Analyzing Select Antibiotics in Commercial Biosolid Fertilizers With Liquid Chromatography Tandem Mass Spectrometry

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INTRODUCTION

The purpose of this research is to apply developed LC-MS/MS methods, such as U.S. EPA method 1694, to OTSC standard operating procedures, in order to characterize and quantify multiple antibiotics potentially found in commercial (Class A) biosolid fertilizer samples, which are regularly tested at OTSC for heavy metal contamination. A narrow field of antibiotic compounds with diverse physiochemical properties was chosen to test optimization of recovery from notoriously complex biosolids. Prior research studies have detected low concentrations of ciprofloxacin, tetracycline, and other pharmaceutical classes in Class A biosolids; it is expected through thorough method development we will be able to analyze them for quantification on LC-MS/MS.

BIOSOLIDS BACKGROUND

Biosolid fertilizers derived from the solids pulled from wastewater influent are known to improve land productivity. Raw sewage solids must meet U.S. EPA standards of pathogen treatment before they are designated as biosolids and applied or sold to the public. Their benefits include stabilizing erosion, sequestering carbon into soils, and reducing greenhouse gases when used to replace synthetic fertilizers. There is concern that pharmaceuticals, personal care products, or other synthetic chemical residues in the biosolids could adversely affect the land and those who apply them or consume goods produced with the aid of these fertilizers. The spread of antibiotic resistant bacteria is also a potential issue with certain biosolids. The majority of these emerging pollutants are not regulated or monitored, and are usually only identified by the EPA through open literature reviews and sewage sludge surveys.

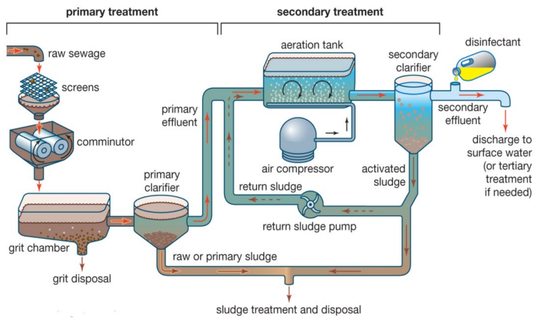


Fig. 1 Typical wastewater treatment process (Fig: Encyclopedia Britannica)

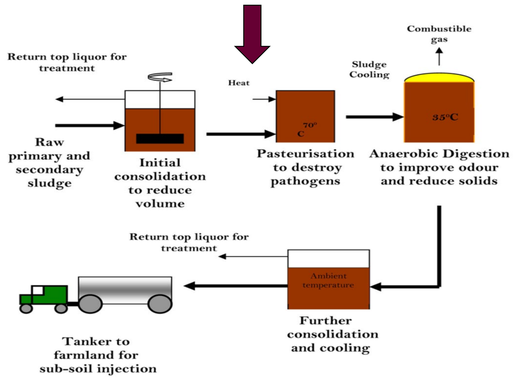


Fig. 2 Typical Biosolid processing (Fig FWR Sewage Treatment Co.)

In wastewater treatment plants, many drug residues are retained by the activated sludge, or, in their more hydrophilic form, remain in the water phase and return to the aquatic environment (**Fig. 1**).

There are differing standards of processing and acceptable levels of various contamination depending on the final purpose of the sludge. During biosolid processing, anaerobic digestion reduces the amount of final solids and inactivates most of the pathogens present in the sludge (**Fig. 2**). However, levels of most pharmaceuticals, personal care product residues, and industrial chemicals entering the wastewater system are not regulated in waters or soils, nor are there standardized methods to test for most of these compounds. Hence, many laboratories develop in house techniques of testing for these chemical residues.

ANTIBIOTIC SELECTION

Table 1. Selected physiochemical data for common antibiotics

Antibiotic	AB Class	Molecular weight	pKa	Solubility (mg/mL)	Log Kow (lipophilicity)	Notes
Ciprofloxacin	Fluoroquinolone	331.3	3.0, 6.1, 8.7	< 1	0.3	Most fluoroquinolones are hydrophilic, insensitive to hydrolysis and increased temperature, susceptible to photo-degradation. They contain amino group in the heterocyclic ring.
Sulfamethazine aka Sulfadimidine	Sulfonamide	279	2.1, 7.5	1.5	0.89	Sulfonamides contain one basic amine and one acidic sulfonamide group. Two pKa values: positively charged at pH 2 and 5, and negatively charged at alkaline conditions above pH 5. Know to have poor soil sorption; mobile in soils and easily leach into waters.
Tetracycline	Tetracycline	444.4	3.3, 7.7, 9.7	0.23	-1.3	Tetracyclines chelate onto metal ions to form stable bivalent & trivalent cation complexes in soils and sludge: not highly mobile in these matrices. They have 3 pKa values: cationic, zwitterionic, or anionic species. Light-sensitive, and stable in acids but not bases.

