

# ***Microbial Degradation of Pharmaceutical Compounds in Municipal Wastewater Effluent***

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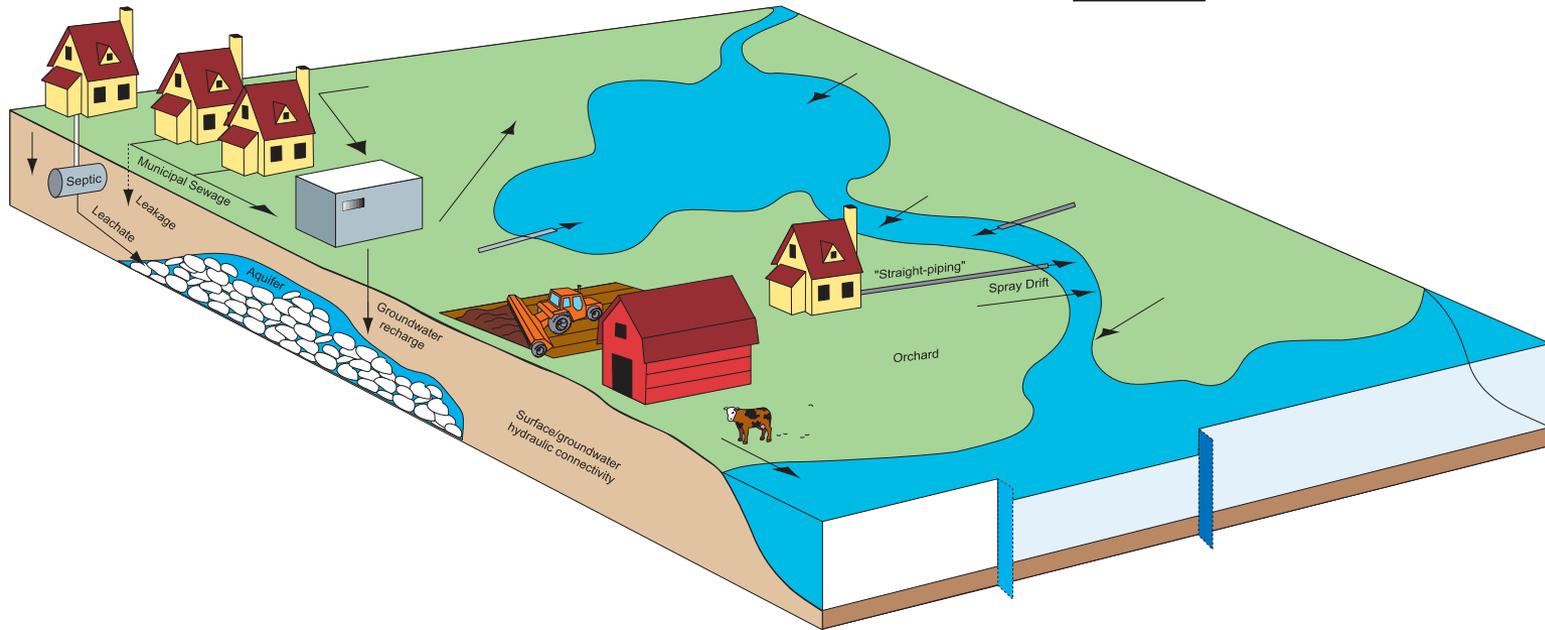


# Overview

- Background of pharmaceuticals in the environment
- Project Objectives
- Project Status
  - Detection methods developed
  - Sample locations identified
  - Enrichment Cultures performed

# Origins and Fate of PPCPs in the Environment

Pharmaceuticals and Personal Care Products



## ***Overall Project Objectives and Scope***

Reduce or eliminate pharmaceuticals emitted from municipal wastewater treatment (MWTP) facilities through enhanced microbial degradation integrated into current operating MWTP systems.

