GFORSS LEARNING

Food Safety Laboratory Capacity Building

Evaluation 3 – Answer Key

1. Please list two (2) strategies that use solvents only in HPLC to improve the separation of compounds

- Solvents are the mobile phase used in HPLC for separation of compounds or analytes. Hence, the polarity and composition of the solvent used helps to improve separation of compounds. Thus, one strategy to improve the separation of compounds is to change or alter the solvent (mobile phase) composition or constituents either in isocratic or gradient run to increase the value of the ratio of the capacity factors for closely eluting peaks thereby improving separation.
- Another strategy is to consider the miscibility of the solvents to be used whether in isocratic run or gradient run. In an isocratic run, the actual composition of an immiscible solvent will vary depending on the depth of the bottle containing the solvent while in gradient run, an immiscible solvent that could be used must be separated by a transition into a solvent with same compatibility.

2. Why is there a difference in the quality of water used between HPLC-FLD and HPLC-MS?

The difference in the quality of water used between HPLC-FLD and HPLC-MS is dependent on the type of detector used. The principle of FLD is fluorescence while MS is ionization. Therefore, distilled (ultra pure) water can be used on HPLC-FLD without interference or diminishing the florescence of the sample but reverse is the case for HPLC-MS if distilled (ultra pure) water is used because ions present in the distilled water would be ionized and could either suppress or enhance the MS signal. Hence, the quality of water used in HPLC-MS is deionized water that is free from ions.

3. Please match the terms in the three columns to describe how you may use the Actions in column 1 and parameters in column 2 to reach the outcome from column 3. Please use each parameter and outcome only once (note: there are many correct answers here)

Action	Parameter	Outcome
Increase	Column length	**Improve separation**
Decrease	Particle size	**Reduces analysis time**
Increase	Solvent polarity	**Shorten isocratic run**
Decrease	Acidity	**Improve ionization**
Increase	Column temperature	**Reduce pressure in LC**
Decrease	% organic solvent	**Increase sensitivity**

4. We generally agree to let laboratories select the best fit-for-purpose methods for their laboratory based on performance parameters (as opposed to imposing methods). Please describe two (2) acceptance criteria that apply to a method using HPLC. (Note: your answer should list 2 acceptance criteria with real numbers, the reference for these criteria and a brief explanation of why this criteria matters)

Potential List: Trueness, applicability, limit of detection, limit of quantitation, precision, repeatability intra-laboratory and inter-laboratory reproducibility, recovery, selectivity, sensitivity, linearity and many others

5. Please complete the sentences below by selecting one of the options and explaining why.

a. Multi-residue methods are validates using (the same / different) acceptance criteria as single residue methods

Answer: Same

Why: Each individual residue of the multi-residue method must meet the acceptance criteria or requirements of the single residue method in terms of recovery, residual standard deviation etc. Also, there is need for verification of all residues in the multi-residue method, if the method is adapted to accommodate more residues

b. Analytes can be included in a multi-residue method if they have similar (polarity / pH / hydrophilicity / collision energy / miscibility)

Answer: hydrophilicity

Why: This is because all the analytes of interest in the sample will undergo the same preparation method, clean up or purification procedure without loss of any analyte of interest as well as same measurement technique

c. The dwell time in MS (increases / decreases) when more analytes are added to a multi-residue method

Your answer: Increases

Why: It would enable the MS to produce high signal to noise ratio that is needed for better separation of the analytes and also to produce narrow and high peaks.

d. I am in the United States. I need to perform a matrix-extension method validation to analyze spinach for pesticide residues because the multi-laboratory validation for the official method was done with lettuce (true / false)

Your answer: False

Why: Spinach and lettuce falls under the same high water content commodity group, typical commodity categories of leafy vegetables and fresh herbs and under same typical representative commodities.

6. Please describe the difference between high resolution mass spectrometry (HRMS) and regular mass spectrometry that justifies why laboratories are using this instrumentation to screen for unknowns.

