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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Liquid Chromatography, Thermo Scientific EASY-nLC 1200 LC System** | | |  |  | | **Version #: 2** | **Author: Akhilesh Pandey Lab** | | **Date: 05/01/2016** |  | |

# 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 chromatographic method parameters. It is specific to the operation of the Thermo Scientific EASY-nLC 1200 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: Thermo Scientific EASY-nLC 1200 LC system (Thermo Fisher Scientific; cat. # LC140)

# Materials

* Injection loop: 20 µL Dionex nanoViper sample loop (Thermo Fisher Scientific; cat. # 6826.2420)
* Autosampler: Autosampler integrated in Easy-nLC 1200 LC system (Thermo Fisher Scientific; cat. # LC140)
* Trap Column: 100 µm I.D. x 2 cm nanoViper column packed with Acclaim PepMap RSLC C18, 5 µm 100 Å (Thermo Fisher Scientific; cat. # 164564)
* Analytical Column: EASY-spray column, 75 µm I.D. x 50 cm nanoViper column packed with Acclaim PepMap RSLC C18, 2 µm 100 Å (Thermo Fisher Scientific; cat. # ES803)
* Autosampler 96-well plate: PCR plate, 150 µL max. well volume (Eppendorf; cat. # 951020401)
* Autosampler 96-well plate cover: Adhesive free film (Excel Scientific; cat. # ZAF-PE-50)

# Reagents

* Water: Optima LC/MS-grade (Fisher Scientific; cat. # W6-4)
* Acetonitrile: Optima LC/MS-grade (Fisher Scientific; cat. # A955-4)
* Formic Acid: Pierce Formic Acid, LC-MS grade (Pierce; cat. # 28905)

# Solutions

* Mobile phase A: 0.1% formic acid in water
* Mobile phase B: 0.1% formic acid in 95% ACN

# Procedure

1. Sample Pickup
   1. Draw Speed = 20 [µL/s]
   2. Wash Volume = 100.000 [µL]
   3. Rinse Between Reinjections = No
   4. Inject Volume = 10.000 µL
2. Pre-column equilibration
   1. Max Pressure = 500 Bar
   2. Flow Rate = unspecified
   3. Volume = 25 µL
3. Analytical column equilibration
   1. Max Pressure = 750 Bar
   2. Flow Rate = unspecified
   3. Volume = 6 µL
4. Sample Loading
   1. Max Pressure = 500 Bar
   2. Flow gradient = Isocratic; 100% Mobile phase A
   3. Load volume = 25 µL
5. Gradient method:
   1. Flow rate = 300 nL/min
   2. Temperature = 45°C
   3. Timetable

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| --- | --- | --- |
| Retention (min) | Flow (nL/min) | %B |
| 0:00 | 250 | 5 |
| 02:00 | 250 | 7 |
| 52:00 | 250 | 30 |
| 55:00 | 250 | 100 |
| 60:00 | 250 | 100 |

# Referenced Documents

N/A