Initially target three pharmaceuticals:

–Ciprofloxacin

Molecular weight  
= 331.3

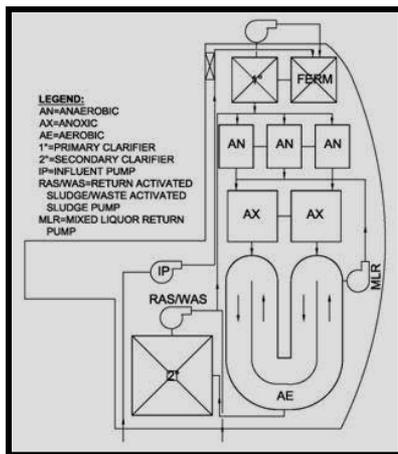
–Carbamazepine

Molecular weight  
= 236.27

–Atorvastatin

Molecular  
weight = 558.6

# Collaborators

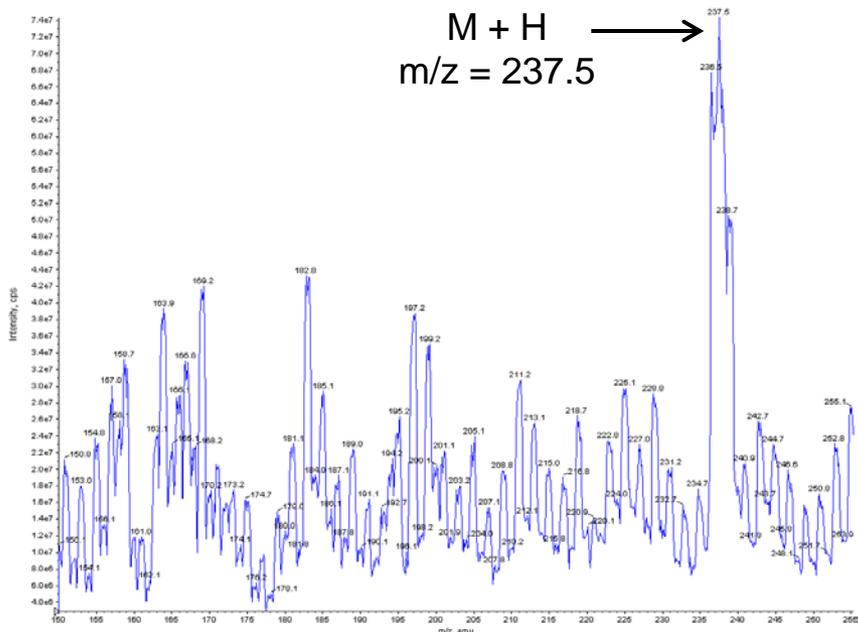


4,800 gal Pilot Plant at City of Moscow MWTP

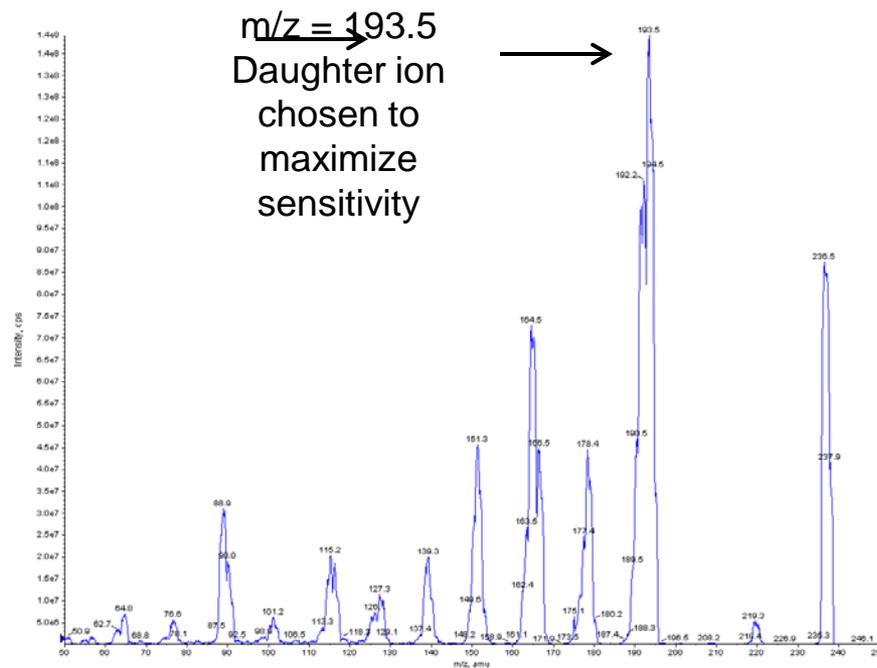


Rapid Fire High-Throughput Mass Spectroscopy

