A case study: Crop (lettuce, spinach, and carrots) uptake of three macrolide antibiotics (azithromycin, clindamycin and roxithromycin) and other drugs

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Introduction

Increasing demands on scarce water resources in the southwestern part of the United States has forced water authorities to look for alternative water sources. Recent studies have shown that human-use macrolide antibiotics (azithromycin, clindamycin, and roxithromycin) are environmentally available in wastewaters, source waters, and biosolids. Some water authorities use treated wastewater effluent for injection into groundwater aquifers, for the purpose of pumping it out later and re-use as drinking water (with further treatment). Other municipalities use treated wastewater effluent for non-potable water reuse, e.g., use on golf courses, municipal green spaces, and crops. This type of reuse has been practiced in several parts of the United States for more than 30 years.

The research presented in this poster will focus on the fate of drugs into food crops via root migration. First, we conducted a controlled greenhouse study, where we exposures three crops (i.e., lettuce, carrots, and spinach) grown in soils that were irrigated with water that had been spiked with known amounts of three antibiotics: azithromycin, roxithromycin and clindamycin.

To groundtruth our methodology, we applied the methods to crop samples (carrots, watermelon, tomatoes, cantaloupe) that were grown in fields that use treated municipal wastewater effluent for irrigation of food crops. This treated wastewater effluent was from a medium population southwestern city (~ 1 million population, July 2008). The treated wastewater effluent had previously been characterized, and was known to contain the macrolide antibiotic azithromycin, the over-the-counter drug pseudoephedrine, the illicit drug methamphetamine, and an industrial flavoring agent n,n'-dimethylphenethylamine (n,n'-dmpea, an isomeric compound to methamphetamine).

Study Design

Two plant uptake studies were conducted, one a controlled greenhouse study, and the other a field study.

In the first study three crops (lettuce (*Lactuca sativa*), spinach (*Spinacia oleracea*), and carrots (*Daucus carota sativus*), grown in sand, were irrigated with varying concentrations of the three macrolide antibiotics (azithromycin, clindamycin and roxithromycin). The concentrations were selected relative to concentrations found in waste streams and were 0, 0.1, 1, 10, 100, and 1000 ng/L. After dissolving the antibiotics in methanol we diluted to 1000 ng/L with Colorado River water. All other concentrations were achieved by serial dilutions with Colorado River water.

In the second study, which was conducted at an university research farm field, several crops: lettuce, spinach, carrots, tomatoes (*Lycopersicon esculentum*), peppers (*Capsicum annuum*), melons (*Cucumis melo*) and watermelons (*Citrullus lanatus*)) were irrigated with treated wastewater effluent, alongside a well water as a control comparison. All crops were sampled at maturity. After harvest, the plants were dissected and separated into leaf and root, and where appropriate fruit, then freeze-dried.

METHODS/RESULTS

SAMPLE PREPARATION, EXTRACTION AND CLEANUP

Plant and Soil preparation

After collection, plant and soil samples were dried. Crop samples were freeze dried for a week, or longer, until no moisture was present. Soil samples are poured into a clean 2 liter beaker and also air dried for 1 to 3 days, dependent upon moisture content.

The dried samples, both plants and soils, are then placed into 25 ml zirconium oxide/steel jacketed grinding jars, along with one zirconium oxide grinding ball and are ground using a high impact ball mill (mixer mill 301, Retsch Inc, Newtown, PA) for 3 minutes at a frequency of 20.0 s^{-1}.

Pressurized Liquid Extraction (PLE)

Both plant and soil samples are extracted using an Accelerated Solvent Extractor (ASE 200, Dionex Corp., Sunnyvale, CA USA). Two solvents are used in extraction in order to extract fractions of samples based on their polarity. A mixture of Methyl Tert-Butyl Ethyl (MTBE):Methanol (90:10) is flushed up to 80% of the cell volume (22 mL stainless steel cells) with the solvent at 50°C and 1500 psi. After a static period of 15 minutes the solvent is purged into a clean collection vial. After the first solvent is purged a similar procedure is repeated with methanol/1%acetic acid, and is flushed up to 80% of cell volume, at temperature of 80°C and 2900 psi. After a static period of 15 minutes the eluant is purged into a clean collection vial. The MTBE extract is placed into a Turbovap® tube and the extract is allowed decrease by ½ (using a Turbovap® at 4 to 7 psi N_2), then the methanol/acetic acid extract is added to the MTBE extract until a sample volume of 5 mL is reached for plant/root extracts before cleanup is performed. The soil extracts are further concentrated to 0.5 mL before analysis by LC-ITMS/MS, as no further cleanup is necessary.

Cleanup of extracts

Soil extracts needed little to no cleanup effort before analysis by HPLC-ITMS/MS.

Plant and root extracts were washed with hexane, first at a sample volume of 5 ml and then at 1 ml. The number of hexane washes varied from one sample to another, but the washes were done as many times as necessary in order to clean the sample of any undesirable compounds such as chlorophyllic compounds, and waxy materials. The extracts are solvent exchanged with methanol/1% acetic acid and concentrated to 0.5 mL before analysis by LC-ITMS/MS.

