

Tracing the sources of macrolide antibiotics and illicit drugs into the Colorado River Basin

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Overview

- Research objectives & approach
- Why concern?
- Emerging contaminants screened
- Sampling sites
- Extraction method
- Detection method
- Application of methods to samples
 - Final data
- Conclusions



Research Objectives

- To characterize environmental sources of drugs (macrolide antibiotics, OTCs and illicit drugs) into the Lower Colorado River basin
 - Distribution
 - Ambient concentrations
 - Trends (spatial)
- To evaluate data for exposure analysis scenarios for risk assessors

Research Approach

- Collect water samples (source and waste) from nineteen different sites along the LCRB – upper basin (Glenwood Springs, Colorado) to lower basin (New River, CA)
- Extract samples using solid-phase extraction (SPE) technology
- Analyze sample extracts using liquid chromatography-electrospray-ion trap mass spectrometry (LC-ES/ITMS)
- Evaluate data looking for spatial trends and sources
 - If possible look at temporal trends where data exists



Why should we be concerned?

Increases in antibiotic-resistant bacteria are found in wastewater effluents T. Schwartz et al. "Real-time PCR detection of *Pseudomonas aeruginosa* in clinical and municipal wastewater and genotyping of the ciprofloxacin-resistant isolates," *FEMS Microbiology Ecology* 57, 158-167 (2006)

One-fifth of the antibiotics were predicted to be very toxic to algae. Almost 1/3 of all of the antibiotics were predicted to be very toxic to fish, and more than half are toxic ($EC_{50} < 10 \text{ mg L}^{-1}$) to fish. The qualitative risk assessment ranking relative to **probability and potential severity for human and environmental health effects is: antibiotics > sex hormones > cardiovascular > antineoplastics**. H. Sanderson et al. "Toxicity classification and evaluation of four pharmaceutical classes: antibiotics, antineoplastics, cardiovascular and sex hormones," *Toxicol.* 43, 27-40 (2004)

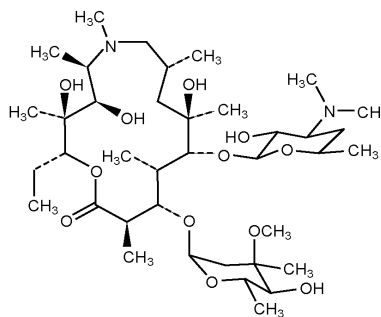
Ofloxacin was the only genotoxic compound and sulfamethoxazole, ofloxacin and lincomycin were mutagenic. As for environmental risk, **the macrolides were found to be the most harmful for the aquatic environment.** M. Isidori et al. "Toxic and genotoxic evaluation of six antibiotics on non-target organisms," *Sci. Tot. Environ.* 346, 87-98 (2005)



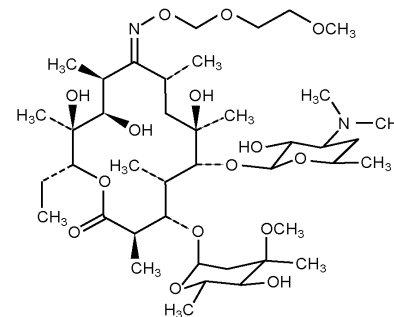
Pharmaceuticals, OTCs, Illicit drugs

Macrolide antibiotics

- Azithromycin
- Roxithromycin
- Clindamycin
- Clarithromycin



Azithromycin mw = 748 Da



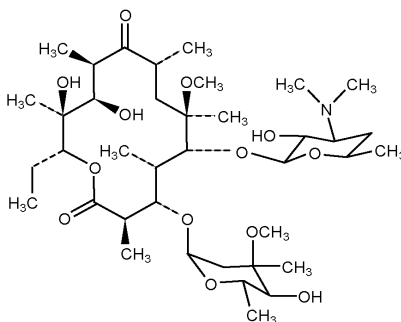
Roxithromycin mw = 837 Da

Over-the-Counter

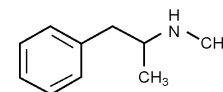
- Pseudoephedrine

Illicit drugs

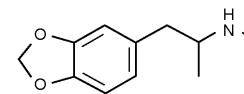
- Methamphetamine
- Ecstasy (MDMA)



Clarithromycin mw = 747 Da



Methamphetamine mw = 149 Da



MDMA mw = 193 Da

Other

- n,n'-dimethylphenethylamine (flavouring agent, same molecular mass as methamphetamine)

