**HPLC Dry Lab & Questionnaire**

**Dry Lab Scenario: Part 1-Semi-quantitative mixture analysis**

A standard 1:1:1 volume mixture of benzene, toluene and ethyl benzene was prepared by delivering 5 mL of each component into a 100 mL volumetric flask and diluting to the mark with methanol to make the standard PE 5% Reference mixture

The standard mixture was then loaded into the 20 uL injector port of the Perkin-Elmer (PE) Nelson 410 LC (serial # 41 BN 2121405) equipped with a model 295 PE UV-VIS detector. A C-18 reverse phase, 2 inch, guard column was used to effect separation in a 30:70 mixture of water:methanol carrier solvent. The carrier flow rate was set a 1 mL/min and the analyzing wavelength was 260 nm using the FAST acquire spectrometer setting with A(min)=0.002. Average pump pressure is ~900 psi.

The following retention times and peak areas (Abs\*s units) are observed:

**Table 1: Retention Times (tr) and HPLC Peak Areas (A) Observed for 5% v/v Reference M ixture of Benzene/Toluene/Ethyl Benzene (1:1:1) Standard Using Conditions Described**

**tr(min) A(Abs\*s)**

**0.636 626068**

**0.807 856639**

**0.997 812537**

An unknown extraction containing some or all of the components in the standard is obtained next and a 20 uL sample of this unknown is loaded and run under the same conditions as applied to the 5 % solution of **Benzene/Toluene/Ethyl Benzene (1:1:1) Standard.**

The following retention times and peak areas (Abs\*s units) are observed:

**Table 1: Retention Times (tr) and HPLC Peak Areas (A) Observed for Unknown Extract in Methanol Obtained Using Conditions Described**

**tr(min) A(Abs\*s)**

**0.641 30021**

**0.800 76550**

**1.004 456032**

**Use the data provided above to estimate:**

**The absolute uL of each of the above components in the unknown 20** μ**L sample used.**

**Dry Lab Scenario: Part 2- Sensitivity Check**

A series of 4 standard dilutions (Dilute Mixtures #1-4) of ethyl benzene have been prepared in methanol with the % volumes listed in Table 3. The observed area A (Abs\*s) of their HPLC peaks at tr~ 1 minute are recorded using the conditions described in Part 1. Note that the lowest % volume has been measured 3 times so that a standard deviation can be computed.

**Table 3: Observed HPLC Peak Areas, A, for Various Dilute Concentrations of Ethyl Benzene in**

 **Methanol Using Conditions Described in Part 1 of Scenario**

|  |  |  |
| --- | --- | --- |
| **Dilute Mixture #** | **% ethyl benzene (v/v)** | **Aobs(Abs\*s)** |
| **1** | **0.050** | **8610** |
| **1** | **0.050** | **8334** |
| **1** | **0.050** | **8590** |
| **2** | **0.100** | **17001** |
| **3** | **0.200** | **33987** |
| **4** | **0.300** | **50980** |

**Use the data to estimate the absolute detection limit, DL(ng), of the HPLC to ethyl benzene under the conditions applied.**

The documentation in Experiment #6 to should be used to guide you in carrying out the analysis for both Part 1 and Part 2 of this scenario. Note that you are to hand in just the RESULTS section of what would have been Lab #6. (See page 6 of Lab #6).

**Chem 6614 Instrumental Methods of Analysis HPLC Technique Questionnaire 10 pts**

**Your name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

1. What pushes the carrier in an HPLC ? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
2. Why is the UV range of the detector most useful in HPLC analysis of organics ?

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1. A fixed solvent mixture HPLC run is referred to as a(n) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ run.
2. Why is a blunt syringe employed when loading a sample into the HPLC ?

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1. What device is used to remove the sinusoidal pressure variations intrinsic to HPLC ?

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. What is being referred to in the HPLC column specification “300 $Å$”\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
2. What kind of carrier solvent is appropriate in a normal phase HPLC run ?\_\_\_\_\_\_\_\_\_\_\_\_\_\_
3. Name two ways to improve (or at least change) the `SSS’ (Sharp-Symmetric-Separated)

character of an HPLC chromatogram.

1)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

2)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. Why is HPLC more prevalent than GC in pharmaceutical labs for separating and analyzing mixtures ? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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