

# Development of LC/MS/MS Methods for the Analysis of Chemicals on U.S. EPA's Contaminant Candidate List

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*Disclaimer: Mention of trade names or commercial products does not constitute endorsement or recommendation for use.*

**Office of Research and Development**  
National Exposure Research Laboratory

# Acknowledgements

## Waters Corporation

- ◆ **For their support of this research through the loan of the Waters Open Architecture UPLC On-line SPE system to the EPA under the Personal Property Loan Agreement Loan No. 05-006-10.**

## **CCL & UCMR Background**

- ◆ **The 1996 amendments to the SDWA required EPA to establish a Drinking Water Contaminant Candidate List (CCL)**
- ◆ **The first CCL was published in 1998 - updated every 5 years**
- ◆ **Nationwide occurrence data needed on CCL chemicals**
- ◆ **EPA's OGWDW collects occurrence data under the Unregulated Contaminant Monitoring Regulation (UCMR)**
- ◆ **EPA methods for drinking water contaminants are needed for UCMR**
- ◆ **CCL 3 was published in October 2009 and contains 104 chemicals or chemical groups**

# Analytical Methods

## National Exposure Research Laboratory

### ❖ **LC/MS/MS Drinking Water Methods**

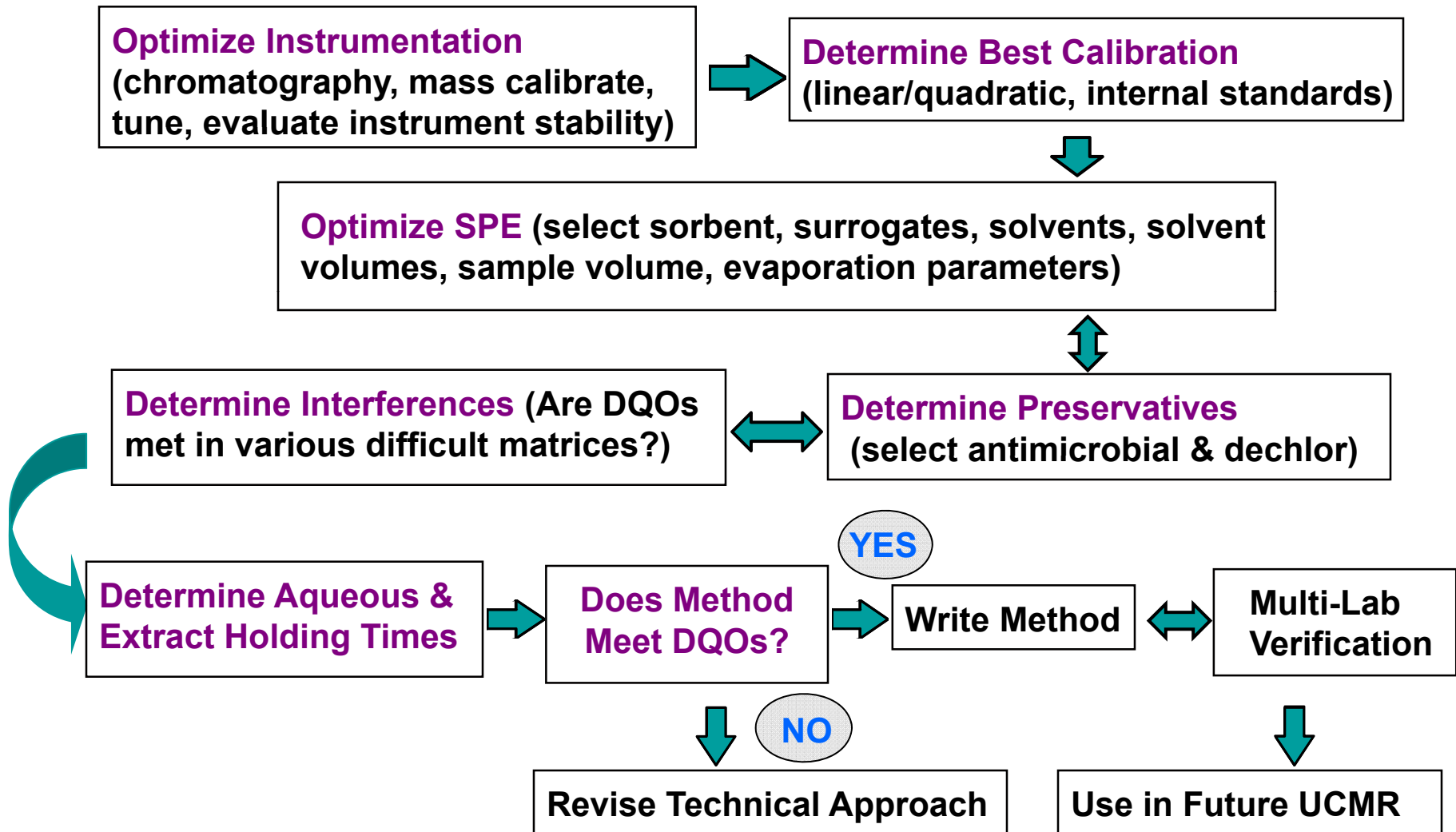
- ✓ Method 535 – 12 acetanilide degradates (6 CCL)
- ✓ Method 537 – 14 perfluorinated alkyl acids (2 CCL)
- ✓ Method 538 – 11 chemicals (4 CCL)

### ❖ **LC/MS/MS methods under development**

- ✓ Method 540 – ~17 chemicals (8 CCL)
- ✓ On-line SPE-LC/MS/MS for Method 540 analytes
- ✓ SPE-LC/MS/MS method for microcystins