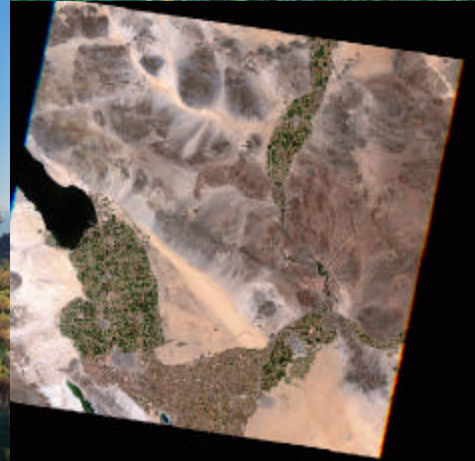
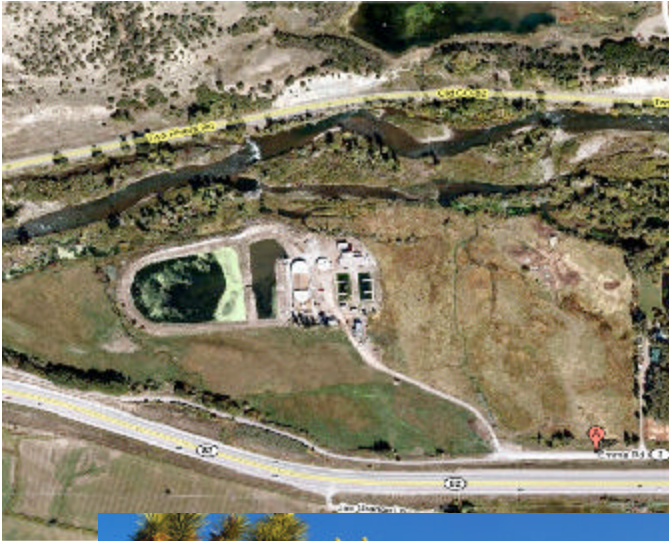


Sampling sites

- Glenwood Springs, Colorado (WWTP)
- Roaring Fork, Colorado
- Colorado River (Glenwood Springs)
- Grand Lake, Colorado
- Colorado River (Fruita), CO
- Green River, UT
- Colorado River (Moab), UT
- Virgin River (St. George), UT (WWTP)
- Virgin River, NV (below Mesquite)
- Cedar Pocket, AZ
- Little Colorado River (Cameron), AZ
- Colorado River (Lee's Ferry), AZ
- Las Vegas Wash (below Lake Las Vegas), NV
- Gila River, AZ
- Tucson, AZ (WWTP)
- Colorado River (Crystal Springs), AZ
- Lake Havasu, AZ
 - Three WWTPs
 - Lake Havasu
- Colorado River (Yuma), AZ
- Colorado River (Imperial Diversion Dam), AZ
- New River, CA

Glenwood Springs WWTP

Satellite view courtesy of GOOGLE maps



Satellite view courtesy of NASA landsat image

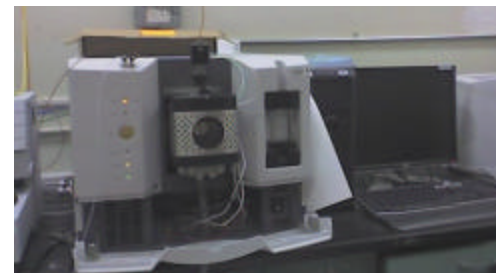
Yuma - Colorado River &
New River – Salton Sea

Extraction

Automated solid phase extraction (SPE)

- 500 mL of water sample is measured out, pH is measured and approx 700 μL of 12N HCl is added, or until $< \text{pH } 3$ is achieved, 2 g NaCl
- Autotrace SPE extractor (Caliper Life Sciences)
 - SPE cartridges: Oasis MCX (Waters Corp) are prepped with 5 mL methanol, 5 mL water, 5 mL water/1% acetic acid at 1 mL/min
 - Samples are loaded at 7 mL/min flow
 - 50 mLs DI H₂O added to sample flask for rinsing
 - After sample loading dried for 15min (N₂)
 - Elute with
 - 5 mL 91% MTBE:8% methanol:1% acetic acid and
 - 5 mL 99% methanol:1% acetic acid at 1 mL/min flow
- Sample extracts are transferred to 50 mL concentrator tubes and concentrated, using approx 5 to 10 psi N₂ flow, to 0.5 mL for analysis [Caliper (formerly Zymark) TurboVap™]

