Challenges in Antibiotics Analysis in Environmental Samples

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Excreted antibiotics from human and veterinary use are introduced into the environment because they are not completely eliminated in treatment systems

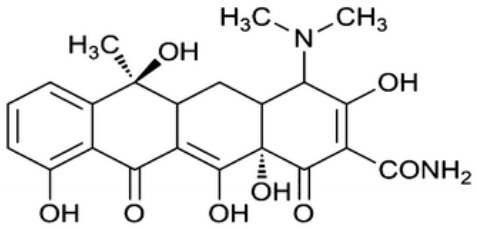
What Antibiotics Should We Analyze For?

Class	Annual Total (Kg)
Tetracyclines	6,514,779
Ionophores (e.g. monensin)	4,434,657
Penicillins	828,721
Macrolides	563,251
Sulfonamides	384,371
Aminoglycosides	270,342
Lincosamides	236,450
Cephalosporins	28,337
Others (e.g. Polypeptides, Streptogramins)	1,527,646

US Food and Drug Administration, **2015**. *Summary Report: Antimicrobials Sold or Distributed for Use in Food- Producing Animals.*

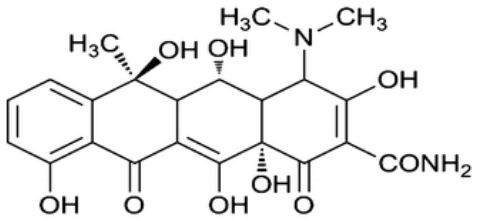
Importance of Transformation Products: Tetracyclines

(a)



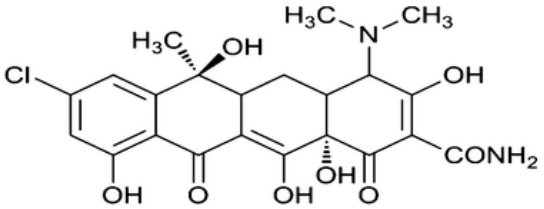
Tetracycline

(b)