<http://www.epa.gov/nerlcwww/ordmeth.htm>

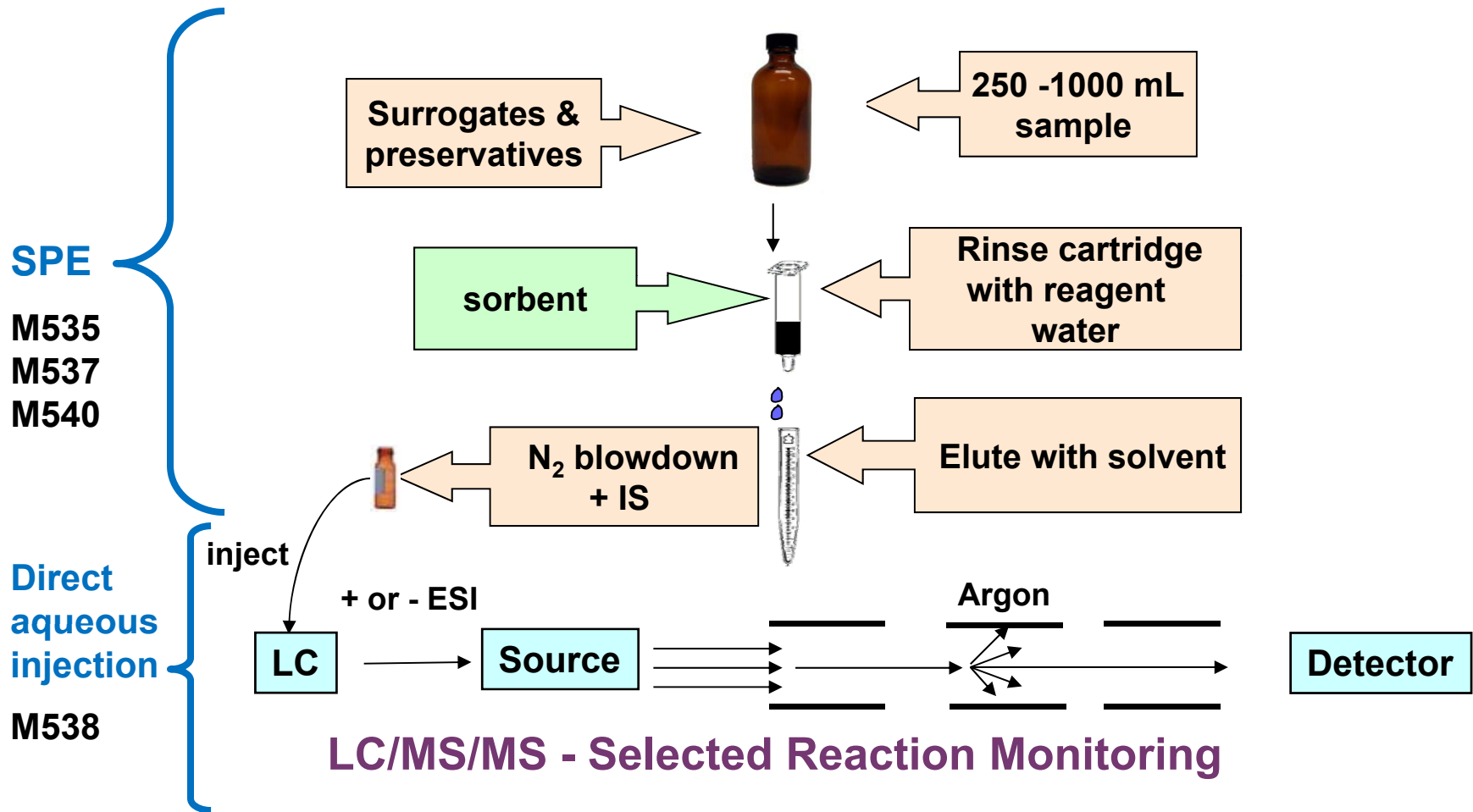
# Method Development Process



## Method Development Goals

- ◆ **Determine appropriate SUR(s) and IS(s)**
- ◆ **70-130% recovery with <30% RSD**
- ◆ **Preservation**
  - ✓ **Dechlorinating agent**
  - ✓ **Antimicrobial**
  - ✓ **for pH control**
- ◆ **Establish sample and extract holding times – ideally  $\geq 14$  days**
- ◆ **Establish detection limits (DLs) and lowest concentration minimum reporting levels (LCMRLs) below health reference levels (HRLs)**

# Generic Analytical Procedure





## Off-line (Method 540) vs On-line SPE-LC/MS/MS



## CCL Chemicals Currently in Method 540 (off-line SPE)

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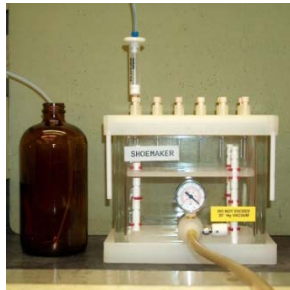
- 4,4'-methylenedianiline
- 3-hydroxycarbofuran
- bensulide
- clethodim
- fenamiphos
- tebuconazole
- tebufenozide
- thiodicarb
- **fenamiphos sulfone**
- **fenamiphos sulfoxide**
- **methomyl**

**8 CCL3 chemicals**  
**3 CCL3 degradates**

# Off-line vs. On-line SPE-LC/MS/MS

## Off-line SPE

manual  
economical manifold  
≤24 samples/24 hr period  
250 - 1000 mL samples  
standards not extracted

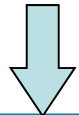


Time consuming steps

Solid phase  
Extraction (SPE)



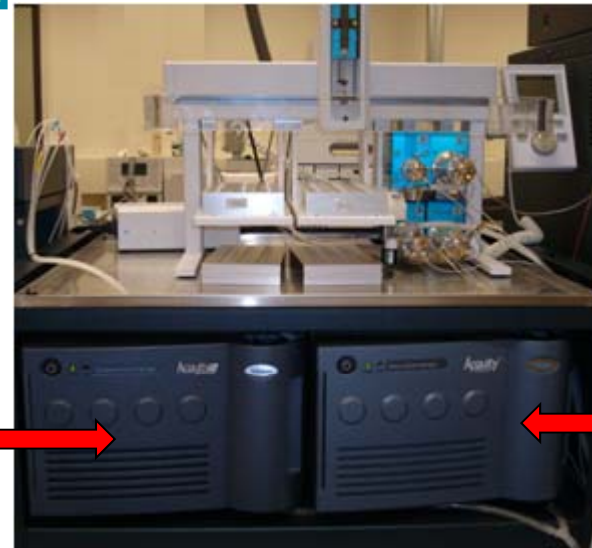
Potentially 2 Methods  
Off-line SPE Method  
On-line SPE Method



LC/MS/MS

## On-line SPE

fully automated  
extra Quat pump and valves  
>70 samples/24 hr period  
1-20 mL samples  
procedural standards