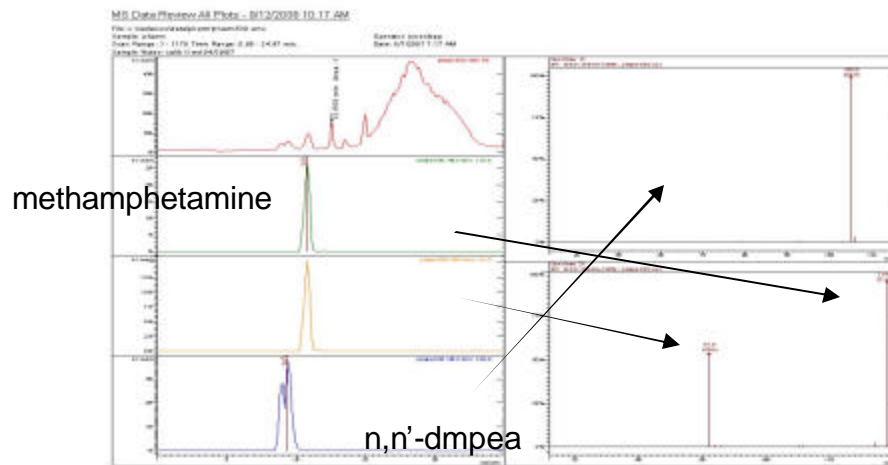
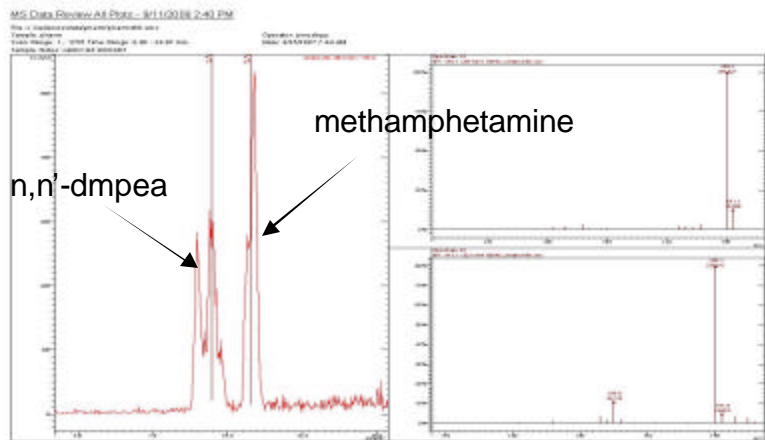
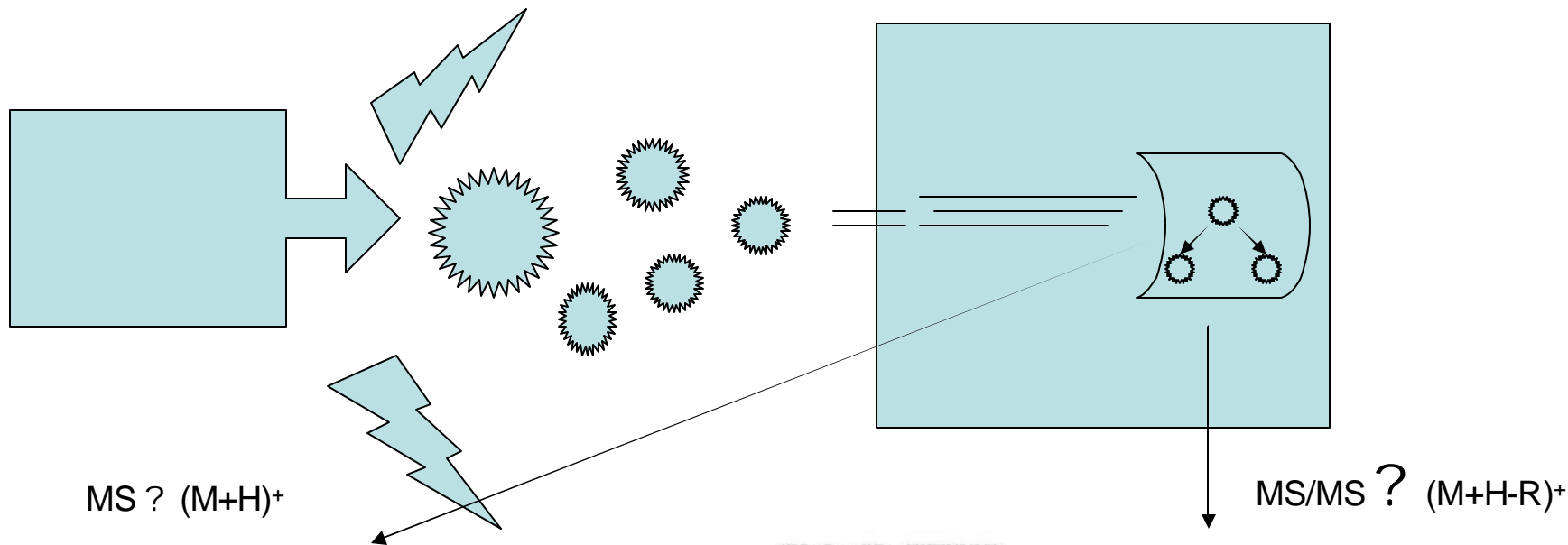