# Agilent Rapid Fire analysis

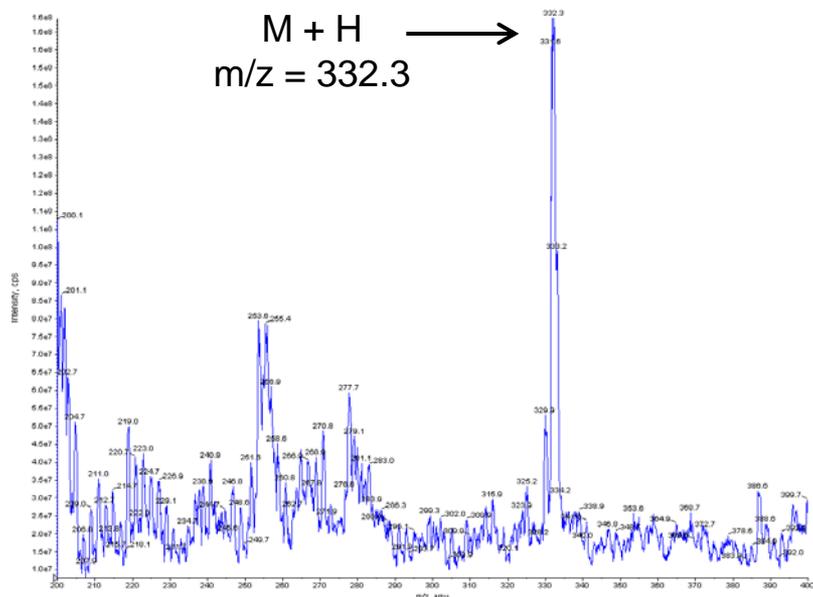


Carbamazepine Parent Ion (Q1) Scan



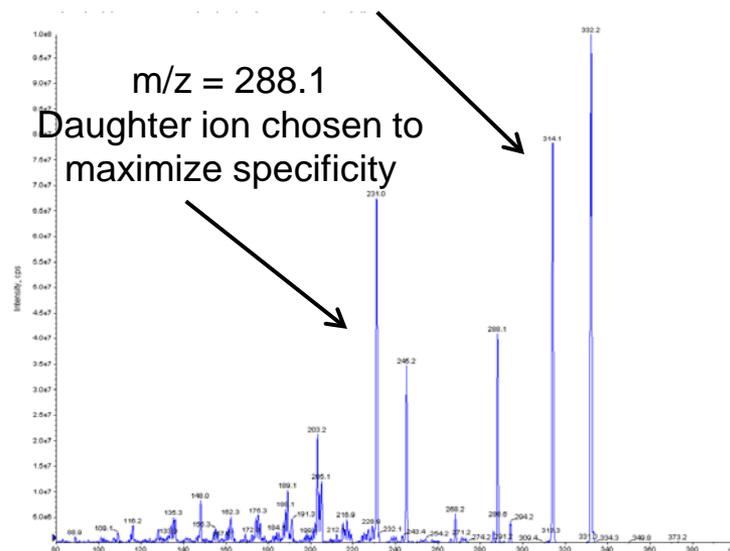
Carbamazepine Daughter Ion (Q3) Scan

# Agilent Rapid Fire Analysis



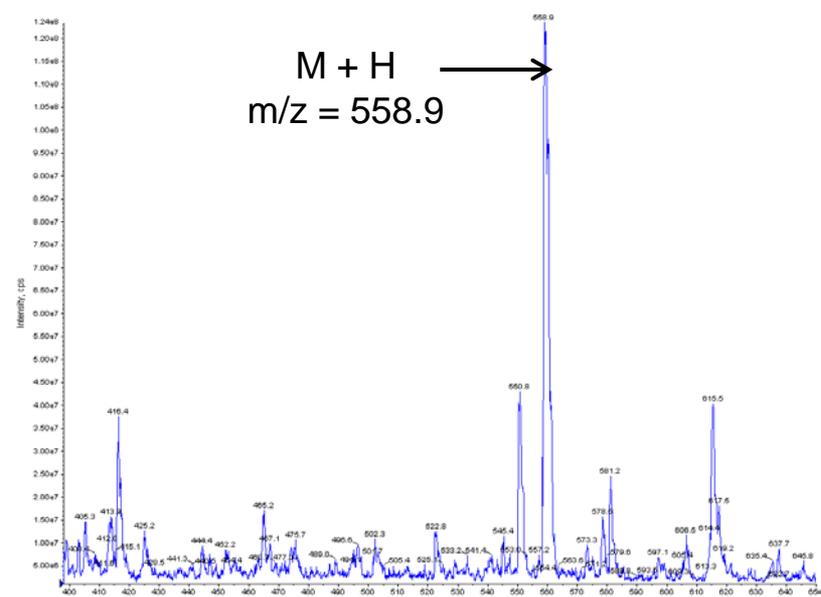
Ciprofloxacin Parent Ion (Q1)  
Scan

$m/z = 314.1$   
 Daughter ion chosen to  
 maximize sensitivity

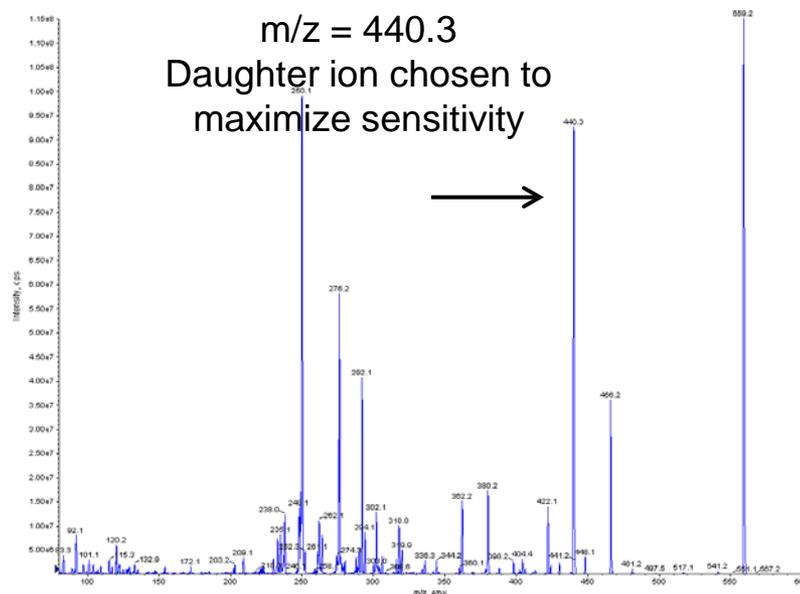


Ciprofloxacin Parent Ion (Q3)  