Quaternary pump

Binary pump

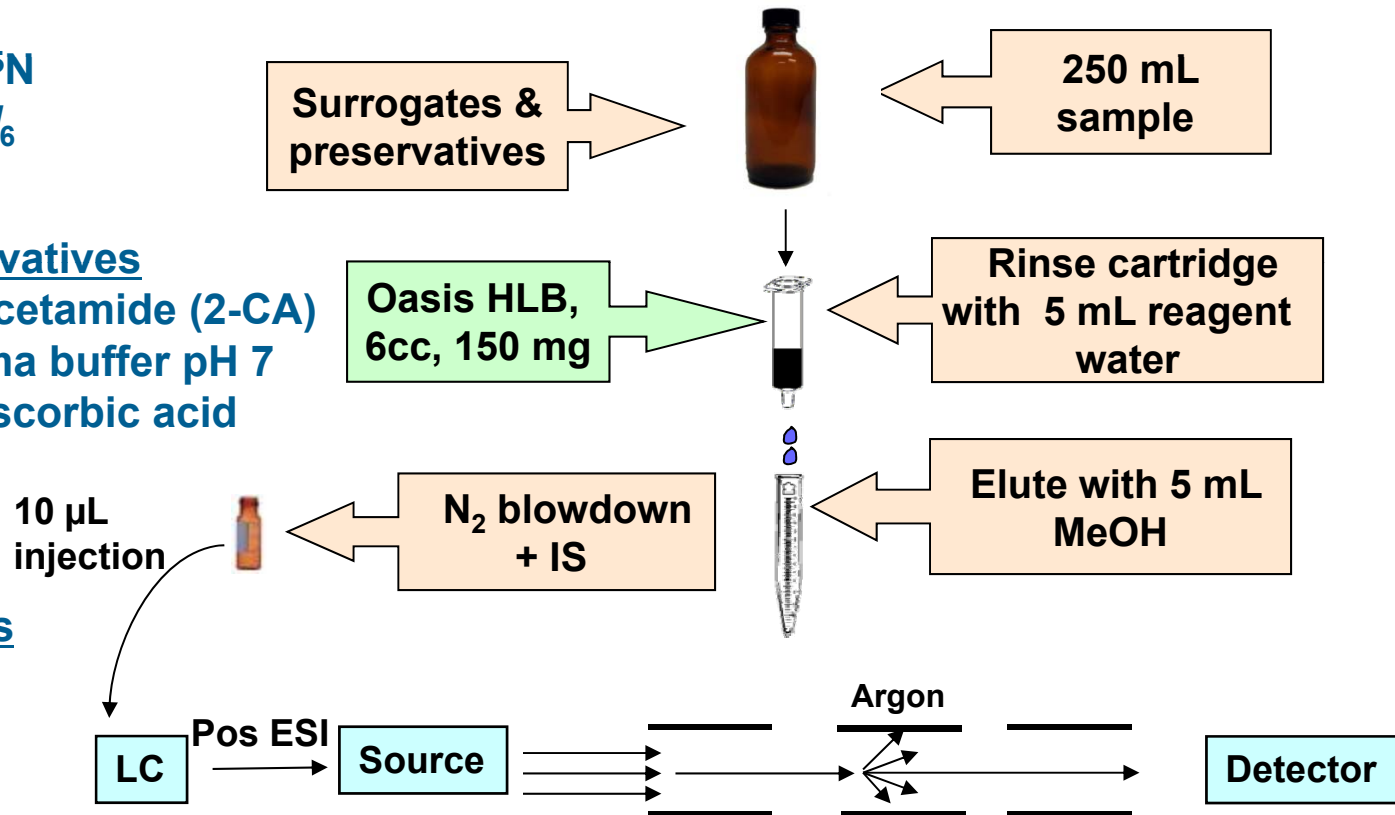
Concentration, elution, separation

# Off-line SPE – LC/MS/MS Procedure

**Surrogates**  
methomyl-<sup>13</sup>C<sub>2</sub>, <sup>15</sup>N  
tebuconazole-*d*<sub>6</sub>

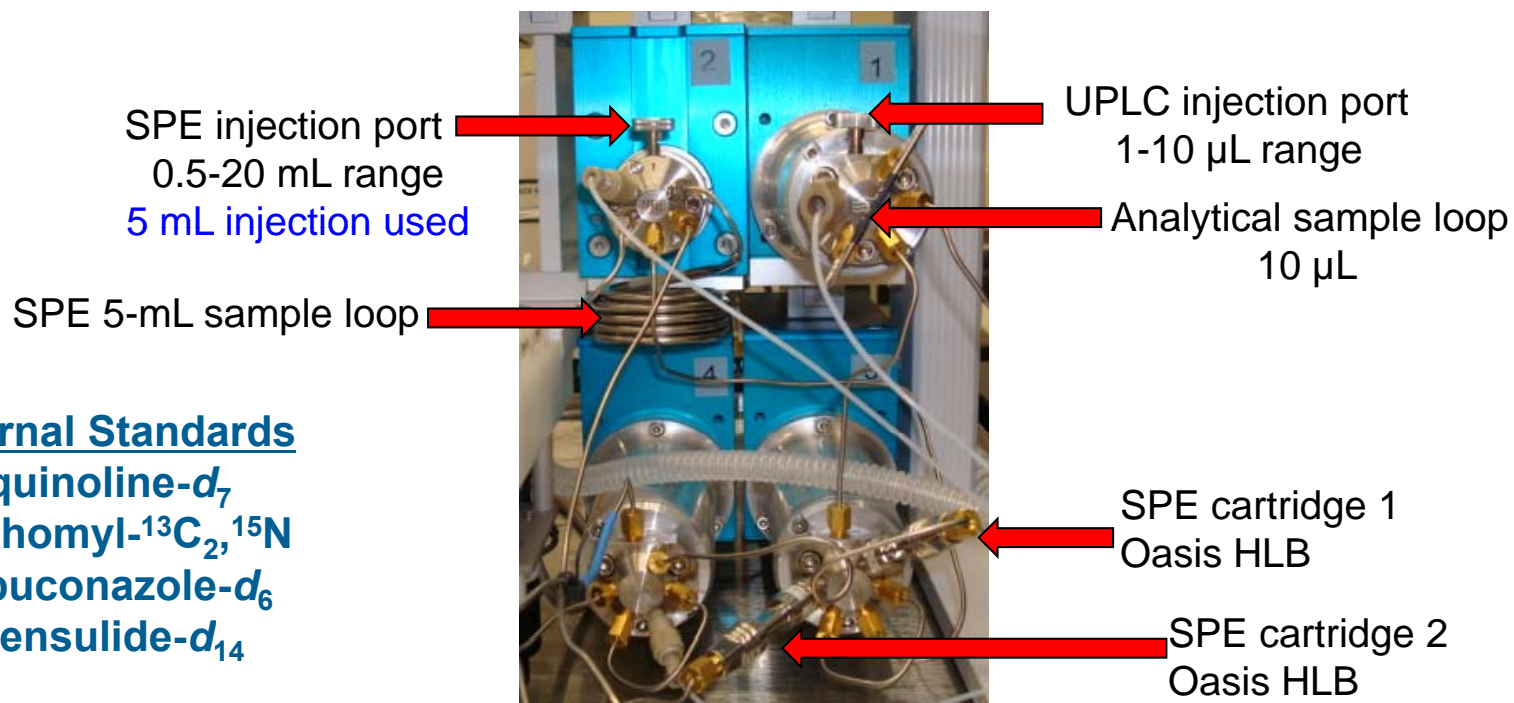
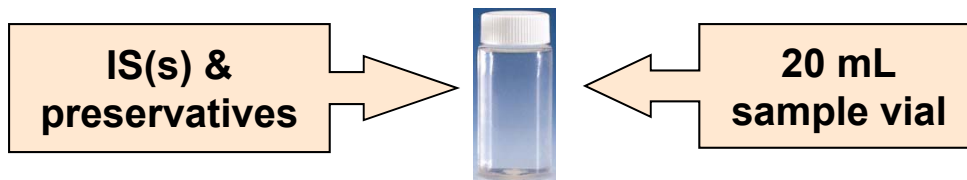
**Preservatives**  
2 g/L 2-chloroacetamide (2-CA)  
7.75 g/L Trizma buffer pH 7  
100 mg/L ascorbic acid