Detector - Ion Trap Mass Spectrometer

- Varian 500MS (Varian Corp., Palo Alto, CA):
 - positive - electrospray ionization mode; source temp: 200°C; needle voltage: 5kV
- All samples were analyzed in the MS/MS mode and product ions monitored for confirmation and quantitation.

Analyte CAS #	Molecular weight (amu)	Precursor ions	Product ions	LOD ng, on-column
Azithromycin (83905-01-5)	748.5	749.5 (M+H) ⁺	591.4 (M+H-C ₈ H ₁₆ O ₂ N) ⁺	0.5
Roxithromycin (80214-83-1)	836.5	859.5 (M+Na) ⁺	755.4 (M+Na-C ₄ H ₉ O ₃) ⁺	1
Clarithromycin (81103-11-9)	747.5	748.4 (M+H) ⁺	590.1 (M+H-C ₈ H ₁₆ O ₂ N) ⁺	1
Clindamycin (18323-44-9)	424.2	425.2 (M+H) ⁺	377.2 (M+H-SH-CH ₃) ⁺	1
Methamphetamine (537-46-2)	149.3	150 (M+H) ⁺	119 (M+H-CH ₃ NH ₂) ⁺	1.5
MDMA (69610-10-2)	193	194 (M+H) ⁺	163.0 (M-CH ₃ NH ₂ +H) ⁺	1
Pseudoephedrine (90-82-4)	165.2	166 (M+H) ⁺	148.2 (M+H-H ₂ O) ⁺	0.5
n,n-dimethyl-phenethylamine (1126-71-2)	149.2	150 (M+H) ⁺	105 (M-N(CH ₃) ₂) ⁺	0.5



Chromatography – liquid – reverse phase

- LC – Binary 212 pump (Varian Corp) with autosampler

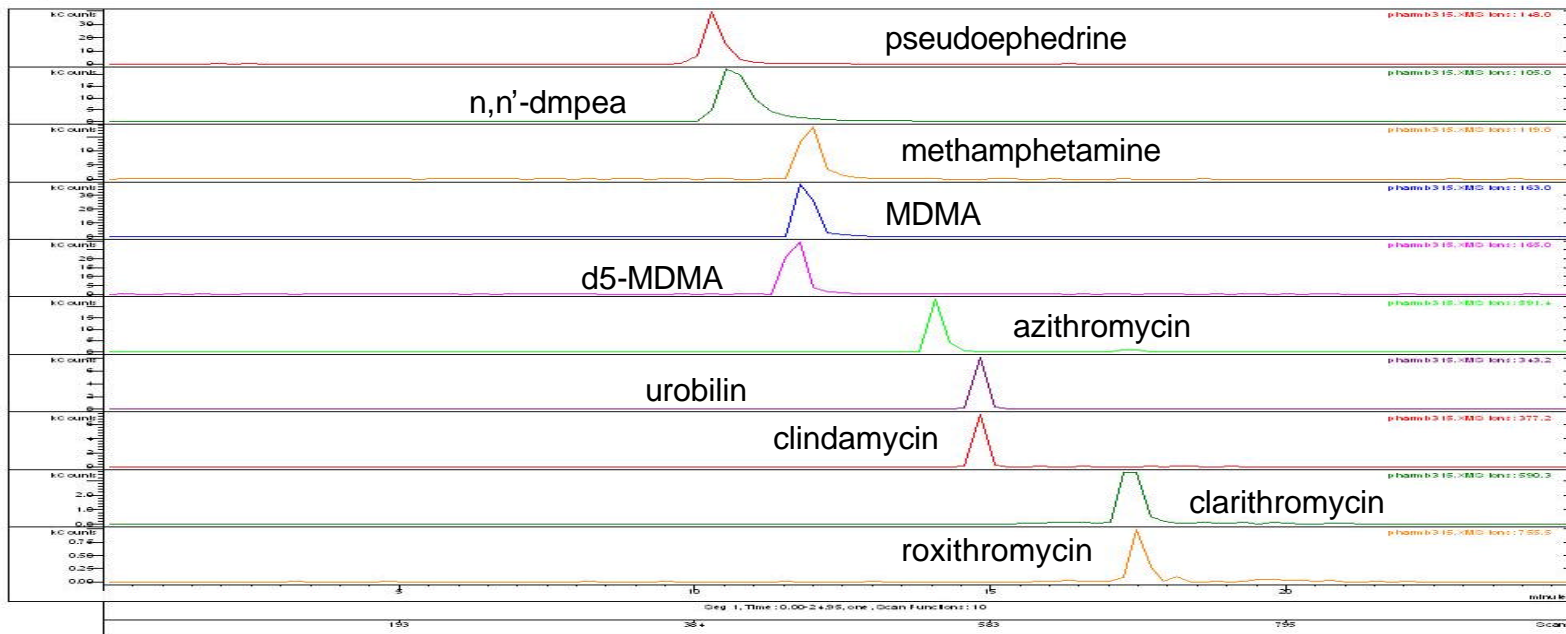
- Initial LC conditions Column: Varian Pursuit XRs 3 μ m C₁₈ 100 x 2 mm (Varian Inc., Lake Forest, CA), with a Varian guard column (MetaGuard 2.0mm Pursuit XRs 3 μ m C18) on the front end.