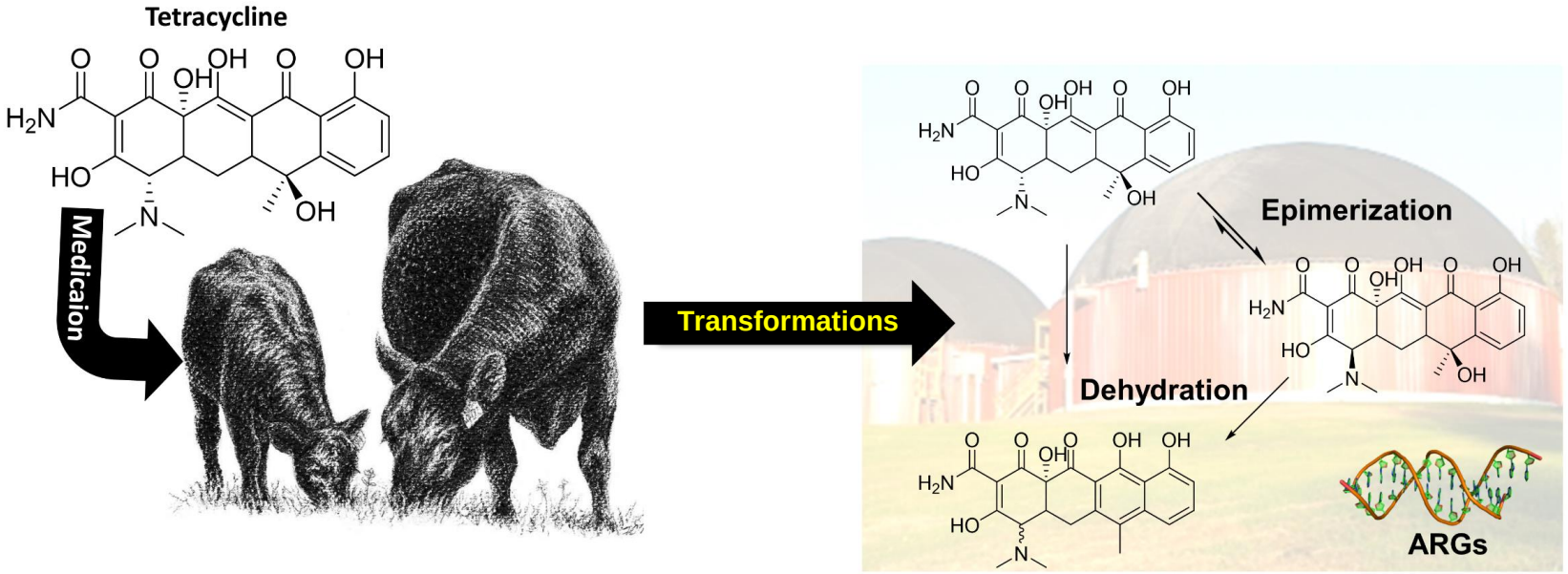


Oxytetracycline

(c)

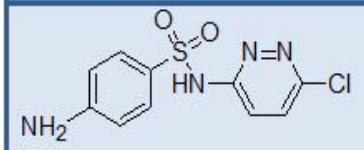


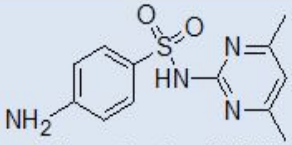
Chlortetracycline

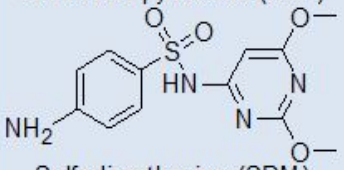


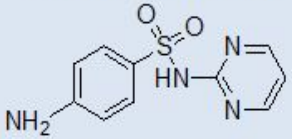
Importance of Metabolites: Sulfonamides

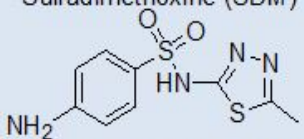
Sulfonamide Antibiotics

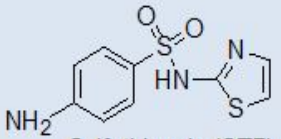

Sulfachloropyridazine (SCP)

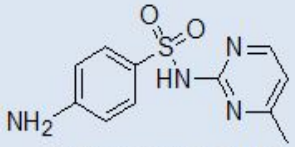

Sulfamethazine (SMZ)

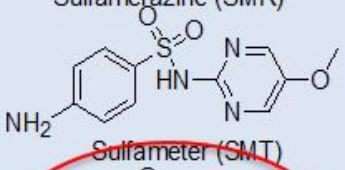

Sulfadimethoxine (SDM)

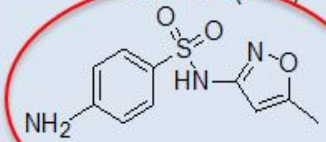

Sulfadiazine (SPD)


Sulfamethizole (SMI)

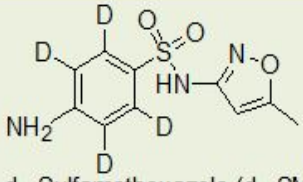

Sulfathiazole (STZ)

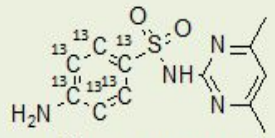

Sulfamerazine (SMR)