**Internal Standards**  
bensulide-*d*<sub>14</sub>  
carbofuran-<sup>13</sup>C<sub>6</sub>



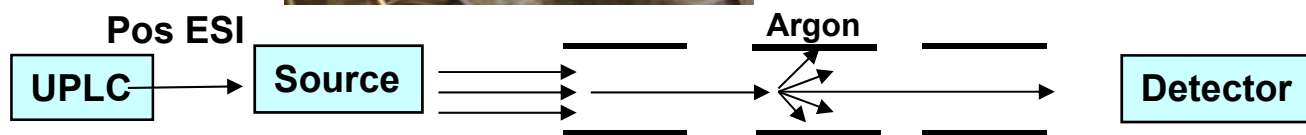
## LC/MS/MS - Selected Reaction Monitoring

# On-line SPE – LC/MS/MS Procedure



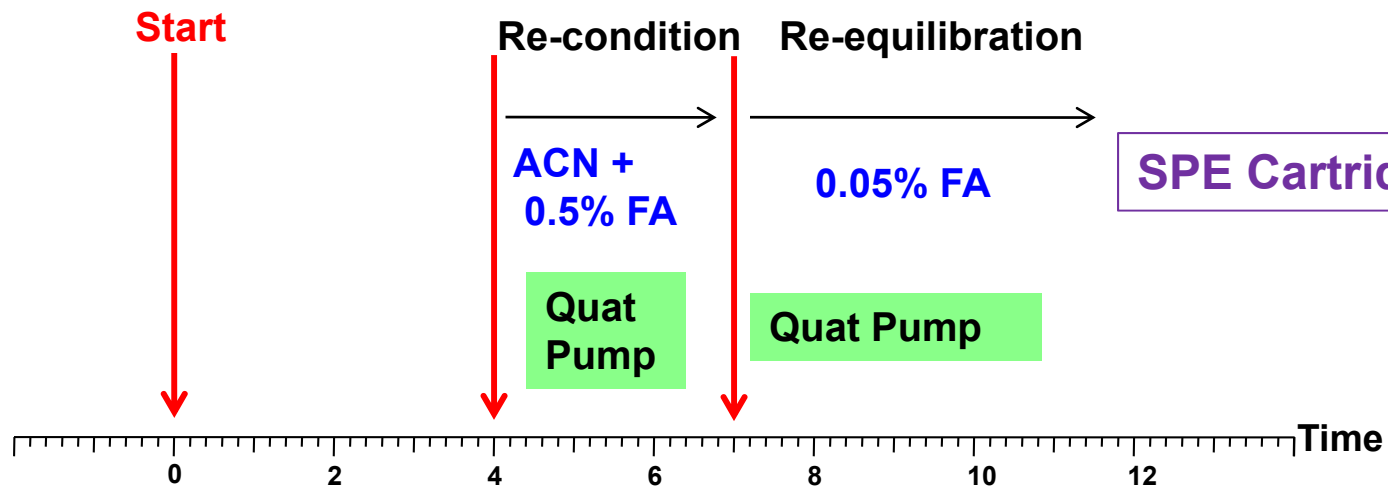
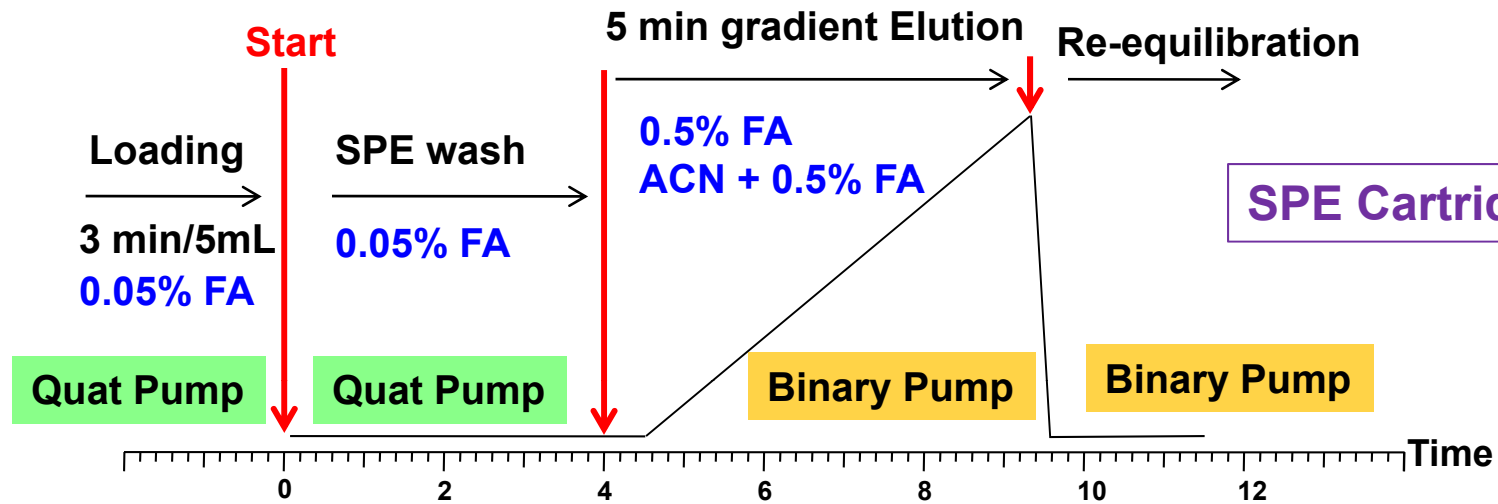
## Internal Standards