Initial LC conditions with standard C₁₈ column

Chromatogram Plots

File: c:\varian\sw\data\pharm\pharm315.xml
 Sample: pharmb
 Scan Range: 1 - 986 Time Range: 0.00 - 24.95 min.
 Sample Notes: Calib std iii 121707

Operator: joneslepp
 Date: 12/19/2007 2:45 PM



Experimental

New LC and new conditions

- Column: Ascentis Express C₁₈ (Supelco-Aldrich, Bellefonte, PA) 2.7 um particle size, 3 cm x 2.1 mm, fused-core technology, with a Varian guard column (MetaGuard 2.0 mm Pursuit XRs 3µm C₁₈) on the front end.
- Gradient elution conditions were as follows

Time (min)	Mobile phase A %	Mobile phase B %
0	100	0
2	100	0
5	30	70
10	30	70
13	100	0
15	100	0

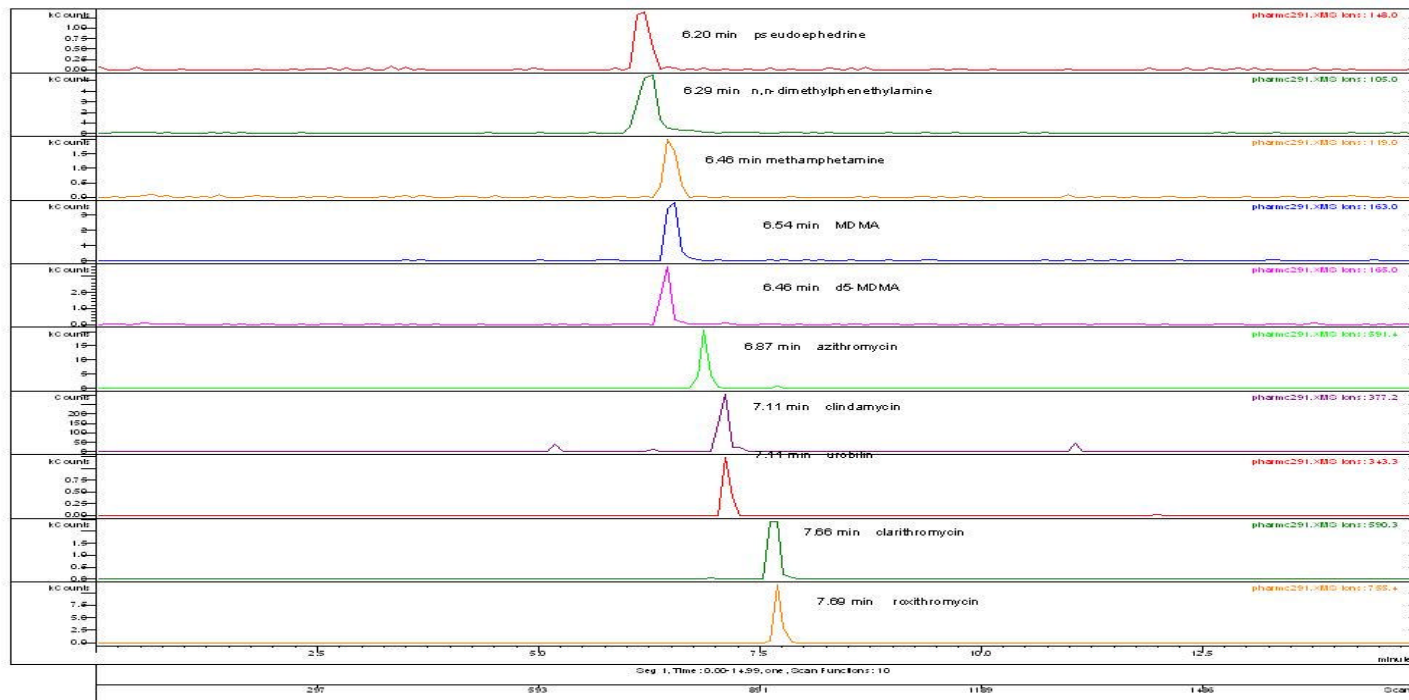
- Mobile phase A: de-ionized water/0.5% formic acid
- Mobile phase B: 82% methanol/18% acetonitrile/0.5% formic acid.