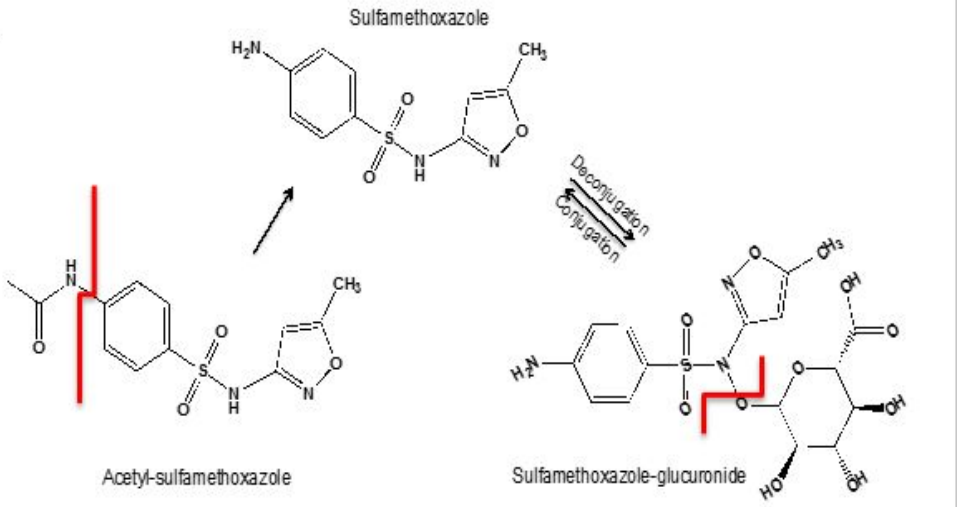

Sulfameter (SMT)


Sulfamethoxazole (SMX)

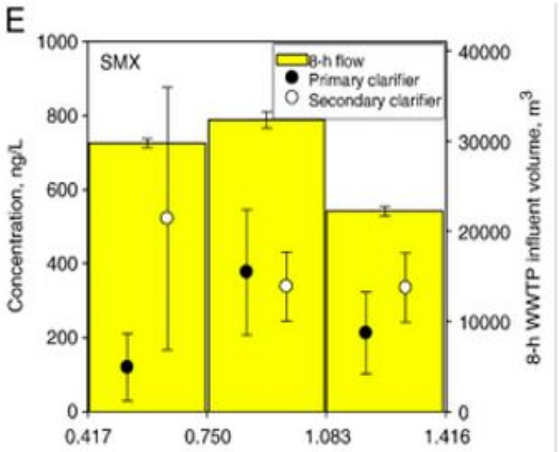
Surrogate Standards


d_4-Sulfamethoxazole (d_4-SMX)


Phenyl-$^{13}C_6$-Sulfamethazine (SMZ)



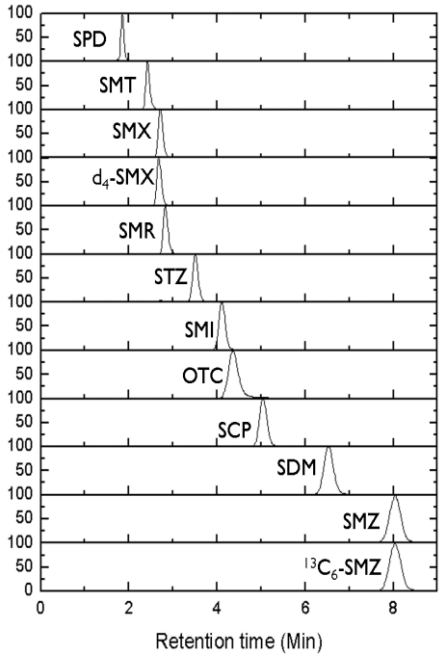
Increase in concentration after wastewater treatment



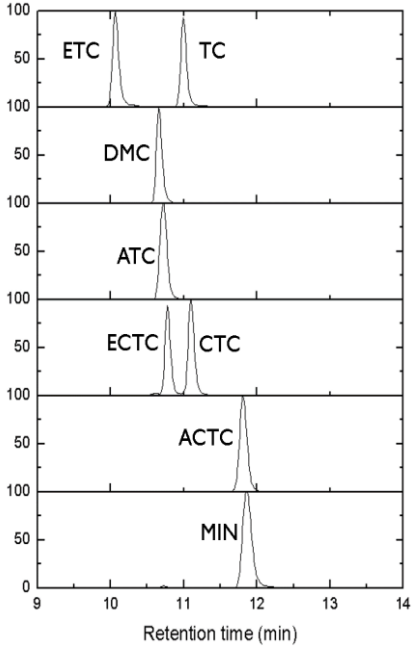
Analytical Techniques

Liquid chromatography/mass spectrometry (ion-trap, triple quad, Q-ToF, Orbitrap) vs. Biological Assays (ELISA, microbial activity assay)

