Current Method Development Activities (Commercial Lab Perspective)

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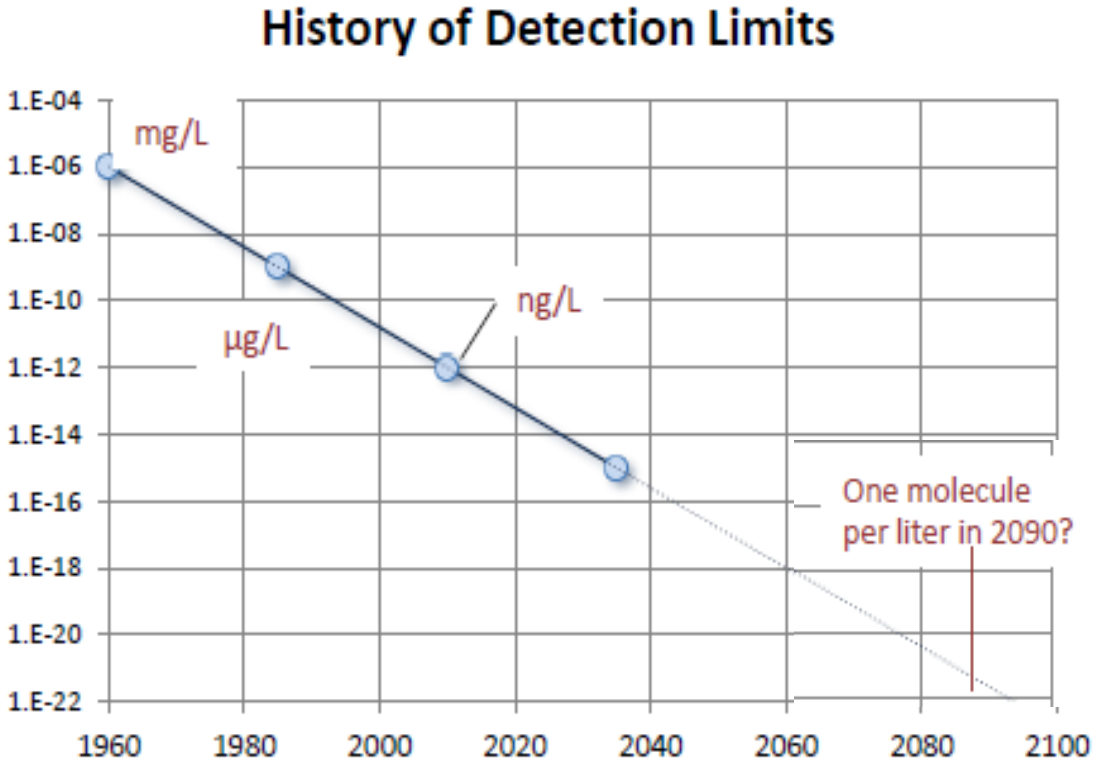
Presentation Outline

- Opportunities
- Why LC-MS-MS?
- Examples
  - PPCPs by on-line pre-concentration
  - PFCs by on-line
  - Pesticide screen
  - Endothall at 0.1 ppb
- Conclusions
- Questions

There Is No Such Thing as Zero….