MASS SPECTROMETRIC ANALYSIS

Liquid chromatography

The separations were performed using an Ascentis Express C18 (fused-core technology) (Supelco-Aldrich, Bellefonte, PA) 2.7 um particle size, 3 cm x 2.1 mm, coupled to a Varian guard column (MetaGuard 2.0 mm Pursuit XRs 3µm C18). Gradient elution conditions were as follows: Mobile phase A 100%, hold for 2 min, 3 min gradient to 30% A:70% B, hold for 5 min, then 3 min gradient to 100% A, hold for 2 min, end run, 5 min equilibration time between analyses. Mobile phase A: de-ionized water/0.5% formic acid; mobile phase B: 82% methanol/18% acetonitrile/0.5% formic acid

HPLC-ESI-ITMS

Mass spectrometric data were acquired with a Varian 500MS (Walnut Creek, CA USA), configured with a liquid chromatograph and an electrospray ion source. The 500MS was run in the positive ionization mode, the voltage applied to the ES needle was approximately 5 kV (dependent upon the optimized response of the ions of interest), the drying gas was set at 20 psi and 200°C, the housing chamber at 50°C, the nebulizer gas at 40 psi, the spray shield at 600V, and capillary voltages were set dependent upon the optimized response of the product ions of interest. Because of the extremely large amounts of interfering materials co-extracted with the pharmaceuticals, the analyses were performed using the MS/MS mode (collision induced dissociation - CID) for both identification and quantitation of the macrolides and illicit drugs.

Analyte CAS #	Molecular weight (amu)	Precursor ions	Product ions	LOD ng on-column
Azithromycin (83905-01-5)	782.5	785.5 $(M+H)^+$	785.4 $(M+H-C_8H_{17}O_4)^+$, $(M)^+$	0.5
Roxithromycin (80214-83-1)	836.5	837.5 $(M+H)^+$	701.4 $(M+H-C_9H_{19}O_3)^+$	1
Clarithromycin (81103-11-9)	747.5	748.5 $(M+H)^+$	158.1 $(M+H-C_{31}H_{56}O_{13})^+$, $(M)^+$	1
Clindamycin (18323-44-9)	424.5	425.2 $(M+H)^+$	377.2 $(M+H-SO_2CH_3)^+$	1
Methamphetamine (537-46-2)	149.2	150 $(M+H)^+$	119 $(M+H-CH_5N)^+$, $(M)^+$	1.5
MDMA (69810-18-3)	193	194 $(M+H)^+$	163.1 $(M-CH_5N)^+$, $(M)^+$	1
Pseudoephedrine (90-82-4)	165.2	166 $(M+H)^+$	148.1 $(M+H-H_2O)^+$	0.5
n,n'-dimethyl phenethylamine (3426-70-2)	149.2	150 $(M+H)^+$	105 $(M-N(CH_3)_2)^+$	0.5

Analytical Challenges

During the development and execution of this methodology for plants we encountered various analytical difficulties, both in the extraction phase and the detection phase.

Extraction difficulties

•Waxy and fatty materials are co-extracted from root matrices, and are not fully removed during hexane washes.

•Numerous hexane (n = 3 or 4) washes are required to remove chlorophyllic and waxy materials from leafy and root extracts.

Detection challenges

•Injection of plant and root extracts build up deposits on spray shield, causing loss of sensitivity. Necessitating cleanup of spray shield after every 2nd injection of sample extracts.

•Injection of some plant and root extracts temporarily bind to the column, even with guard column, causing non-detects. This necessitates reverse-flow of high organic solvents then water through the column for cleanup before use again.

RESULTS

Sample type (X = spiked compared to unspiked water)	Extraction recoveries (% recovery)							
	AZ	RXY	CLA	CLI	METH	MDMA	n,n'-DMPEA	PSEUDO
lettuce leaf (RXY)	6	140	52	41	88	55	51	85
lettuce root (RXY)	3	305	72	77	109	65	60	60
spinach leaf (RXY)	5	44	19	48	43	40	30	37
spinach root (CLI)	29	167	85	16	42	28	78	56
carrot root (CLI)	7	191	15	39	41	32	39	34

Concentrations of pharmaceuticals detected, ng/g					
Sample type	AZ	RXY	CLI	n,n'-DMPEA	METH
Greenhouse Lettuce leaf Roxithromycin Lettuce leaf Roxithromycin duplicate		>LOQ** >LOQ			
Greenhouse Spinach leaf Clindamycin Spinach leaf Clindamycin duplicate			107 134*		
Greenhouse Carrot Clindamycin			53		
Tucson effluent Green bell pepper				58	
Tucson effluent Cantaloupe Tucson effluent Cantaloupe duplicate				60 47	
Tucson effluent Watermelon (8314) Tucson effluent Watermelon duplicate				41 128	
Tucson effluent Watermelon (8347) Tucson effluent Watermelon duplicate				362 186	
Tucson effluent spinach Tucson effluent spinach duplicate				53 48	
Bermuda roots biosolids amended field Bermuda roots duplicate	220 1				171 139

AZ = azithromycin, RXY= roxithromycin, CLA= clarithromycin, CLI= clindamycin, METH= methamphetamine, MDMA= ecstasy, n,n'-DMPEA = n,n'-dimethylphenylethylamine, PSEUDO= pseudoephedrine, * = background subtracted, ** LOQ for Roxithromycin = 10 ng on-column, 2* = analyte possible ionic build up of contaminant on spray shield and HPLC column

CONCLUSIONS

- We detected no uptake of azithromycin in any of the plant/root samples from either the greenhouse or Tucson effluent field crops. However, we did detect azithromycin and methamphetamine in bermuda roots sampled from a field that has been treated for several years with biosolids from a large California wastewater treatment plant.
- There were traces of uptake of clindamycin into the spinach leaves and possibly lettuce root, however we did not have enough root sample to perform a duplicate extraction/analysis.
- Trace amounts of roxithromycin were detected in lettuce roots. Carrots showed the greatest amount of uptake of roxithromycin, 110 ng/g, from the 1000 ng/L of roxithromycin watered into the carrot plots.
- All of the plants, except the carrots, from the field crops watered with Tucson wastewater effluent showed uptake of n,n'-dimethylphenethyl amine, an industrial chemical used in manufacturing, food industry, etc.

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