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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Liquid Chromatography, Eksigent NanoFlex, trap and elute** | | |  |  | | **Version #: 1** | **Author: Paulovich lab** | | **Date: 8/17/2015** |  | |

# Purpose

The purpose of this document is to describe the liquid chromatography (LC) method for quantitative analysis.

# Scope

This procedure encompasses the setup of the LC and method parameters. It is specific to operation of the dual column chip system using an Eksigent Ultra system coupled to nanoflex cHiPLC.

# 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: NanoLC-2D plus (Eksigent, 950-00061)
* Eksigent NanoFlex chip system.

# Materials

* Injection loop: 1 uL peeksil, 100 um (Eksigent, 200-00208)
* Column Compartment: cHiPLC-nanoflex (Eksigent, 950-00070)
* Trap column: 200 μm x 0.5 cm ChromXP C18-CL 3 μm 120 Å (Eksigent, 804-00006)
* Analytical column: 75 μm x 15 cm ChromXP C18-CL 3 μm 120 Å (Eksigent, 804-00001)
* Water, HPLC grade (H2O) (Fisher, W5-1)
* Acetonitrile, HPLC grade (ACN) (Fisher, A998-1)
* Formic Acid (FA) (EDM, 11670-1)

# Solutions

Mobile phases. Must be degassed every week:

* Gradient 1, mobile phase A: 0.1% FA in H2O
* Gradient 1, mobile phase B: 0.1% FA in 90% ACN
* Gradient 2, mobile phase A: 0.1% FA in H2O
* Gradient 2, mobile phase A: 0.1% FA in 90% ACN)

# Procedure

1. Autosampler method:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Step #** | **Operation** | **Value** | **Parameter** | **Speed** | **Height** | **Description** |
| 1 | Output |  | 1-OFF |  |  | Wait for Gradient 1 ready to start |
| 2 | Output |  | 2-OFF |  |  | Wait for Gradient 2 ready to start |
| 3 | Valve |  | Injector Load |  |  | Valve Position Control |
| 4 | Aspirate | 10.5 | Reagent-1 | 1 | 3 | Aspirate specified volume |
| 5 | Wait | 00:00:05 |  |  |  | Pause for specified time |
| 6 | Aspirate | 0 | Reagent-1 | 1 | 3 | Aspirate specified volume |
| 7 | Aspirate | 4.2 | Sample | 1 | 9 | Aspirate specified volume |
| 8 | Wait | 00:00:05 |  |  |  | Pause for specified time |
| 9 | Aspirate | 0 | Sample | 1 | 10 | Aspirate specified volume |
| 10 | Aspirate | 0 | Reagent-3 | 1 | 5 | Aspirate specified volume |
| 11 | Aspirate | 5.3 | Reagent-1 | 1 | 5 | Aspirate specified volume |
| 12 | Wait | 00:00:05 |  |  |  | Pause for specified time |
| 13 | Output |  | 1-ON |  |  | Start LC run on channel 1 |
| 14 | Output |  | 2-ON |  |  | Start LC run on channel 2 |
| 15 | Valve |  | Injector Inject |  |  | Switch AS injector valve to Inject position (1-2) |
| 16 | Marker |  | Inject |  |  | Pause for specified time |
| 17 | Wait | 00:10:00 |  |  |  | Pause for specified time |
| 18 | Valve |  | Injector Inject |  |  | Switch AS injector valve to Load position (1-6) |
| 19 | Dispense | 20 | Waste | 3 | 0 | Dispense specified volume |
| 20 | Needle Wash | 50 | Port 1 |  |  | Perform needle wash |
| 21 | END |  |  |  |  |  |

1. Gradient 1 method:
   * 1. Flow rate (L/min): See timetable
     2. Temperature (C): 40
     3. Run Conditions:
        + 1. Pre-run
          2. Flush column for 0.1 minutes using 100% initial flowrate conditions.
     4. Timetable

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Time (min)** | **% Mobile phase A composition** | **% Mobile phase B composition** | **Flow rate (L/min)** | **Event** |
| 0 | 99 | 1 | 10 | Valve Load |
| 2 | 99 | 1 | 10 | Valve Inject |
| 2.1 | 50 | 50 | 2 |  |
| 65 | 50 | 50 | 2 |  |
| 65.1 | 99 | 1 | 10 |  |
| 75 | 99 | 1 | 10 | Valve Load |
| 50 | 99 | 1 | 10 |  |

1. Gradient 2 method:
   * 1. Flow rate (nL/min): 300
     2. Temperature (C): 40
     3. Run Conditions:
        + 1. Pre-run
          2. Flush column for 0.1 minutes using 100% initial flowrate conditions.
     4. Timetable for column 1 elution:

|  |  |  |  |
| --- | --- | --- | --- |
| **Time (min)** | **% Mobile phase A composition** | **% Mobile phase B composition** | **Event** |
| 0 | 99 | 1 |  |
| 3 | 99 | 1 |  |
| 10 | 90 | 10 |  |
| 40 | 75 | 25 |  |
| 55 | 60 | 40 |  |
| 65 | 40 | 60 |  |
| 66 | 10 | 90 |  |
| 69 | 10 | 90 |  |
| 70 | 99 | 1 |  |
| 70 | 99 | 1 |  |