Scan

# Agilent Rapid Fire Analysis



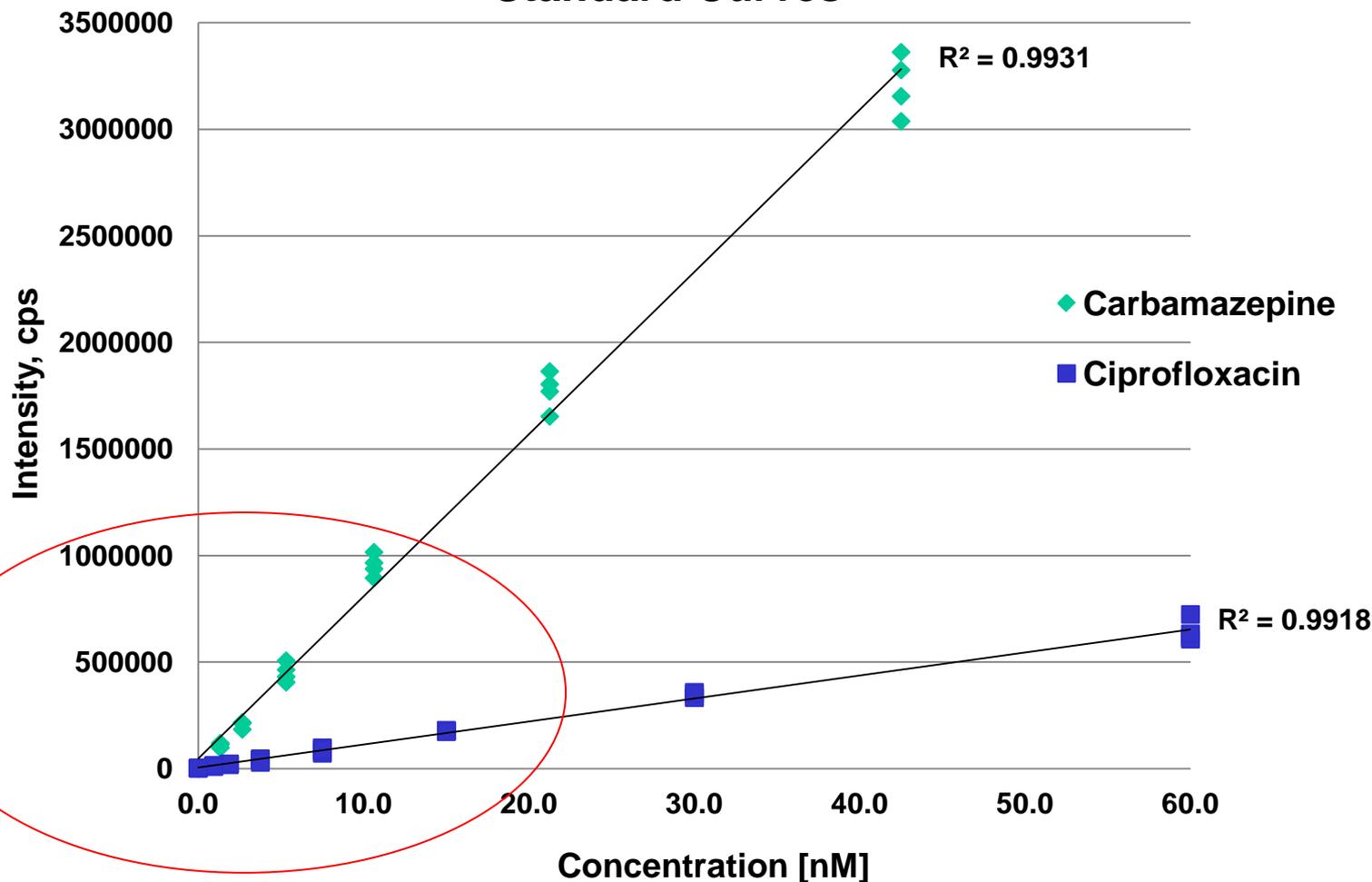
Atorvastatin Parent Ion (Q1)  
Scan



Atorvastatin Daughter Ion (Q3)  
Scan

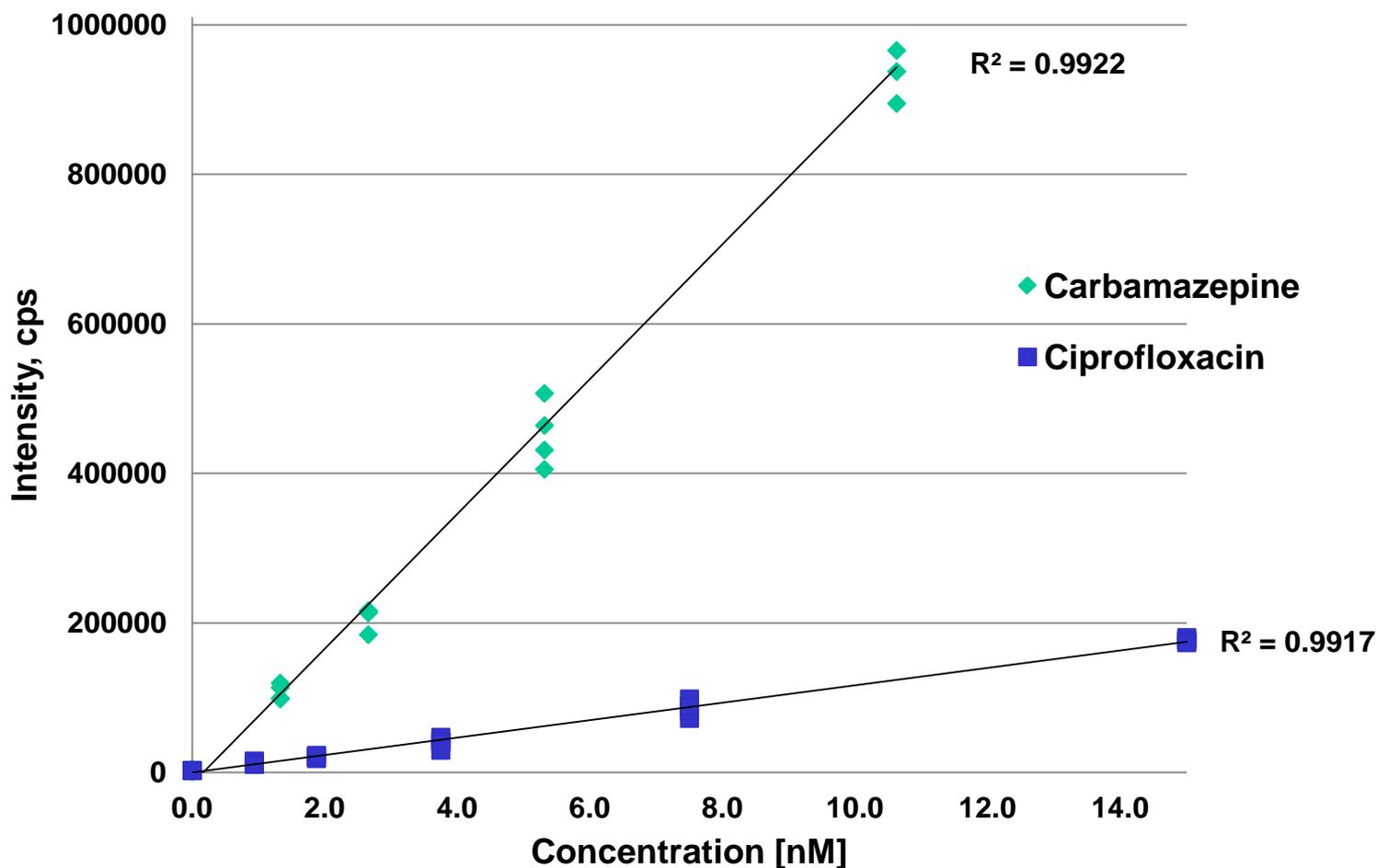
# High Throughput Mass Spectroscopy

## Agilent Rapid-Fire Mass Spectroscopy Standard Curves



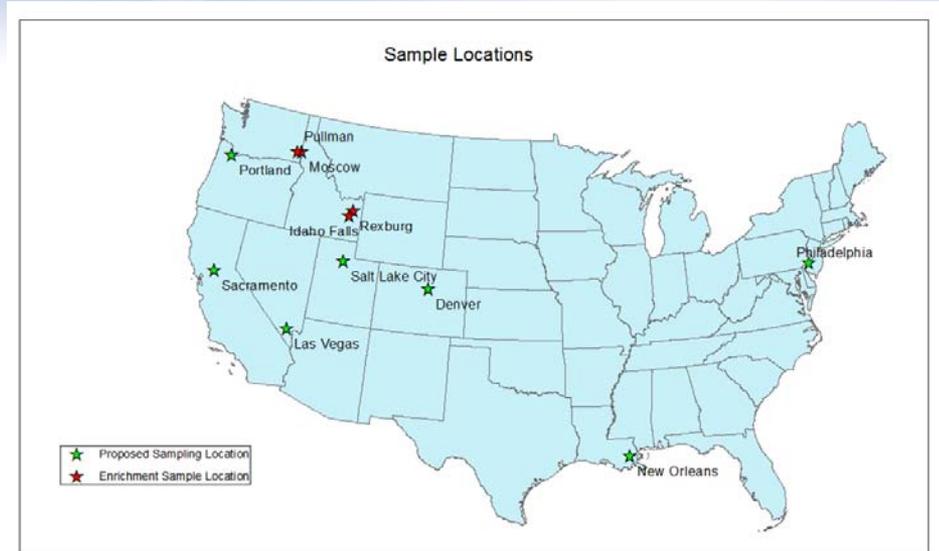
# High Throughput Mass Spectroscopy

## Agilent Rapid-Fire Mass Spectroscopy