- quinoline- $d_7$
- methomyl- $^{13}C_2, ^{15}N$
- tebuconazole- $d_6$
- bensulide- $d_{14}$



## LC/MS/MS - Selected Reaction Monitoring

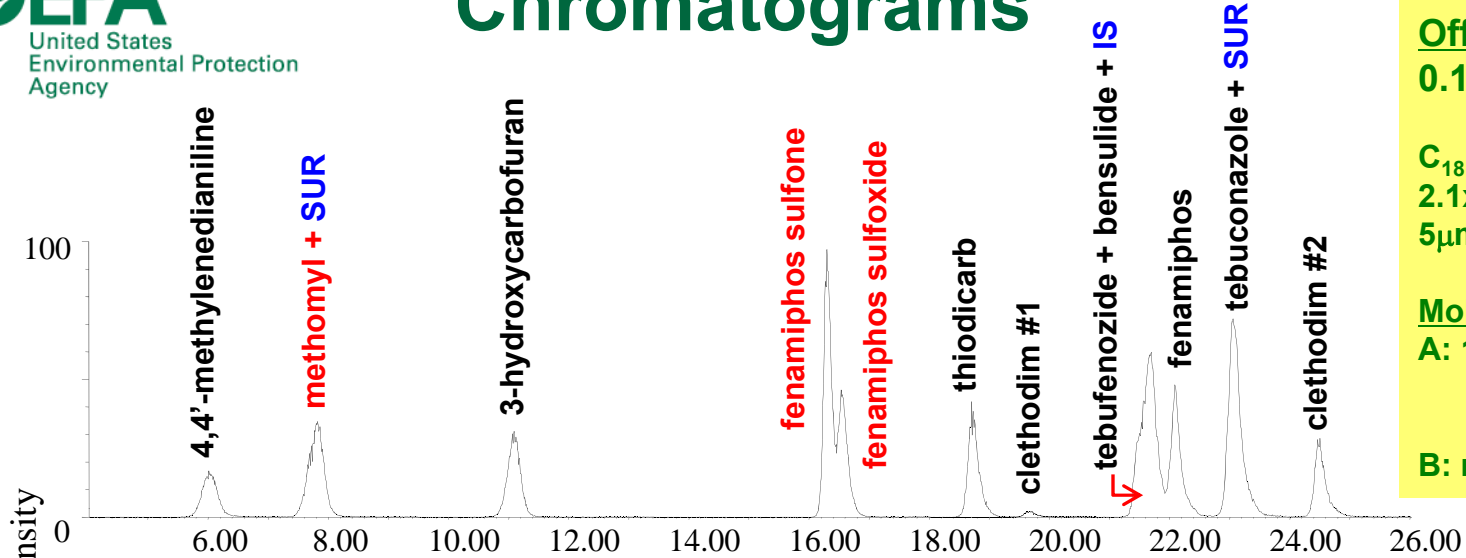
# On-line SPE Events



## Preliminary Method Studies

- ◆ **Optimize LC/MS/MS parameters**
- ◆ **Aqueous sample pH buffer optimization**
  - Some analytes not stable under acidic or basic conditions during sample holding time
- ◆ **LFB & LFM recoveries and precision for off-line and on-line SPE**
- ◆ **Preliminary holding time studies**
- ◆ **Preliminary DLs**