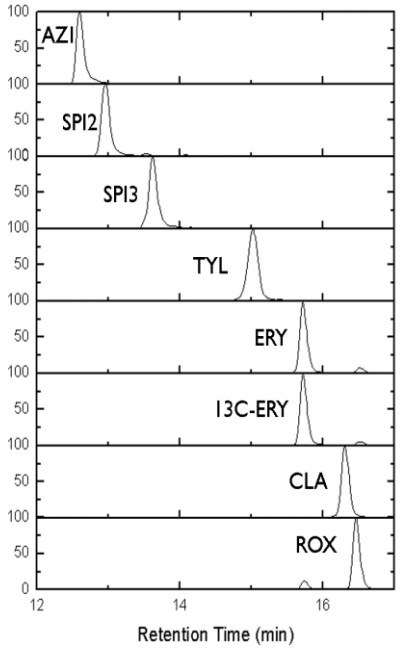
sulfonamides

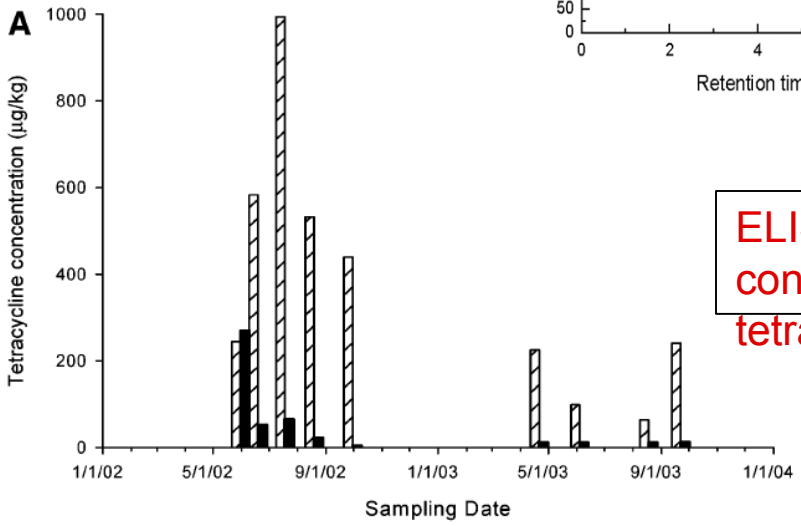


Tetracyclines



Macrolides



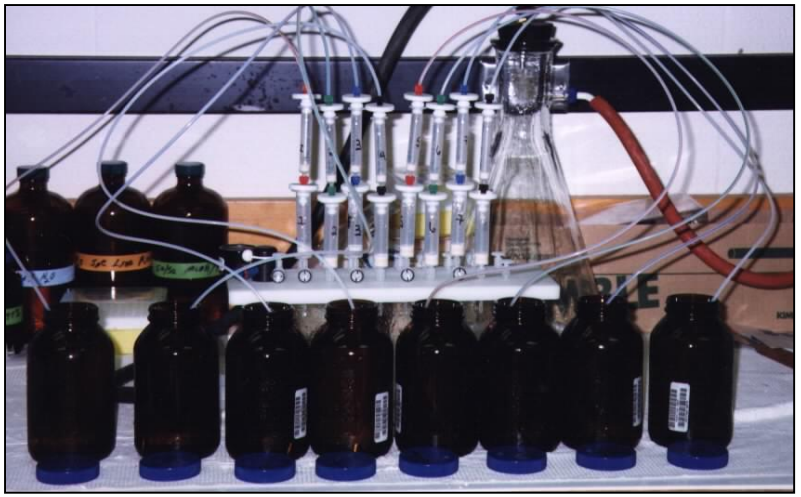


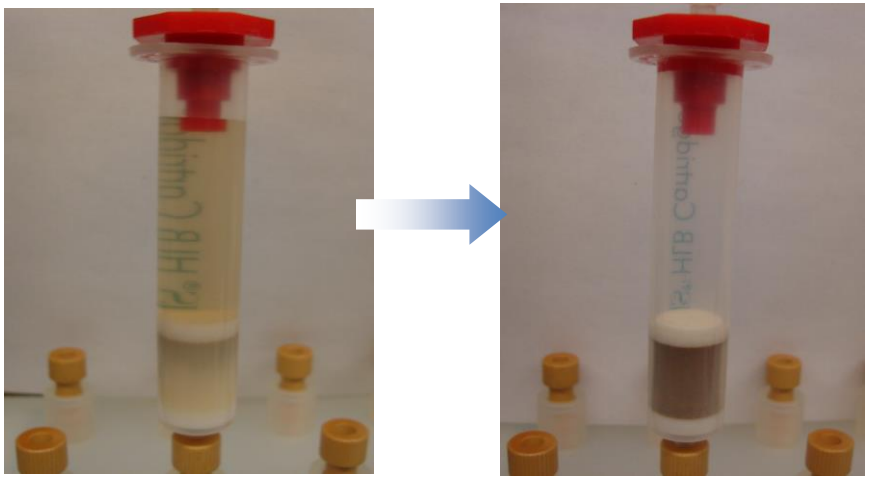
ELISA Method (striped bar) gave higher concentrations than LC/MS method (solid bar) for tetracyclines in soil

Aga, D. S.; O'Connor, S.; Ensley, S.; Payero, J. O.; Snow, D.; Tarkalson, D., Determination of the Persistence of Tetracycline Antibiotics and Their Degradates in Manure-Amended Soil Using Enzyme-Linked Immunosorbent Assay and Liquid Chromatographyâ"Mass Spectrometry. *Journal of Agricultural and Food Chemistry* ***2005, 53, (18), 7165-7171.***

