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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Liquid Chromatography, Eksigent NanoFlex, dual column** | | |  |  | | **Version #: 1** | **Author: Paulovich lab** | | **Date: 5/1/2012** |  | |

# 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-Ultra 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)
* 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)

# Reagents

* 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
* Loading Pump: 0.1% FA in H2O

# Procedure

1. Autosampler method:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Step #** | **Operation** | **Value** | **Parameter** | **Speed** | **Height** | **Description** |
| 1 | External Events |  | Wait for Gradient 1 Ready |  |  | Wait for Gradient 1 ready to start |
| 2 | External Events |  | Wait for Gradient 2 Ready |  |  | Wait for Gradient 2 ready to start |
| 3 | External Events |  | Start Gradient 1 |  |  | Start LC Gradient 1 |
| 4 | External Events |  | Start Gradient 2 |  |  | Start LC Gradient 2 |
| 5 | Wait | 00:25:00 |  |  |  | Pause for specified time |
| 6 | Valve |  | Injector Load |  |  | Valve Position Control |
| 7 | Aspirate | 11 | Reagent-1 | 1 | 3 | Aspirate specified volume |
| 8 | Wait | 00:00:05 |  |  |  | Pause for specified time |
| 9 | Aspirate | 0 | Reagent-1 | 1 | 3 | Aspirate specified volume |
| 10 | Aspirate | 2 | Sample | 1 | 3 | Aspirate specified volume |
| 11 | Wait | 00:00:05 |  |  |  | Pause for specified time |
| 12 | Aspirate | 0 | Sample | 1 | 3 | Aspirate specified volume |
| 13 | Aspirate | 2 | Reagent-1 | 1 | 3 | Aspirate specified volume |
| 14 | Wait | 00:00:05 |  |  |  | Pause for specified time |
| 15 | Aspirate | 0 | Reagent-1 | 1 | 3 | Aspirate specified volume |
| 16 | Valve |  | Injector Inject |  |  | Switch AS injector valve to Inject position (1-2) |
| 17 | Dispense | 15 | Waste | 3 | 0 | Dispense specified volume |
| 18 | Needle Wash | 250 | Port 1 |  |  | Perform needle wash |
| 19 | END |  |  |  |  |  |

1. Gradient 1 method:
   1. Flow rate (nL/min): 500
   2. Temperature (C): 40
   3. Run Conditions:
      1. Pre-run
      2. Flush column for 3 minutes using 50% initial flowrate conditions.
   4. Timetable

|  |  |  |
| --- | --- | --- |
| **Time (min)** | **% Mobile phase A composition** | **% Mobile phase B composition** |
| 0 | 50 | 50 |
| 2 | 10 | 90 |
| 7 | 10 | 90 |
| 8 | 97 | 3 |
| 50 | 97 | 3 |

1. Gradient 2 method:
   1. Flow rate (nL/min): 300
   2. Temperature (C): 40
   3. Run Conditions:
      1. Pre-run
      2. Flush column for 3 minutes using 200% initial flowrate conditions.
   4. Timetable for column 1 elution:

|  |  |  |  |
| --- | --- | --- | --- |
| **Time (min)** | **% Mobile phase A composition** | **% Mobile phase B composition** | **Event** |
| 0 | 97 | 3 | Valve Inject |
| 3 | 93 | 7 |  |
| 30 | 75 | 25 |  |
| 37 | 60 | 40 |  |
| 38 | 40 | 60 |  |
| 40 | 40 | 60 |  |
| 41 | 97 | 3 |  |
| 50 | 97 | 3 |  |

* 1. Timetable for column 2 elution:

|  |  |  |  |
| --- | --- | --- | --- |
| **Time (min)** | **% Mobile phase A composition** | **% Mobile phase B composition** | **Event** |
| 0 | 97 | 3 | Valve Load |
| 3 | 93 | 7 |  |
| 30 | 75 | 25 |  |
| 37 | 60 | 40 |  |
| 38 | 40 | 60 |  |
| 40 | 40 | 60 |  |
| 41 | 97 | 3 |  |
| 50 | 97 | 3 |  |