|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| peak ID | tr(min) | A(Abs\*s) | V% | absolute Vo(uL) | γk =A/V (Abs\*s/uL) |  |
| benzene | 0.636 | 626068 | 5 | 1 | 626068 |  |
| toluene | 0.807 | 856639 | 5 | 1 | 856639 |  |
| ethyl benzene | 0.997 | 812537 | 5 | 1 | 812537 |  |
| reference mixture data | |  |  |  | reference peak sensitivity | |

Unknown mixture analysis using derived γk

%Pk = 100\*Vk/20

0.240

0.447

2.80

|  |  |  |  |
| --- | --- | --- | --- |
| peak ID | tr(min) | Ax (Abs\*s) | Vk=Ak/γk (uL) |
| benzene | 0.641 | 30021 | 0.0479 |
| toluene | 0.8 | 76550 | 0.0894 |
| ethyl benzene | 1.004 | 456032 | 0.561 |

Unknown extract data

|  |  |  |  |
| --- | --- | --- | --- |
| mixture | V% | V(uL) absolute in 20 uL sample | Aobs(Abs\*s) |
| 1 | 0.05 | 0.01 | 8610 |
| 1 | 0.05 | 0.01 | 8334 |
| 1 | 0.05 | 0.01 | 8590 |
| 2 | 0.1 | 0.02 | 17001 |
| 3 | 0.2 | 0.04 | 33987 |
| 4 | 0.3 | 0.06 | 50980 |

Ethyl benzene Sensitivity check data =V

Analysis for D.L.

The standard deviation of the 0.01 uL A(Abs\*s) =s=153.9~154

To compute D.L. we use the recipe:

D.L. = 3\*s/m where m is the slope of the fit of A(Abs\*s) vs V(uL)

∴D.L. =3\*154/849480=5.439\*10-4 uL

Conversion to L=> 5.439\*10-10 L = 5.439\*10-7 mL

The density of Ethyl Benzene is given as 0.866 g/mL so the equivalent mass of ethyl benzene detected is:

0.866 g/mL \* 5.439\*10-7 mL =4.71\*10-7 g =481 \*10-9 g= 471 ng

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

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

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

\_\_\_\_most organic compound absorb strongly in the uv, making it a good wavelength range to fix the analysis at\_\_

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

\_\_\_\_\_\_avoids tearing the inlet system’s Teflon pressure gaskets\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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

\_\_\_pulse damper\_\_\_\_

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

character of an HPLC chromatogram.

1)\_\_\_\_\_\_\_change solvent mix and schedule\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

2)\_\_\_\_\_\_\_\_\_change column\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. Why is HPLC more prevalent than GC in pharmaceutical labs for separating and analyzing mixtures ? \_\_\_\_many pharmaceutical and biological compounds cannot withstand the high temperatures used in standard GC. The use of room temperature HPLC where the solvents can dissolve, but not destroy these kinds of problems explains the prevalence of HPLC in pharmaceutical labs\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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