Sample Preparation and Clean up

SOLIDS:
Lyophilize, pulverize, sonicate with organic solvent





500 mL sample
(pH = 2.0 ± 0.2)

+ 4 mL 12.5% Na_2EDTA
Adjust pH to 4.0 ± 0.2

Solid Phase Extraction
(Oasis® HLB Cartridges)

Conditioning:
- 6mL methanol
- 10mL 0.1% EDTA water

Loading:
- 0.5 – 1.0 mL/min
- Rinsed 5% MeOH
- Dried under vacuum

Elution:
- 10 mL (9:1) EtAc/MeOH
- Acid/base rinses
- 10 mL 2% NH_4OH in MeOH

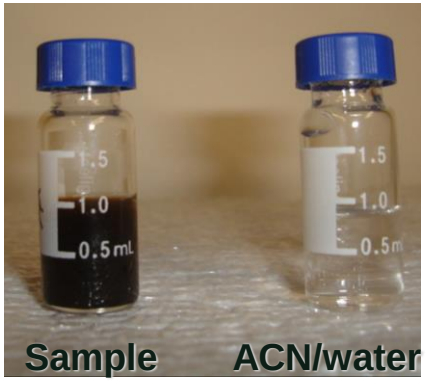
Dried to 0.2mL under N_2

Sample Extracts
(Reconstituted to 0.5 with 95/5 water/ACN)

