GFORSS LEARNING 

Food Safety Laboratory Capacity Building

Evaluation 2 – Answer Key

1. Please describe the concept of "fit-for-purpose" for an analytical method in a regulatory food safety laboratory.

The fit-for-purpose approach is the assessment and capacity of a test method to show the aptness of a given methodology to provide answers or test results to a set of requirements as regulatorily mandated. This concept or approach covers the methodology to be used, the range of equipment, technologies, and facilities necessary to achieve the desired outcome. This means for any analytical result to be fit for its intended purpose, it must be sufficiently reliable to enable the user to make the correct technically and administratively decisions. Thus, the selected method performance must be validated, and the uncertainty estimated within a given level of confidence.

2. What differentiates direct and reverse phase liquid chromatography?

Direct phase liquid chromatography using a polar stationary phase (e.g Silica) and a non-polar mobile phase (hexane) the reverse phase liquid chromatography uses a non-ploar stationary phase (C18) with polar mobile phase. The non-polar molecule elutes faster than the polar molecule for direct liquid chromatograph while the opposite is same for reverse phase liquid chromatograph.

3. Please describe the difference between an isocratic and a gradient run in LC?

Isocratic utilizes a single mobile phase composition or uses a time invariant-eluent composition through out the LC. Gradient elution uses changes the polarity of the mobile phase though the LC. The change in the solvent strength is normally a function of time. Gradients can be binary, ternary and quaternary solvent mixtures in which solvents are blended to achieve the proper strength.

4. In GC equipped with a vaporization injector, what is the part that needs cleaning most often, and describe its purpose.

The injector liner. This is to remove the accumulation of minor deposits or residues from samples especially during routine analysis. This further prevents the transfer of dirt into part of the GC which are harder to clean which will eventually cause leaks in the system.

5. Please list at least one characteristic of a sample that makes it amenable to be analyzed using the following detectors:

a. Fluorescence

- Fluorescent compound
- Ability to emit higher wavelength radiation or fluorescence when excited by shorter wavelength energy

b. UV/VIS or PDA (you can choose)

- Should be Chromophoric
- Able to absorb light at specific wavelength

c. Mass spectrometer

- Ionizable compounds
- Molecular mass weight (charge-to-mass ratios)

d. ECD

- Electron-absorbing Halogenated hydrocarbons
- High electronegativity

6. How can you reduce the back pressure in a liquid chromatograph (please provide at least 2 options)

a. Degassing or removing bubbles from the mobile phase

b. Backflushing the HPLC column

c. Using guard columns or inline filters (changing of columns or inline filters should be part of the

d. Filtering mobile phases with a microfilter prior to running the chromatograph

e. Filtering samples prior to injection

7. Please describe at least one impact of increasing the column length in chromatography (gas or liquid)

a. Improves or increases the resolution due to more or better interaction between the molecules, mobile phase and stationary phase. The "added time" effect.

b. Increases column efficiency.

8. Please describe at least one advantage and one disadvantage of using a column with smaller particles in LC.

Advantage:

- Separates more effectively and improves resolution.
- Improves the S/N ratio due to the narrower and tall peaks is produces.

Disadvantage

- Relative increase in mobile phase back pressure due to the flow of the mobile phase through very small interstitial spaces.

9. Please list 3 types of additives that can be used in LC and their purpose.

Acetic acid (glacial)

- Establish and maintain acidic conditions of the mobile phase

Formic acid

- Provides a suitable environment for the ionization of the analytes in the MS and to maintain/control of pH to ensure reproducible retention times.

TFA (Trifluoroacetic acid)

- Used in separation of biological molecules where it functions an ion-pairing reagent and its ability to equilibrates swiftly makes its suitable for gradient elution.

10. Please explain why standards must be analyzed as part of our routine analytical batches

Due to the likelihood of the response of the instrument to change, drift, or shift, it is important to ensure correct qualitative and quantification of analyte or contaminant especially in regulatory purposes. This requires that the retention times of the standards ran on the same instrument, using the same experimental parameter (e.g column, column temperature, mobile phase, flow rate etc) and on the same day matches the samples analyzed in a batch(s).