Standard Curves



# Sample Locations

- Idaho Falls, ID - local
- Rexburg, ID - local
- Moscow, ID - local
- Pullman, WA – local
- Denver, CO – antibiotics
- Salt Lake City, UT – anecdotal high use of anti-depressants
- Portland, OR – antibiotics, wet climate, strict regulation
- Las Vegas, NV – carbamazepine, arid climate
- Sacramento, CA – no testing, strict regulation
- New Orleans, LA – statins, recent disasters, southern U.S.
- Philadelphia, PA – antibiotics, statins, eastern U.S.



## ***Enrichments Samples-Idaho Falls MWTP***



Samples were collected at:

- Activated Biofilter (top/bottom)
- Aeration Basins
- Anaerobic Digester
- Solids Holding Lagoon



# *Enrichment Samples-Rexburg MWTP*



Samples were collected at:

- Activated Biofilter
- Oxidation Ditch (anoxic & aerobic stages)
- Anaerobic Digester



## ***Enrichment Samples-Moscow/Pullman MWTP***

- Moscow samples were collected at:
  - Aerobic zone
  - Anoxic zone
  - Anaerobic zone
- Pullman samples were collected at:
  - Anaerobic zone
  - Digester



## ***Primary Enrichment Procedure***

- 5ml of raw sample
- 45 ml of synthetic media
- 50 ul of a 20 ug/ml stock of drug
  - final concentration of 20 ng/L ciprofloxacin or carbamazepine
- incubated at room temperature and 45°