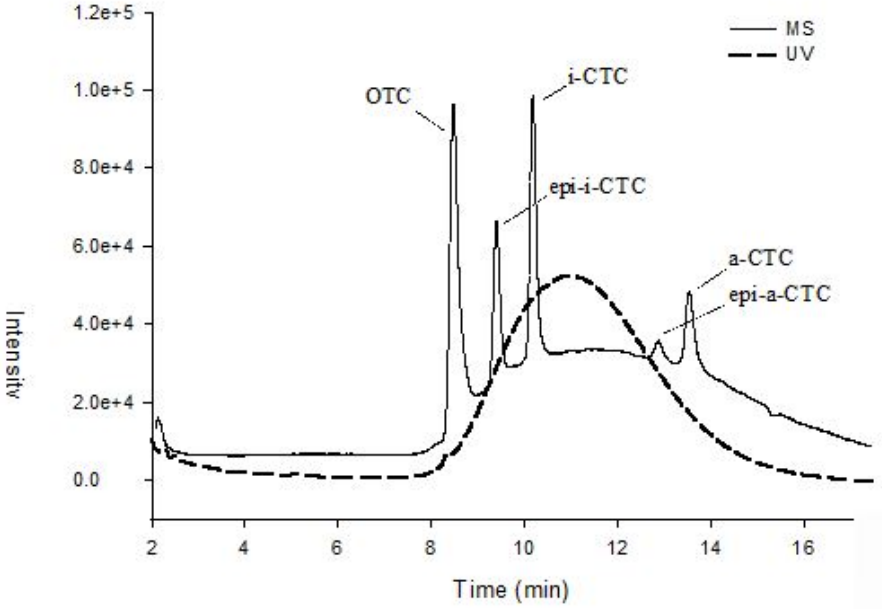
Split

200 µL
(Native + 10µL d_{10}-CBZ)

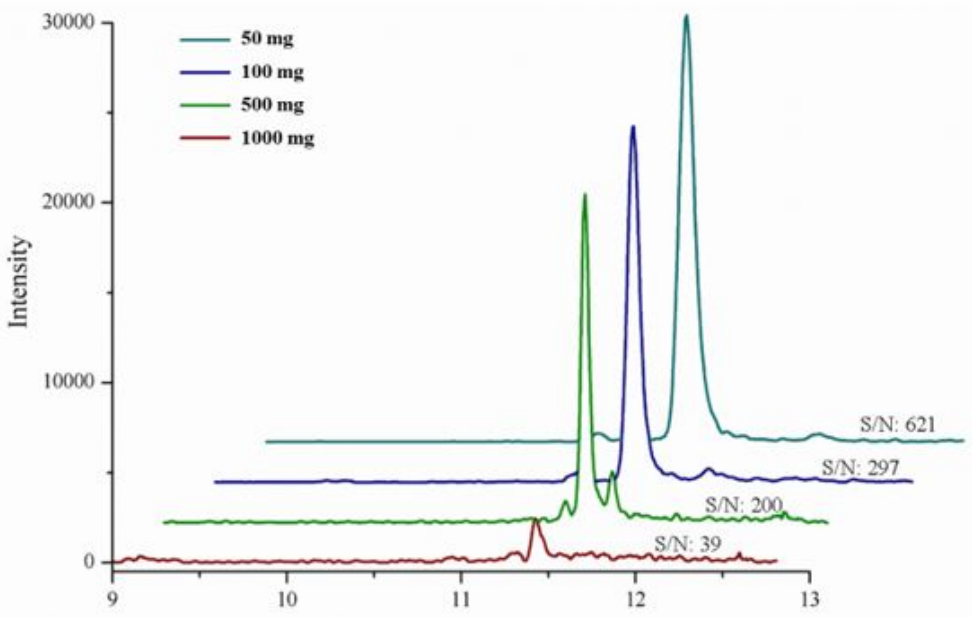
200 µL
(Spiked 10µL- 500 ppb std)