Standard chromatogram fused-core column

Chromatogram Plots

File: c:\varian\data\pharm\pharmc291.xms
 Sample: pharmc
 Scan Range: 1 - 1780 Time Range: 0.00 - 14.99 min...
 Sample Notes: calib_std 082608

Operator: joneslepp
 Date: 8/29/2008 9:05 AM



- Better separation
- Shorter analysis times - < 15 min, 5 min equilibration between analyses
- Easy recovery from “dirty” samples



Results

ng/L (ppt)

Site	Azithro	Roxithro	Clarithro	Clinda	Meth	MDMA	Pseudo
Glenwood Springs, Colorado (WWTP)	336				230	67	490
Colorado River (Glenwood Springs)	No analytes detected						
Roaring Fork, Colorado	No analytes detected						
Grand Lake, Colorado	No analytes detected						
Colorado River (Fruita), CO	No analytes detected						
Green River, UT	No analytes detected						
Colorado River (Moab), UT	No analytes detected						
Virgin River (St. George), UT (WWTP)	270			1007		detected <LOQ	315
Cedar Pocket, AZ							210



Results

ng/L (ppt)

Site	Azithro	Roxithro	Clarithro	Clinda	Meth	MDMA	Pseudo
Little Colorado River (Cammeron), AZ	No analytes detected						
Colorado River (Lee's Ferry), AZ	No analytes detected						
Las Vegas Wash (below Lake Las Vegas), NV					150		
Gila River, AZ	No analytes detected						
Tucson, AZ (WWTP) range	253 [†] (150–625)				286 [†] (137 – 509)		226 [†] (71-532)

†AVG from March to July 2008;

LOQ = Limit-of-quantitation (10 d), LOD = Limit-of-detection (3 d)



Results

ng/L (ppt)

Site	Azithro	Roxithro	Clarithro	Clinda	Meth	MDMA	Pseudo
Colorado River (above Lake Havasu), AZ					nd – <LOQ*	nd – <LOQ*	
Lake Havasu, AZ (Thompson Bay)	No analytes detected						
Lake Havasu WWTPs** Mulberry	nd - 65				nd - 350		400
Island	50 – 260			nd - 970			900
NWR	24 - 200		nd - 40				200 - 600
Colorado River below Yuma WWTP, AZ	196						
Colorado River (Imperial Diversion Dam), AZ						detected <LOQ‡	
New River, CA		detected <LOQ	detected <LOQ		160		100

LOQ = Limit-of-quantitation (10 d), LOD = Limit-of-detection (3 d)

* Samples collected May 2007 to November 2007 – highest value July 2007; ** Samples collected May 2007 to May 2008

‡ Detected, but values are < LOQ, samples collected from Sept 2007 to July 2008.

Conclusions

- Waste water treatment plants are a major source of macrolide antibiotics and OTC
 - macrolide antibiotics are not detected downstream
- Waste water treatment plants and recreational use are sources of illicit drugs
- Small molecules, i.e., pseudoephedrine, MDMA, have capability to travel greater distances than larger macrolide antibiotics



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