STANDARD OPERATING PROCEDURE

Title: Liquid Chromatography, Dionex UltiMate 3000 RSLCnano LC System

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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 25 cm EASY-Spray column packed with Acclaim PepMap RSLC C18, 2 µm (Thermo Fisher Scientific; cat. # ES802)
- 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

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 =	6.000 µL

2. Loading pump profile
    a. Flow rate: 5.000 µL/min

b. Flow gradient: Isocratic; 100% A
c. Injection valve switch: 4.000 min

3. Gradient method:
    a. Flow rate: 500 nL/min
    b. Temperature: 42 °C
    c. Timetable

Retention (min)	*Flow (µL/min)*	*%B*
0.00	0.500	4.00
5.00	0.500	5.00
64.00	0.500	40.00
67.00	0.500	90.00
77.00	0.500	90.00
78.00	0.500	4.00
90.00	0.500	4.00

Referenced Documents

N/A