

Application

Agilent Purification System, LAB EXERCISE

LAB TITLE

Preparative application of Coca Cola.

Lab Objectives

To run the application of Tea, from analysis, to up-scaling, then to preparative collection.

The LAB has one part only:

- 1) Application of Tea

Application

1) Application of Tea

IMPORTANT: Use prep column that was packed last week and use slides

- a) Ensure that you have installed the hardware and the software as per relative SPIFMs and CBT and that the system passes the Test Protocol.
- b) Run a sample of straight Tea on an analytical column according to the conditions in step **g)**.
- c) Optimise the run for preparative scale, but still on the analytical column, by overloading the column.
Note that if there is no more separation, you have overloaded the column too much.
- d) Based on the preparative column that you have been given and the system's configuration, work out the linear scale up factor, list the new values in step **h)** and build a preparative method.
- e) Run the preparative method and report the details of this method in step **i)**.
- f) Analyse the purest fractions and determine the %Area purity:
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- g) Analytical Method Details:

- i) **Run time** 12 minutes
- ii) **Flow rate** 2 mL per minute.....
- iii) **Injection volume** 50 μ L.....
- iv) **Solvent** 100% H₂O for 2 min, then over 5 min to 100% MECH and hold for 2 min, then back to 100% H₂O and hold for 3 min.....
- v) **Wavelength** 205nm.....
- vi) **Caffeine RT** Target is approximately 1.89min.....

Application

h) Scale Up factor (r₂ prep / r₂ analytical) =

Scalable Factors	Absolute Factors
Flow rate	Packing Material
Sample injection Volume	Sample Concentration
Tubing ID	Back Pressure
Fraction Volume	Fraction Concentration
Solvent Consumption	Percent Recovery
Throughput	Peak Height and Area
Detector Flow Cell Size	Gradient Slope
Sample Loop Size	Run Time
Dead Volume	Column Performance
	Fraction Purity
	Temperature

i) Preparative Method Details:

Run time
Flow rate
Injection volume
Gradient
Wavelength
 Delay
 Time 1
Action 1
Tube duration 1
 Time 2
Action 2
Tube duration 2
 Time 3
Action 3
Tube duration 3