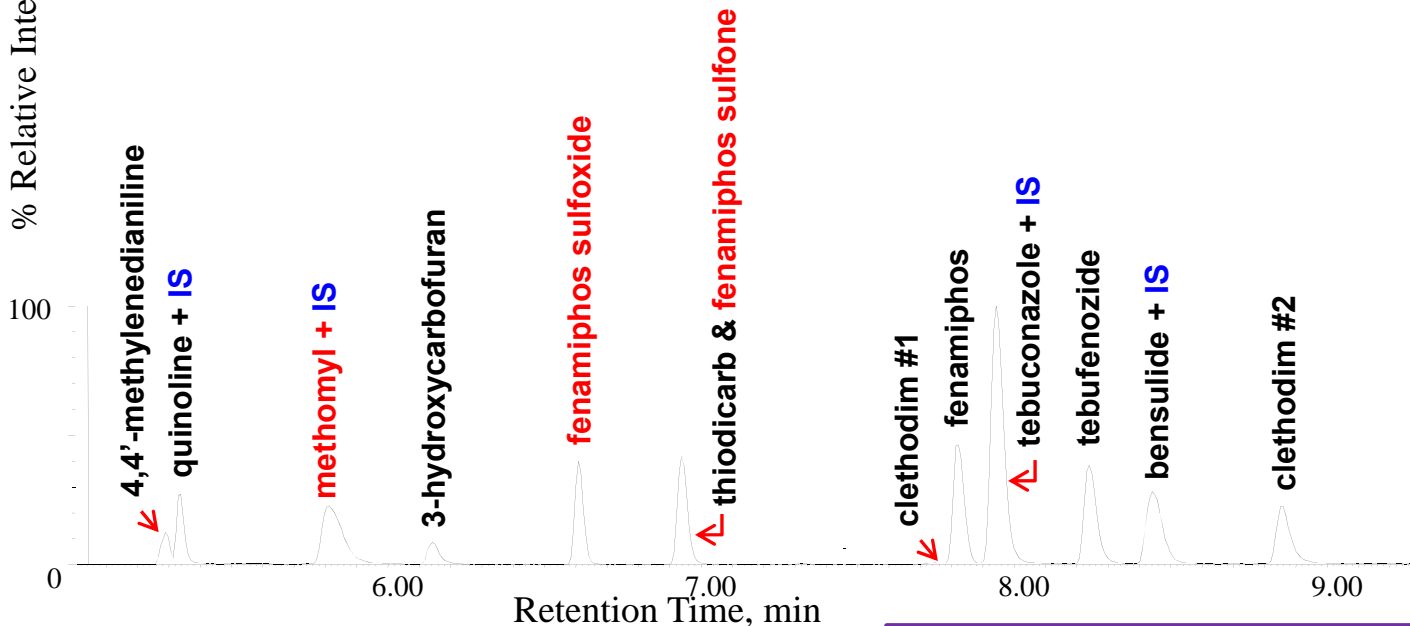
# Chromatograms



**Off-line SPE**  
0.128-0.32 µg/L

C<sub>18</sub> column  
2.1x100 mm  
5µm particles

**Mobile phase**  
A: 10 mM NH<sub>4</sub>HCO<sub>2</sub>  
0.05% HCOOH  
pH 3.4  
B: methanol



**On-line SPE**  
0.040-0.10 µg/L

UPLC BEH C<sub>18</sub>  
2.1x50 mm  
1.7 µm particles

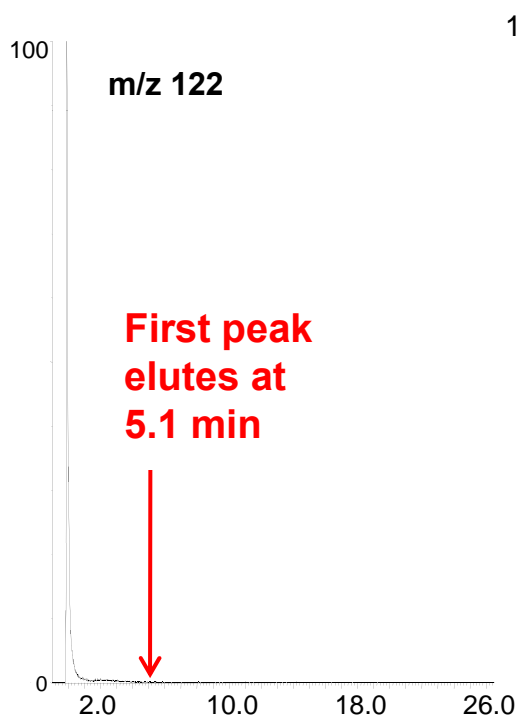
**Mobile phase**  
A: 0.5% FA  
B: ACN+0.5% FA

