STANDARD OPERATING PROCEDURE

Title: Liquid Chromatography, Eksigent Ultra 2D+, trap and elute

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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 Eksigent Ultra system in trap/elute mode.

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: UltraNanoLC-2D plus (Eksigent, 950-00061)
- Eksigent AS2 autosampler.

Materials

- Injection loop: 10 uL peeksil
- Trap column: 300 μm ID x 5 mm LC Packings trap column (Acclaim PepMap 100 C18, 5 μm, 100 Å, 160454, Dionex) in a precolumn holder (160431, Dionex)
- Analytical column: 75 μm ID, 360 μm OD IntegraFrit column (IF360-75-50-N-5, New Objective), packed in-house to 10.0 cm with ReproSil-Pur C18-AQ, 3 μm (Dr. Maisch GmbH, Ammerbuch, Germany)
- Water, Optima® LC/MS, suitable for UHPLC-UV (W6-4, Fisher Scientific)

- Acetonitrile (ACN), Optima® LC/MS, suitable for UHPLC-UV (A955-4, Fisher Scientific)
- 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

Procedure

1. Autosampler method:

Step #	Operation	Value	Parameter	Speed	Height
1	Valve		Injector Load		
2	External Events		Wait for Grad 1 Ready		
3	External Events		Wait for Grad 2 Ready		
	External Events		Wait for Loading Pump Ready		
4	Aspirate	11	Reagent-1	2	3
5	Wait	00:00:02			
6	Aspirate	0	Reagent-1	1	3
7	Aspirate	11	Sample	2	2
8	Wait	00:00:05			
9	Aspirate	0	Sample	1	2
10	Aspirate	0	Reagent-2	1	3
11	Aspirate	2	Reagent-1	1	3
12	External Events		Start Grad 1		
13	External Events		Start Grad 2		
14	External Events		Start Loading Pump		
15	Valve		Injector Inject		
17	Wait	00:05:00			
18	Valve		Injector Load		
19	Dispense	24	Waste	3	0
20	Needle Wash	100	Port 1		
21	END				

2. Loading pump method:
    i. Flow rate (µL/min): See timetable
    ii. Temperature (C): 45
    iii. Run Conditions:
        a. Pre-run
        b. Flush column for 0.1 minutes using 100% initial flowrate conditions.

iv. Timetable

Time (min)	Flow rate (µL/min)	Event
0	20	
10	20	
10.1	5	
18	5	
18.1	20	
20	20	

3. Gradient 1 method (channel 1):
   i. Flow rate (nL/min): 300
   ii. Temperature (C): 45
   iii. Run Conditions:
      a. Pre-run
      b. Flush column for 0.1 minutes using 100% initial flowrate conditions.
   iv. Timetable for column 1 elution:

Time (min)	% Mobile phase A composition	% Mobile phase B composition	Event
0	98	2	Valve Load
1	98	2	Valve Inject
2	86	14	
12	64	36	
13	10	90	
14	10	90	
15	98	2	
20	98	2	