Antibiotic Residues in Dairy Manure

Part 4: Laboratory methods for analyzing antibiotic residues extracted from dairy manure

Methods to analyze environmental samples for pharmaceuticals have advanced significantly since approaches were first developed in the 1980s. Today, *liquid chromatography integrated with tandem mass spectrometry (LC-MS/MS)*, and *enzyme-linked immunosorbent assay (ELISA)* are the most commonly employed.

LC-MS/MS

LC is a technique used to separate a dissolved sample (**Figure 1.A**), based on a compound's affinity for the liquid-phase (solvent it is dissolved in) and sorption to the solid-phase (the column). As the solvent is moved through the column, compounds with high affinity for the liquid-phase flow through the column more quickly (small retention time). Compounds with high affinity for the column flow through more slowly (large retention time). Detectors can be used to measure the intensity (concentration) of different groups of compounds (detected as 'peaks') that elute from the column at the same time.

MS/MS (**Figure 1.B**) is a technique that more finely fractionates these mixed compound peaks to resolve individual chemicals. It consists of a collision cell between two mass spectrometers (denoted as MS-1 & MS-2). Mass spectrometers utilize electromagnets (depicted as grey bars) in vacuum chambers to deflect charged chemicals as they pass. The strength of the electromagnetic field and mass of the compound dictate if the deflection of a particular chemical prevents, slows, or enables the chemical to pass through the chamber.

As a compound peak from the LC enters MS-1, the chemicals in this peak (colored arrows) are ionized (charged) and the electromagnets are powered to enable the selection of the target chemical (green arrow), and to prevent other non-target chemicals from entering the collision cell. In the collision cell argon gas is used to fragment the target chemical into its chemical building blocks (green shapes). The electromagnetic field of the MS-2 is then used, not to select, but to sort these fragments based upon their mass to charge ratio. A detector measures the intensities of these fragments and generates a mass specta - a sort of 'fingerprint' - for each peak (**Figure 1.C**).

While able to detect antibiotics in complex mixtures at very low concentrations (ppb), LC-MS/MS operation is technical and time consuming. To successfully target, identify and quantify specific chemicals based on mass spectra requires sophisticated computational analysis and operator training, the mass spectra of standard chemicals, and knowledge of the target chemical structure and its breakdown products. Operation and interpretation of data is often limited by the lack of standard chemicals, incomplete knowledge antibiotic degradation patterns, and the complexity of mass spectra from environmental samples like manure. While LC-MS/MS is a powerful tool, its expense, technical limitations and long run time have limited its development for antibiotic analysis of manure.

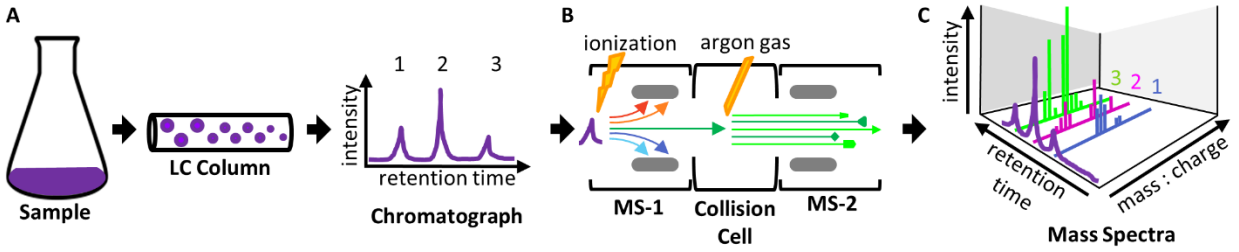

Figure 1. Overview of LC-MS/MS.

ELISA

ELISA is a technique that utilizes multi-well plates to screen for antibiotics in liquid extracts. While multiple ELISA techniques exist, 'competitive ELISA' is typically used for antibiotics. In each well of the plate, antibodies designed to uniquely bind to a specific chemical target (analyte) are immobilized. As a sample is added, analytes in the extract will bind to the antibody, and sample impurities can be washed out. Next, fluorophores (chemicals that emit fluorescent light when excited with a specific wavelength of light) designed to selectively bind to the antibodies are added to bind to the remaining antibodies. The sample is washed and bound fluorophores are excited with the specific wavelength of light and the emission is measured. As the analyte *competes* with the fluorophore for the antibodies, measured fluorescence is inversely related to analyte concentration (**Figure 2**).

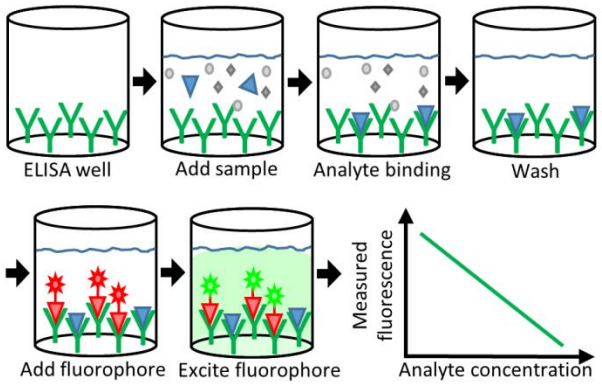

Figure 2. Overview of ELISA.

While ELISA is a rapid test, non-specific and /or weak binding to the antibodies can limit detection limits and applicability to complex samples like manure. If, for example, the sample contains antibiotic breakdown products or sample impurities that have similar chemical structure to the analyte, these compounds may compete with the analyte for antibody bonding sites, and skew analyte measurement. While ELISA results may be highly reproducible in milk, meat, urine and plasma samples, complex manure samples may be more likely to result in un-targeted binding. Research is ongoing to understand the limits of this technique to antibiotic detection in manure.

Other emerging technologies

The most noteworthy development in the detection of pharmaceuticals in environmental samples has been the advent of biosensors. Biosensors are technologies that use living cells or biomolecules with optimal affinity and specificity to an analyte as a receptor. By integrating this receptor with a specific signal transducer the interaction between the receptor and the analyte induces a measureable signal. Biosensors may soon be developed that have LC-MS/MS sensitivity and the throughput of ELISA, or even real-time measurement of environmental samples. While various biosensor designs are showing great promise, their application to antibiotic detection in manure has yet to be demonstrated.

FACT SHEET SERIES
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Part 2: Sampling dairy manure for antibiotic detection
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Part 5: What is known about antibiotic residues in dairy manure?

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