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| STANDARD OPERATING PROCEDURE |
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| **Title: Liquid Chromatography using Accela system for MRM assays** |
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| **Date: 06/10/2016** |  |

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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 Thermo Scientific Accela 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: Accela 1250 Quaternary Low Pump (Thermo Scientific)
* Autosampler: Accela Open Autosampler (Thermo Scientific)

**Materials**

* Injection loop: 10 µL
* Column heater: Hot pocket (Thermo Scientific; cat. # 92016-150)
* Analytical Column: 1.0 mm I.D. x 15 cm Viva C18 3 µm 300 Å (Restek; cat. # 9514361)
* Autosampler vials: Polypropylene 12 x 32 mm screw neck vial with caps and PTFE/silicone septum, 300 µL (Waters; cat. # 186002640)
* 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: Water/0.2 % formic acid
* Gradient pump, mobile phase B: 90% ACN/0.2% formic acid

**Procedure**

1. Autosampler method

 Temperature: 4°C

 Syringe: 100 µL

 Airgap Volume (µl): 3

 Front Volume (µl): 2

 Rear Volume (µl): 2

 Filling Speed (µl/s): 5

 Pullup Delay (ms): 3000

 Inject to: LC Vlv1

 Injection Speed (µl/s): 5

 Pre & Post Inject Delay (ms): 500, 500

 Needle Gap Valve Clean (mm): 3

 Valve Clean Time Solvent 2 (s): 2

 Post Clean Time Solvent 2 (s): 2

 Valve Clean Time Solvent 1 (s): 5

 Post Clean Time Solvent 1 (s): 5

 Stator Wash: 0

 Delay Stator Wash (s): 120

 Stator Wash Time Solvent 2 (s): 5

 Stator Wash Time Solvent 1 (s): 5

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

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| Retention (min) | Flow (µL/min) | %B |
| 0.00 | 50 | 1.00 |
| 5.00 | 50 | 1.00 |
| 30.00 | 50 | 35.00 |
| 35.00 | 50 | 55.00 |
| 38.00 | 50 | 95.00 |
| 45.00 | 50 | 95.00 |
| 46.00 | 50 | 1.00 |
| 60.00 | 50 | 1.00 |