Some of the reasons that justifies why laboratories are using High Resolution Mass Spectrometry (HRMS) to screen for unknowns are:

- Its applicability covers a wide mass range and it has a high mass accuracy and mass resolution that offers a degree of certainty for identified compounds than regular mass spectrometer.
- Its ability to measure mass to charge ratios (exact mass) to four or more decimal places and isotopic distribution unlike regular mass spectrometer that measure to the nearest whole numbers (nominal mass) makes it possible to simultaneously analyze unlimited number of compounds using its full scan data mode that corresponds to specific compounds thus enhancing selectivity and specificity
- Also, in using HRMS, there is no need to run standards of all possible unknowns because of its high mass accuracy which has a spectral library search for identification of unknown compounds unlike the regular mass spectrometer where standards are ran for identification by comparing the precursor and product ion ratio
- Lastly, HRMS promises to be a technique that could help to test for certain new contaminants and also to understand the time these new contaminants begin to come out in food commodities.

7. Please list and describe three aspects of a quality assurance plan, how they are controlled and how they contribute to the assurance of the reliability of the data.

Three aspects of quality assurance plan that should be considered in a laboratory are:

i) Personnel (staff) of the laboratory

Personnel (staff) of the laboratory refers to employee(s) who are engaged by the laboratory management to carry out duties and responsibilities that are in line with the primary objective of the laboratory set up.

- The personnel (staff) of the laboratory can be controlled when the number of personnel (staff) of the laboratory are documented and this documentation should have among other things; formal qualifications of each staff; date of recruitment of each staff and previous experience; verification of each staff qualifications through certificate inspection or other evidence which could be carried out by the personnel department or laboratory management.
- Also, there should be documentation of all training activities (*test and calibrations for customers; sample receiving etc*) for which any staff has undergone and regular re-assessment of each staff's competence in each activity where they have been trained.
- The personnel (staff) contributes to the assurance of the reliability of the data generated from the laboratory because the documentation of their training(s) provide reference to supervisor(s) who assign task to any staff, ensures that such staff has adequate training and competence for the task assigned to him/her and that the training of the staff is up to date. Also, it helps to trace all raw data generated to a staff assigned any task because of the documentation. In other words, it helps to audit raw data generated from the laboratory to any staff assigned specific task based of the type and level of training obtained.

ii) Test and calibration methods used in the laboratory

Test and calibration methods used in the laboratory refers to appropriate methods published by reputable technical organizations (ISO, ASTM, AOAC etc) as national, regional or international standards or methods that meets requirements any person or organization patronizing the laboratory. These methods are documented in the laboratory and made accessible for staff to use.

- These test and calibration methods are controlled when the staff strictly adhere and comply to the steps and procedures contained in these standard methods (ISO, ASTM, AOAC or verified standards published in scientific literatures). Therefore, any report generated from the laboratory must specify the exact method that was used and also indicate where there are any variations or deviations from the standard procedure.
- The reliability of the data generated are assured because the laboratory uses certified reference materials (CRM), spikes, calibration reference, interlaboratory comparison of tests results which are contained in these methods as procedures for validation by accreditation bodies.

iii) Equipment used in the laboratory

Equipment used in the laboratory refers to any item which may affect the validity of measurements or calibrations, including reference standards of measurement, such as standard weights and reference thermometer as defined in ISO 17025 practical guidebook.

- The efficiency of the equipment in the laboratory can be controlled when there is a record or documentation of complete history of every equipment installed or used in the laboratory. Also, when there is a record of detailed checks and calibration done on the equipment before its use as well as detailed records of all repairs, calibration, routine maintenance and performance check of the equipment. Consequently, the functionality of all new equipment installed or to be used should checked against the manufacturer's specifications and checks.
- The equipment can contribute to the assurance of the reliability of the data when installation, usage, servicing and preventive maintenance are done as recommended by the manufacturer.