

Chem 231 Quiz Number 2
Solutions

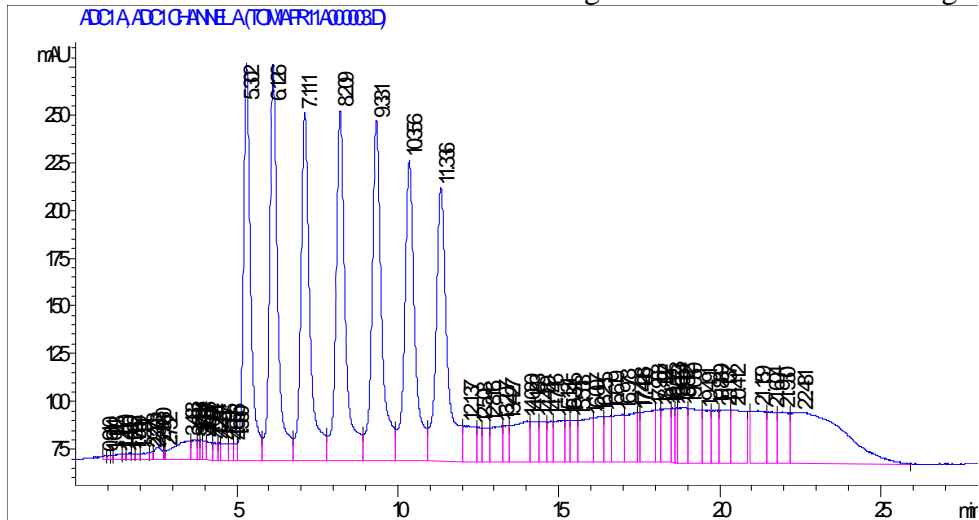
A. Short Answer/Multiple Choice Section (2 points each)

1. What is the name of the Agilent ChemStation program (e.g. Method, except that Method is not the correct answer) that allows you to run multiple samples, one after another using the autosampler. Program Name = SEQUENCE

2. A dye from a plant extract is being analyzed by thin layer chromatography. The chemist does not have a good knowledge of the polarity of the dye. What is an advantage to using thin layer chromatography over HPLC?

- a) higher resolution
b) better detection sensitivity
c) **faster means of finding its polarity**
d) greater peak capacity possible

3. What can be said about how the chromatogram shown below was integrated?



- a) it is pretty well integrated
b) some real peaks were missed by the integrator
c) too many noise peaks were integrated, but the sample peaks are well integrated
d) **the integration parameters were set too sensitive, resulting in integration of noise peaks as well as an artificially low baseline when peaks were integrated**

B. Calculations/Longer Answer. (6 pts)

1. A chemist is testing a method to use normal phase SPE cartridges to separate derivatized sugars from the unreacted derivatizing reagent (2-amino-benzamide or 2-AB). The derivatization reaction is carried out using glucose oligomers ranging from 1 to 4 glucose units (DP1 to DP4) in DMSO. The chemist traps the derivatized sugars (2-ABsugars) on normal phase SPE cartridges as 20 mL of the reaction solution passes through the SPE cartridge while the 2-amino-benzamide passes through. Then, using 4 mL 50% methanol/50% water solvent, the derivatized sugars are eluted. The table below

lists the results of the efficiency tests conducted. Given the relative polarity of the compounds (listed below), answer the following questions:

Most Polar

Least Polar

water < SPE phase < DP4 < DP1 < DP4-2AB < DP1-2AB < methanol ~ DMSO < 2-AB

| Compound | 2AB | DP1 | DP2 | DP3 | DP4 |
|-------------------|-----|------|------|------|------|
| μmol added | 500 | 10.0 | 10.0 | 10.0 | 10.0 |
| μmol pass-through | 489 | 0* | 0* | 0* | 0* |
| μmol eluted | 7 | 9.4 | 9.1 | 8.3 | 6.7 |

Note: DP1 means 10 mol of DP1 was added, but it is only detected as the derivatized product. Pass-through refers to what is collected at the bottom of the SPE column after adding the sample. Eluted refers to what is removed using the 50% methanol, 50% water solvent.

* No compound detected. Assume that any undetected amount is insignificant.

a) Is this method pretty effective at removing the 2-AB reagent from derivatized products?

Yes. Only 1.4 % of 2AB was eluted with the derivatized (2-AB-) sugars. Also, no detectable derivatized sugars passed through the SPE cartridges.

b) Suggest two possible reasons for losses of sugars and a way to tell the difference between the two losses.

1) Inefficient reactions. Reactions do not always go to completion.

2) Not removed from SPE cartridge. This seems more likely, since the most strongly retained products (DP4-2AB) shows the greatest losses.

One could check for either loss, although checking for removal from SPE cartridges would be easier. One could check for these losses by 1) switching conditions to improve SPE removal (use higher % water or larger volume of 50% water/50% methanol) and re-measuring moles eluted, 2) use a secondary removal solvent (e.g. an additional 4 mL of solvent) and check for elution. If one had a supply of reagent product, one could try the extraction on just the product.

To check for reaction losses, one could carry out the reaction under more favorable conditions and look for increases in amount eluted, or try to measure unreacted sugars using another method.