**Quinoline not in off-line SPE method due to poor sensitivity under LC conditions**

# pH Buffer

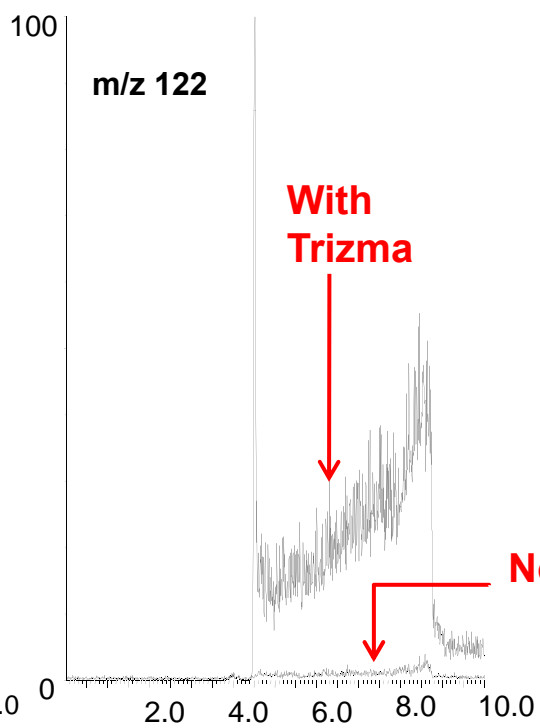
## Off-line SPE

**2-CA**  
**ascorbic acid**  
**Trizma (pH 7)**



## On-line SPE

**2-CA**  
**ascorbic acid**  
**Trizma (pH 7)**



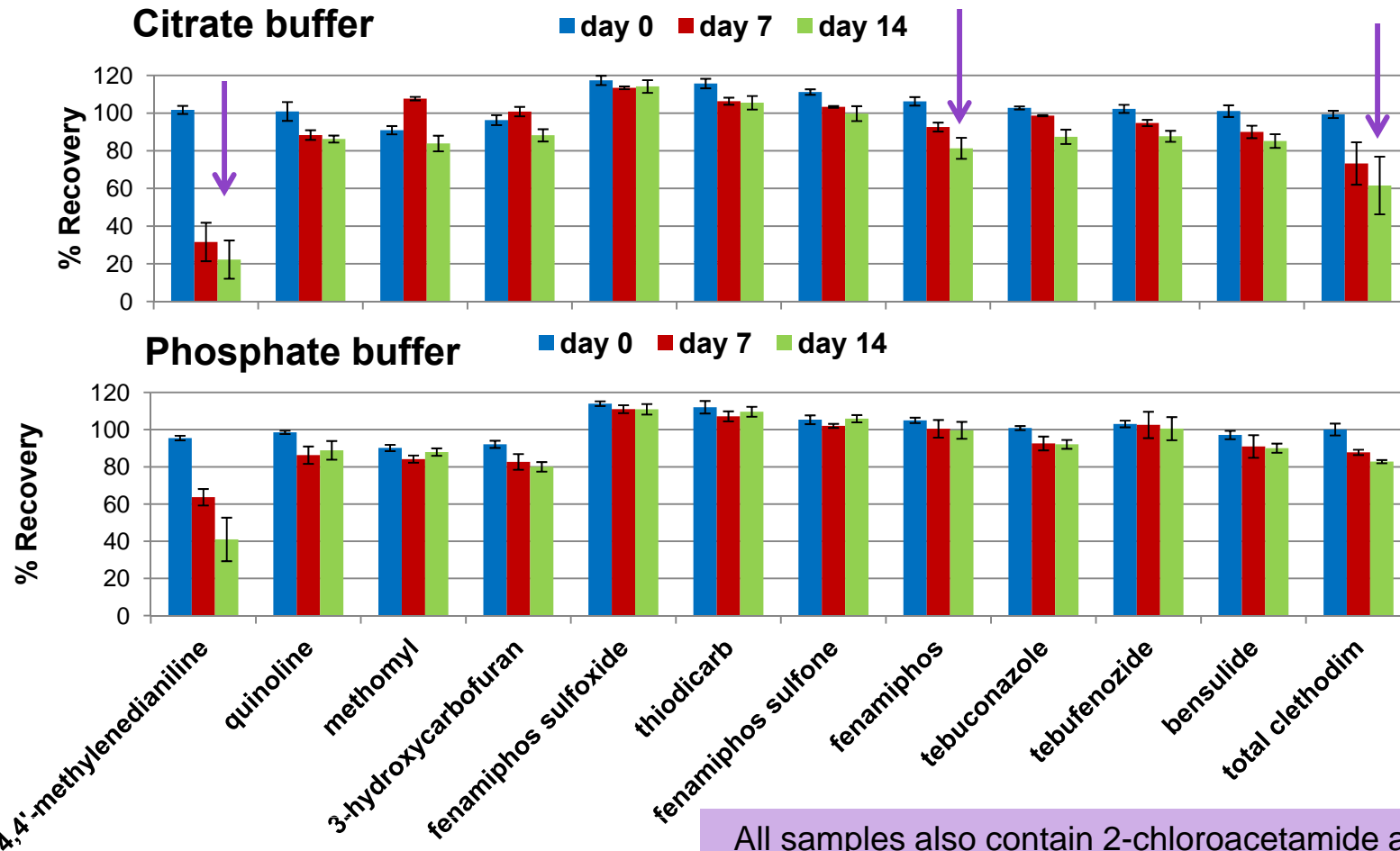
Evaluate other  
neutral pH buffers  
for on-line SPE,  
e.g.:

Citrate (6.2)  
Phosphate (7.1)



# Preliminary Holding Time Studies

## 0.2-0.5 µg/L in tap water (1-mL injection)

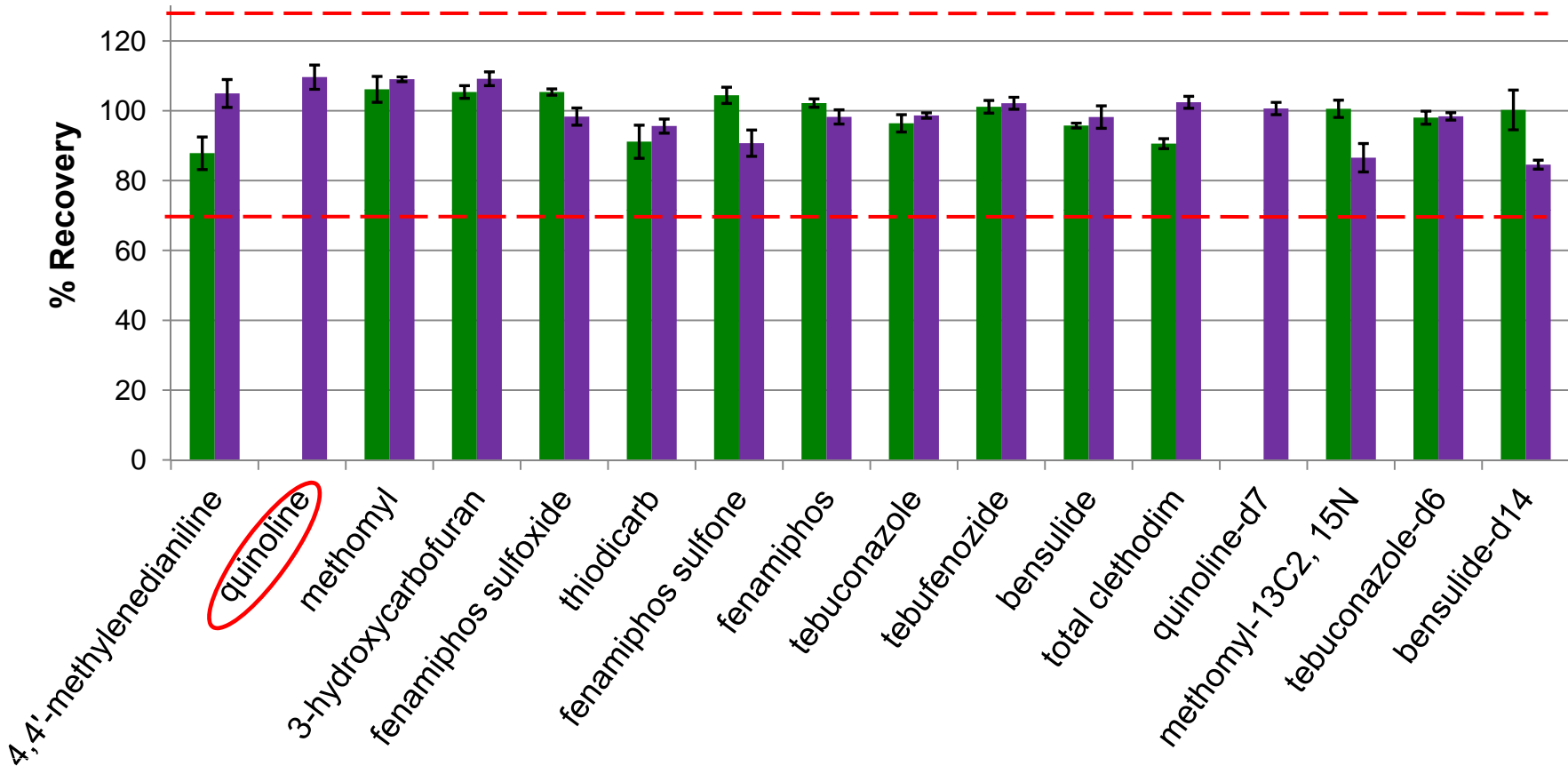


All samples also contain 2-chloroacetamide and ascorbic acid in addition to the indicated buffer

# Mean LFB Recoveries

fortified at 0.128-0.32 µg/L and 0.04 -0.1 µg/L

■ Off-line SPE (n=4)    ■ On-line SPE (n=3)