The antibiotics proposed for analysis are based on research literature detailing consumption rate (human and veterinary), frequency and concentrations found in solid agricultural and environmental samples, and potential toxicity. Tetracyclines, (fluoro)quinolones, and sulfonamides are the top three antibiotic classes found in solid samples, but even individual drugs within the classes display wide ranges of polarity, solubility, and ionizable functional groups (**Table 1**). Unlike neutral organic pollutants, the sorption and mobility of antibiotics in soil is not directly related to their octanol-water partition coefficient (K_{OW}) or solubility due to these ionic properties. To optimize analyte recovery, compounds of differing properties are often extracted and run in separate groups.

INSTRUMENTATION AND PROCEDURE

It is generally agreed that liquid chromatography-mass spectrometry (LC-MS/MS) instrument methods are preferred to achieve sensitivity and accuracy with multi-residue methods for analytes which are less volatile and more polar. Performance criteria, especially analyte recovery and detection limits in the range of ng/L^{-1} for the particular LC-MS/MS used, are critical to evaluate suitability of procedures for producing valid and precise results. To correct for interfering matrix effects common to complex environmental matrices, isotope-labeled internal standards will be used for at lest two of the analytes. An extraction method adapted from EPA Method 1694 is currently being optimized, compatible with our laboratory capabilities and instruments. The procedure involves three rounds of ultrasonication with acetonitrile solvent added to an acidic-buffered biosolid sample. The solvent is dried off, then the sample is reconstituted with water and run through a solid phase extraction clean-up cartridge—the intended result is to concentrate the antibiotic compounds and reduce the distorting matrix effects of high-organic content samples like biosolids.

Fig. 3 Waters Acquity UPLC I-Class FTN and Xevo TQD Mass Spec

METHOD DEVELOPMENT

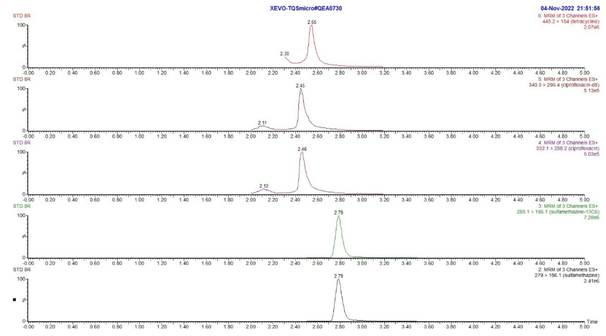
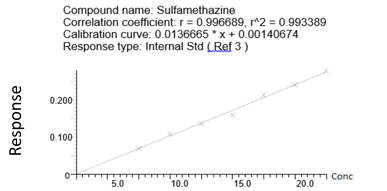
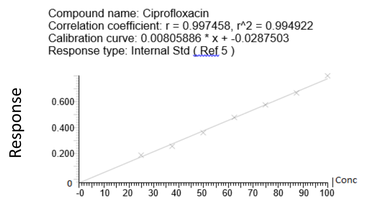


Fig. 4 Chromatograms of quantitation ions for three target antibiotic compounds and two internal standards.





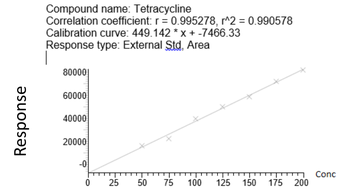


Fig. 5 Recent analyte calibration curve examples demonstrating method sensitivity

Validation will be the next step of the method development, followed by testing dozens of commercial biosolid samples, collected for OTSC, to determine potential detectable quantities of the target antibiotic compounds.

Pure standards of ciprofloxacin, tetracycline, and sulfamethazine are successfully separated by LC and linear calibration curves established. To date, spike-recovery studies optimizing the sample preparation and extraction method have resulted in sulfamethazine recovery between 91-97%, ciprofloxacin between 75-96%, and tetracycline between 45-71% when calculated by comparing the peak area of measured concentration to the peak area of the spiked concentration. After slight adjustments to improve the performance of tetracycline are made, we have high confidence this method will meet all OTSC performance acceptance criteria.

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