

## Antibiotic Residues in Dairy Manure

### Part 3: Laboratory methods for extracting antibiotic residues in dairy manure

#### Introduction

Antibiotic residues must first be extracted (or separated from manure), then purified, so that they can be accurately measured. Just as there is no standard approach to sample manure for antibiotic residues, there is also no standard method for extracting antibiotic residues from manures. The challenge to developing such standards is that depending on the target antibiotic and the exact composition of the manure, the extraction approaches could vary widely. The approach developed by our collaborators<sup>[1]</sup> was optimized to target multiple classes of antibiotics from dairy manure is outlined below.

#### Extraction efficiency & surrogates

Extraction efficiency is the amount of chemical recovered from an extraction divided by the total amount of the chemical in the sample. As no method will reliably extract 100% of an antibiotic residue from manure, it is important to quantify the extraction efficiency so that measurements can be corrected for un-extracted antibiotic that remain in the sample. As the total amount of an antibiotic residue in a sample is unknown, surrogates are used to calculate extraction efficiency. A surrogate is a compound of very similar chemical structure to the target compound's, but is not found in the sample, or is the exact target antibiotic with a unique radioactive label, the latter being the method used by our collaborators<sup>[1]</sup>. Before extraction, a known amount of a surrogate is added to the sample. After extraction and clean-up, the concentration of this surrogate is measured. Based upon the known amount added, the extraction efficiency of the surrogate is determined. The concentration of the target compound is then corrected for extraction loss assuming the target antibiotic extraction efficiency equals that of the surrogate. There

is no exact threshold for acceptable extraction efficiencies, often averaging around 40%, though optimally they can exceed 80%.

#### Extraction

Most antibiotics are large, non-volatile compounds that are best extracted by liquid solvents. The solvent used and conditions of the extraction procedure both strongly influence extraction efficiency. First freeze dried samples are diluted into a pH adjusted solvent-aqueous buffer mixture. Typically, polar solvents like methanol or acetonitrile are used as they have higher affinities for antibiotics than non-polar solvents. Acidic pH is typically used to minimize adsorption of antibiotics to the sample or sample containers. Samples are then mixed with this solvent, centrifuged to separate the liquids from the manure solids. The liquid solvent with extracted antibiotics is then collected into a new tube. Extractions are often repeated to improve recovery rates. Our collaborators use a mixture of acetonitrile and methanol in aqueous buffer acidified to pH 4 to extract manure. Sample are briefly vortex mixed, then ultrasonicated (use of ultrasound energy to agitate) to free antibiotics bound to the manure. This is repeated three times, and extracts are combined prior to clean-up<sup>[1]</sup>.

#### Purification/Clean-up

Impurities are often extracted alongside the target antibiotics and need to be removed ('cleaned-up') prior to extract analysis. Solid phase extraction (SPE) cartridges are the most efficient and effective way to quickly clean-up a sample extract. SPE cartridges are essentially a syringe half filled with a finely porous (~0.5 µm) filter media that selectively separates compounds dissolved in a liquid extract based on their physical and chemical properties. Different SPE cartridges can be

used in tandem to enable the separation of different types of impurities from a target compound at the same time. Our collaborators use two SPE in tandem to remove impurities from cow manure extracts<sup>[1]</sup>. To prevent unwanted sample loss in an SPE, the cartridges first have to be wetted with the solvent (Figure 1.A). Typically, a vacuum is applied via a pump to draw the solvent through the cartridge and into a vacuum flask. Once the cartridges are wet, the sample extract (purple) can be drawn through the cartridges. The approach used by our collaborators is to select the 1<sup>st</sup> SPE cartridge so that it captures a particularly abundant class of impurities, but enables the target antibiotics to pass through.

The 2<sup>nd</sup> SPE cartridge is then selected to capture the target antibiotic and allow other less abundant impurities to pass through (Figure 1.B). Once the targets have been captured onto the 2<sup>nd</sup> SPE, it can be transferred to a new vacuum flask that contains a sample vial. As the ability of the 2<sup>nd</sup> SPE to capture the target antibiotics and allow impurities to pass is solvent dependent, a different solvent can then be used to free the bound target antibiotics, and the vacuum pulls the sample through the SPE and into the sample vial (Figure 1.C). The sample vial can then be removed, capped and is ready for analysis and quantification of recovery rates and antibiotic concentrations (Figure 1.D).

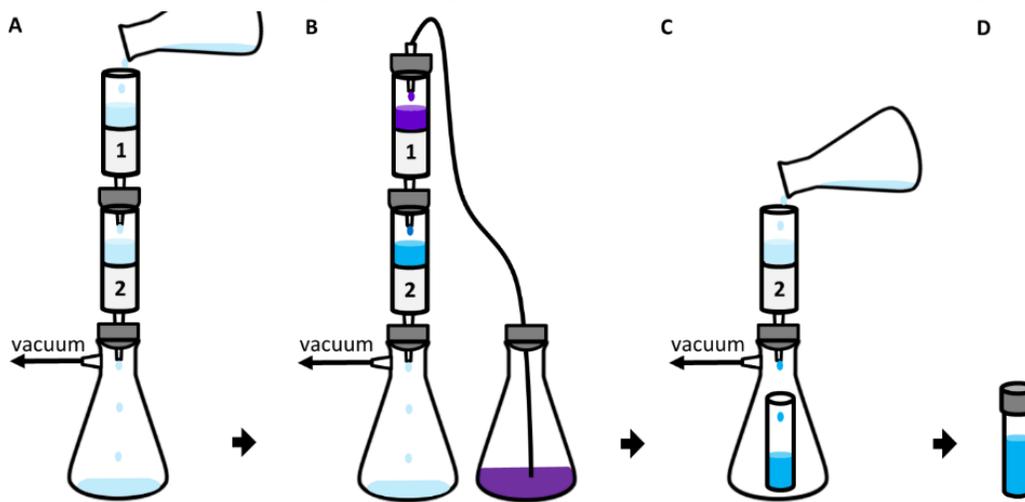


Figure 1. Schematic of sample purification/clean-up using SPE.

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**REFERENCES**

- <sup>[1]</sup> Wallace, J.S. & D.S. Aga. 2016. Enhancing extraction and detection of veterinary antibiotics in solid and liquid fractions of manure. *Journal of Environmental Quality* 45(2):471-479.



This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2016-68003-24601. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.