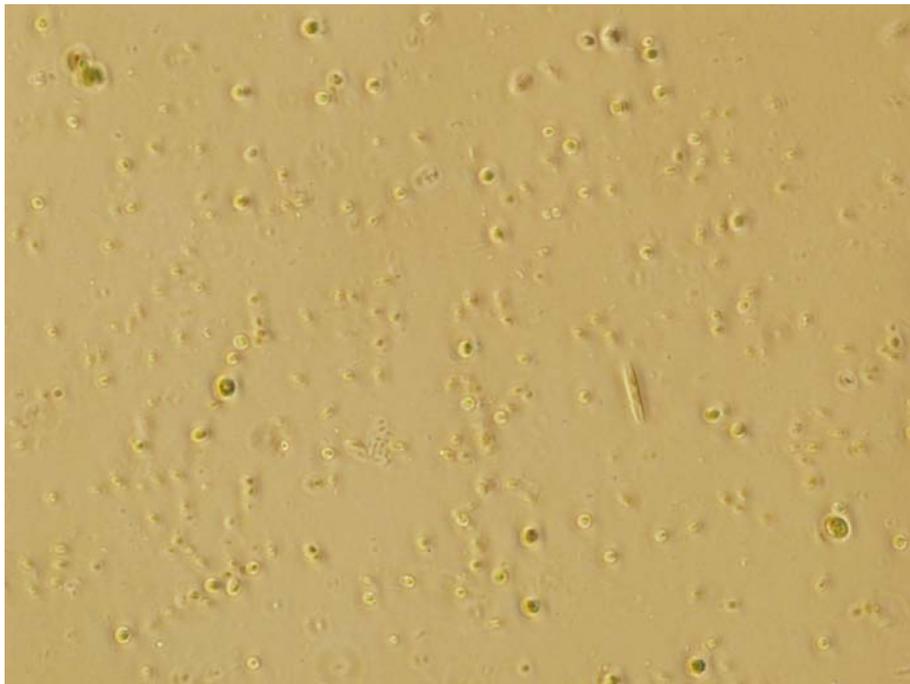


2 wk

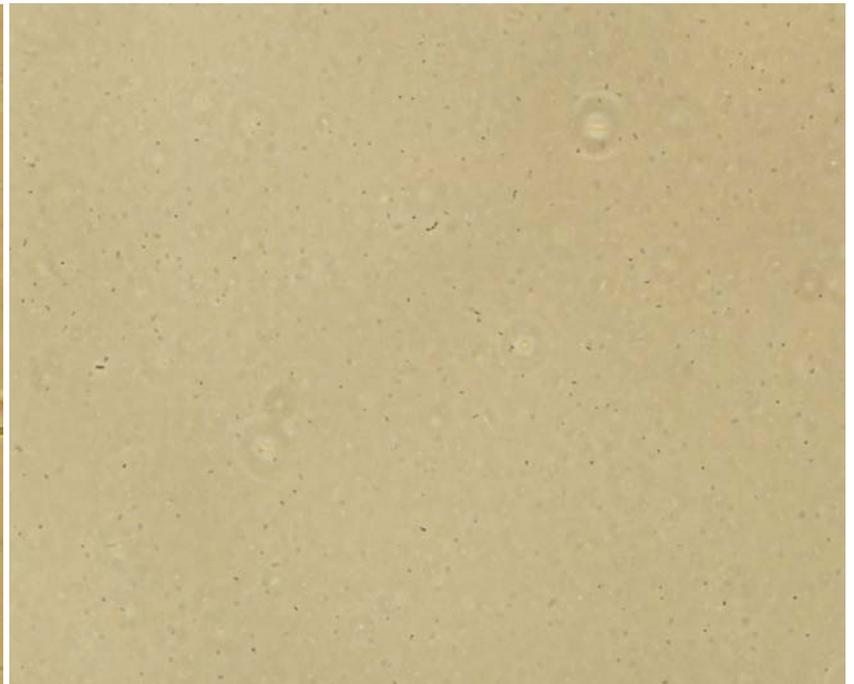


4 wk

## ***Growth- Wet mount: 40X magnification***



Sample 11c 4 wk



Samples 15a 4 wk

## Secondary Enrichment Procedure

- 5ml of select first enrichment samples
- 45 ml of synthetic media
- 50 ul or 100 ul of a 20 ug/ml stock of drug
  - final concentration of 20 ng/L or 40 ng/L ciprofloxacin or carbamazepine

Incubated for at room temperature and 45° and shaken



# Pre-Incubation Detection of Compounds

WWTP Site	Treatment Location	% Carbamazepine	% Ciprofloxacin
Moscow	Aerobic zone	26	51
Moscow	Anoxic zone	23	52
Moscow	Anaerobic zone	31	60
Pullman	Aerobic	31	29
Pullman	Digester	2	9
Rexburg	Digester	26	8
Rexburg	Aeration basins oxygenic	33	34
Rexburg	Aeration basins anoxic	34	33
Rexburg	Activated biofilter	24	45
Idaho Falls	Activated biofilter bottom	31	61
Idaho Falls	Activated biofilter top	28	83
Idaho Falls	Digester	1	32
Idaho Falls	Aeration basins	30	60
Idaho Falls	Activated biofilter return	28	47
Idaho Falls	Solids, post digestion	0	11
Control	none	98	115

## *Summary*

- Developed targeted pharmaceutical detection methods and limits
- Identified 11 potential sampling locations
- Performed primary and secondary enrichments cultures
- Quantified pre-incubation pharmaceutical detection
- Awaiting further results