-grade (Fisher Scientific; cat. # W6-4)
* Acetonitrile: Optima LC/MS-grade (Fisher Scientific; cat. # A955-4)
* Formic Acid: LC-MS Ultra (Sigma-Aldrich; cat. # 14265)

# Solutions

* Loading pump, mobile phase A: 2% ACN/0.1% formic acid in water
* NanoFlow pump, mobile phase A: 2% ACN/0.1% formic acid in water
* NanoFlow pump, mobile phase B: 0.1% formic acid in 90% ACN

# Procedure

1. Autosampler method

Temperature= 5°C

Draw Speed = 200 [nl/s]

Draw Delay = 5000 [ms]

Disp Speed = 2000 [nl/s]

Dispense Delay = 2000 [ms]

Waste Speed = 4000 [nl/s]

Wash Speed = 4000 [nl/s]

Loop Wash Factor = 2.000

Sample Height = 2.000 [mm]

Puncture Depth = 8.000 [mm]

Wash Volume = 150.000 [µl]

Rinse Between Reinjections = No

Low Dispersion Mode = Off

Inject Mode = µL pick-up

Inject Volume = 5.000 µL

1. Loading pump profile
   1. Flow rate: 5.000 µL/min
   2. Flow gradient: Isocratic; 100% A
   3. Injection valve switch: 6.000 min
2. Gradient method:
   1. Flow rate: 250 nL/min
   2. Temperature: 42 °C
   3. Timetable

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| Retention (min) | Flow (µL/min) | %B |
| 0.00 | 0.250 | 4.00 |
| 7.00 | 0.250 | 4.00 |
| 27.00 | 0.250 | 40.00 |
| 30.00 | 0.250 | 90.00 |
| 37.00 | 0.250 | 90.00 |
| 38.00 | 0.250 | 4.00 |
| 45.00 | 0.250 | 4.00 |

# Referenced Documents

1. Thomas SN, Harlan R, Chen J, Aiyetan P, Liu Y, Sokoll LJ, Aebersold R, **Chan DW**, **Zhang H**. Multiplexed Targeted Mass Spectrometry-Based Assays for the Quantification of N-Linked Glycosite-Containing Peptides in Serum. Anal Chem. 2015 Nov 3;87(21):10830-8. doi: 10.1021/acs.analchem.5b02063. Epub 2015 Oct 21. PubMed PMID: 26451657; PubMed Central PMCID: PMC4708883.