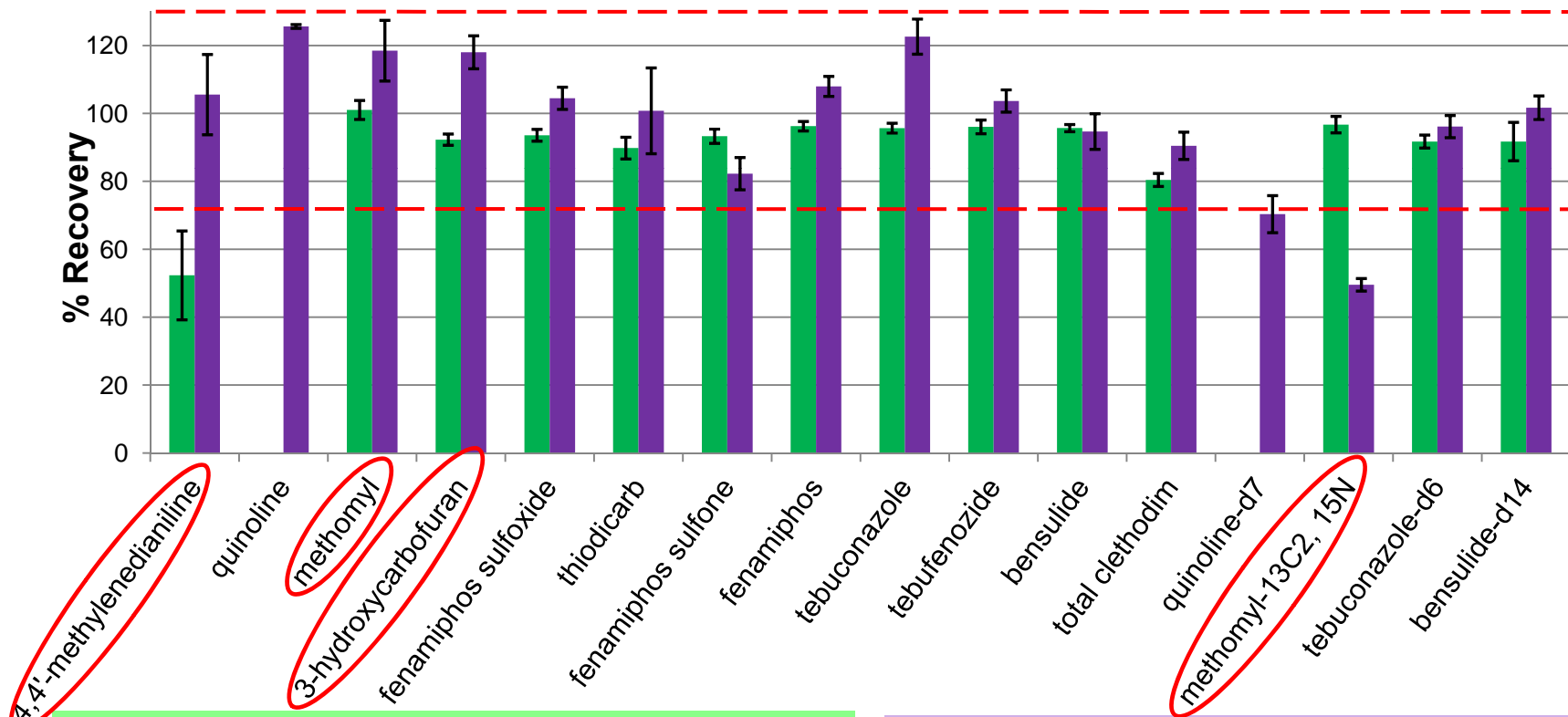


**Quinoline not in off-line SPE method due to poor sensitivity under LC conditions**

# Mean LFM Recoveries

fortified at 0.128-0.32  $\mu\text{g/L}$  and 0.004-0.010  $\mu\text{g/L}$   
chlorinated drinking water from a surface water source

■ Off-line SPE (n=4) ■ On-line SPE (n=3)



In off-line SPE, methylenedianiline's recovery is dependent on TOC concentration (2.9 mg/L)

By external calibration, methomyl % Rec is 60%, 3-hydroxycarbofuran is 57% & methylenedianiline is 72% - breakthrough using on-line SPE!

## Health Reference Level (HRL) and Preliminary DLs

Analytes	HRL, ng/L	DLs, ng/L	
		Off-line	On-line
quinoline	10	ND	1.1
4,4'-methylenedianiline	22	0.34	0.51
3-hydroxycarbofuran	42	0.22	0.65
fenamiphos	700	0.17	0.11
thiodicarb	1,900	0.41	0.49
bensulide	35,000	0.75	0.42
clethodim total	70,000	0.20	0.29
tebufenozide	126,000	0.24	0.11
methomyl	175,000	0.17	0.43
tebuconazole	210,000	0.40	0.12
fenamiphos sulfone	ND	0.28	0.35
fenamiphos sulfoxide	ND	0.26	0.66

Preliminary DLs for both methods are below the HRLs for all analytes

ND = Not determined

250 mL sample  
0.17-0.75 ng/L

5 mL sample  
0.11-1.1 ng/L

## Conclusions

- ◆ **Preservatives will be different for off-line and on-line SPE methods**
- ◆ **LFB recoveries meet 70-130% goal and precision goal of <30% RSD using both the off-line and on-line SPE methods for all analytes**
- ◆ **LFM recoveries meet 70-130% goal and precision goal of <30% RSD using both the off-line and on-line SPE methods, except for methomyl, 3-hydroxycarbofuran and 4,4'methylenedianiline**
- ◆ **At least a 14-day aqueous holding time can be achieved for the on-line SPE method using a phosphate buffer instead of the Trizma buffer (off-line SPE)**
- ◆ **Preliminary DLs were below the HRLs for all the analytes using both the off-line and on-line SPE methods**
- ◆ **Comparable DLs were achieved using the fully automated on-line SPE with a 5 mL sample size to the 250 mL off-line SPE samples**

## Future Work

### Off-line SPE

- ◆ **Multi-laboratory study**
- ◆ **Publish final method**

### On-line SPE

- ◆ **Continue optimization of SPE conditions**
- ◆ **Gather final method data including LCMRLs and final holding time study**
- ◆ **Multi-laboratory study**
- ◆ **Publish final method**