Interference from Co-extracted Matrix



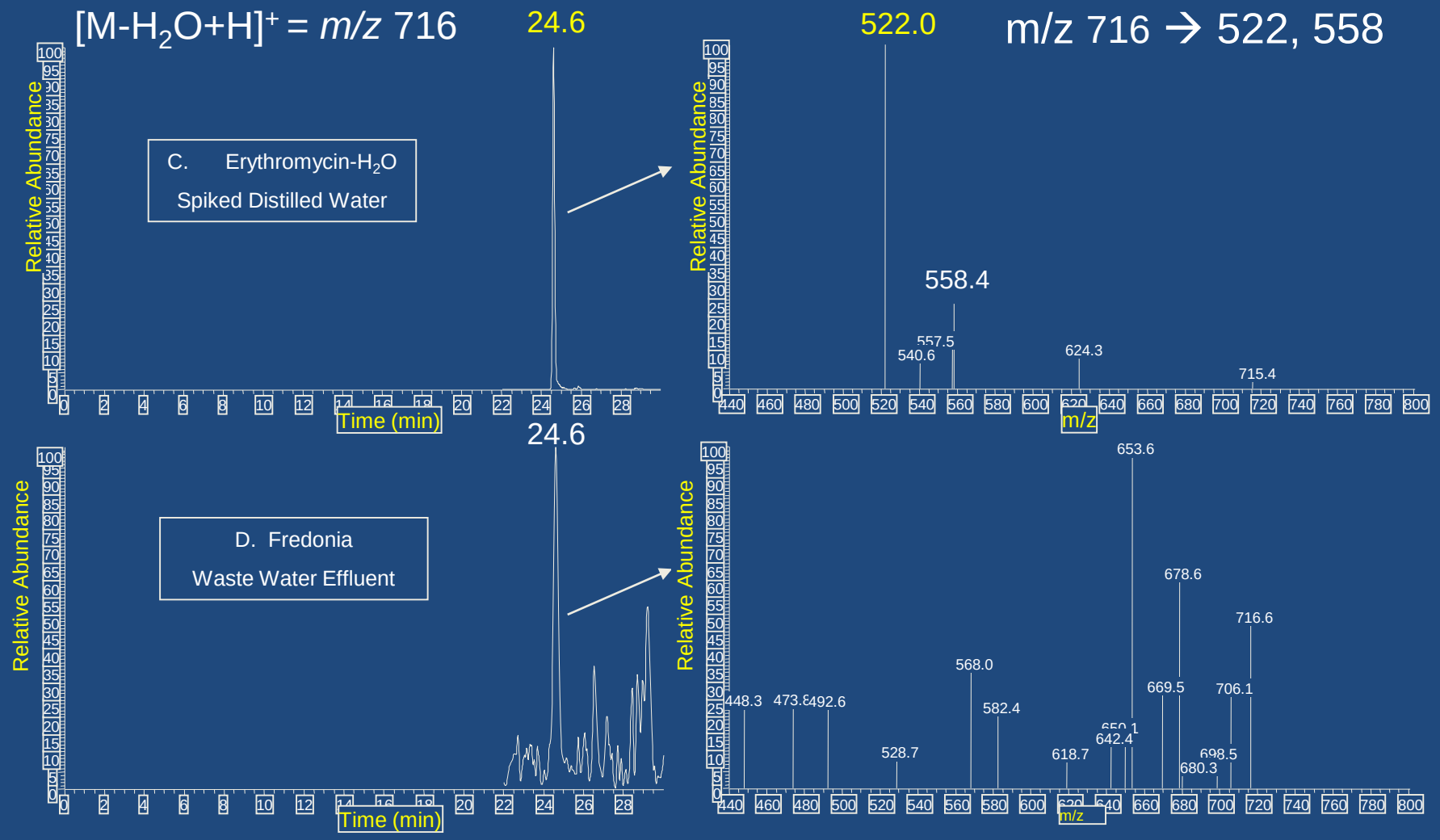
O'Connor, S.; Locke, J.; Aga, D. S., Addressing the challenges of tetracycline analysis in soil: extraction, clean-up, and matrix effects in LC-MS. *Journal of Environmental Monitoring* **2007**, *9*, 1254-1262.



Wallace, J.S.; Aga, D.S., Enhancing extraction and detection of veterinary antibiotics in solid and liquid fractions of manure. *Journal of Environmental Quality* **2016**, *45*, 471-479 .

Avoiding “false positives” using tandem mass spectrometry

Batt, A. L.; Aga, D. S., Simultaneous analysis of multiple classes of antibiotics by ion trap LC/MS/MS for assessing surface water and groundwater contamination. *Analytical Chemistry* **2005,** *77* (9), 2940-2947.



Sample Collection: Which samples are important?