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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Liquid Chromatography using Shimadzu Nexera system for MRM assays** | | | **Version #: 1.1** | **Author: Hui Zhang Laboratory – Johns Hopkins University** | | **Date: 06/10/2016** |  | |

# 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 Shimadzu Nexera HPLC 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: Nexera X2 UHPLC system (Shimadzu Scientific)

# Materials

* Injection loop: 50 µL
* System controller (Nexera, model # CBM-20A CL; Shimadzu Scientific)
* LC Pump A & B (Nexera, model # LC-30AD CL; Shimadzu Scientific)
* Autosampler (Nexera, model # SIL-30ACMP CL; Shimadzu Scientific)
* Column oven (Nexera, model # CTO-30A CL; Shimadzu Scientific)
* Solvent Degasser (Nexera, model # DGU-20A 5RCL; Shimadzu Scientific)
* Analytical Column: 1.0 mm I.D. x 15 cm Viva C18 3 µm (Restek; cat. # 9514361)
* 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

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

# Procedure

1. Autosampler method

Temperature: 4°C

Sampling speed: 5.0 µL/sec

Rinsing speed: 35 µL/sec

Rinsing volume: 100 µL

Rinse mode: Before and after aspiration

Rinse dip time: 5 sec

Inject volume: 5 µL

1. Gradient method:
   1. Flow rate: 50 µL/min
   2. Column compartment temperature: 50 °C
   3. Timetable:

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| Retention (min) | Flow (µL/min) | %B |
| 0.00 | 50 | 3.00 |
| 5.00 | 50 | 3.00 |
| 45.00 | 50 | 35.00 |
| 50.00 | 50 | 45.00 |
| 53.00 | 50 | 90.00 |
| 60.00 | 50 | 90.00 |
| 61.00 | 50 | 3.00 |
| 70.00 | 50 | 3.00 |