- The fastest-growing area of techno-science in water is analytical technology

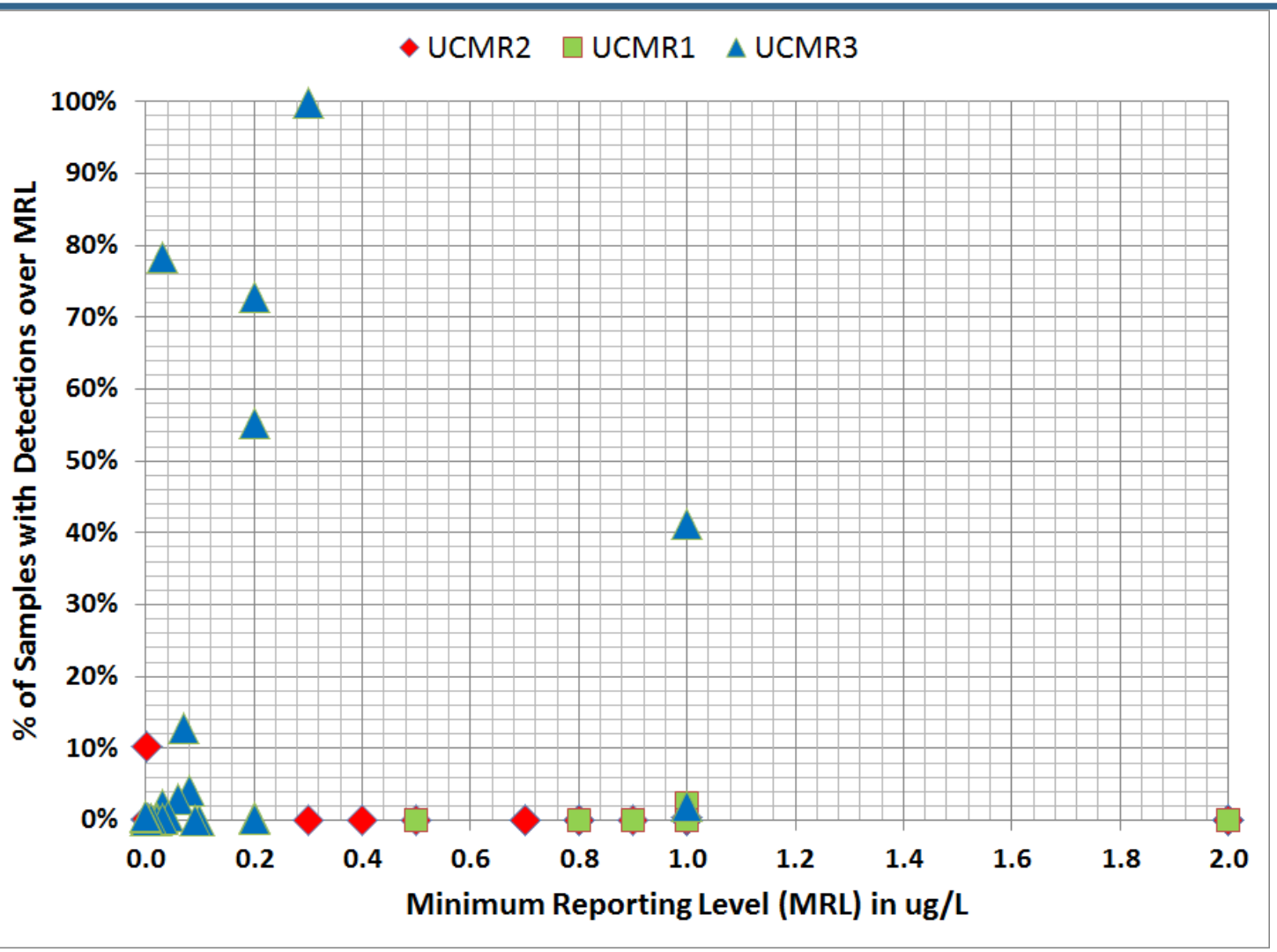


Moore's Law:
No. of transistors on
A microchip doubles
Every 2 years

Trussell's Law:
The Detection Limit for trace organics doubles (drops 2 fold) every 2.5 years

From Trussell, Clarke Prize Lecture 2013

What We Find Depends on How Low We Look



Data from EPA's Unregulated Contaminant Monitoring Rules.

Detection frequencies increase as Minimum Reporting Levels get lowered.

Figure From Roberson and Eaton, JAWWA 2014

AND You Only Find What You Actually Look For

Source Water CECs

- Barium
- PFBS
- PFOA
- Strontium
- PFHpA
- PFHxA
- PFNA
- PFOS
- PFBA
- PFDA
- PFHxS
- PFPeA
- Atrazine
- Carbamazepine
- Sulfamethoxazole
- 3,4,4'-Trichloro carbanalide
- Caffeine
- Carbamazepine
- Lithium
- Triclosan
- E1 (Estrone)
- Metoprolol
- *A. fumigatus*
- Benzotriazole methyl-1H
- Caffeine
- Carbamazepine
- N,N-diethyl-meta-toluamide (DEET)
- Giardia
- Aciclovir
- Atrazine
- Vanadium
- *Legionella pneumophila*
- Metformin
- Carbamazepine
- Galaxolide (HHCB)
- Methocarbamol
- Metolachlor
- PFUnDA
- Sulfamethoxazole
- Tri(2-butoxyethyl) phosphate
- Adenovirus
- Caffeine
- Meprobamate
- Tramadol
- Tri(2-chloroethyl) phosphate

These are compounds that showed up more than 1/3 of the time in a 25 plant study.

Did NOT include artificial sweeteners.

The only one of these that we might consider to be "emerging" would be the benzotriazole; although some of the PFCs are intriguing.

Treated Water CECs

- Barium
- PFBS
- PFHxA
- PFOA
- Strontium
- PFPeA
- PFHpA
- PFNA
- PFOS
- PFBA
- Atrazine
- PFHxS
- PFDA
- Bromoform
- Lithium
- Benzotriazole methyl-1H
- Triclosan
- Atrazine
- Caffeine
- Isophorone
- Metolachlor
- PFUnDA

Glassmeyer et al, SETAC 2013

PPCPs – Again You Have to Have the Targets Right

Chemical	Class	Overall Detection Frequency*	Concentrations (ng/L)			
			Maximum		Minimum	
Pharmaceuticals						
Alprazolam	anti-anxiety	40%	**1.4**	**J**	0.5	U
Amphetamine	stimulant	7%	**6.0**	**NJ**	2.5	U
Benzoylecgonine	cocaine metabolite	40%	**1.9**	**J**	0.5	U
Betamethasone	anti-inflammatory	7%	**3.2**	**J**	2.7	UJ
Carbamazepine	anticonvulsant	80%	**454**	**J**	2.9	UJ
Dehydronifedipine	nifedipine† metabolite	20%	**4.5**	**J**	1.2	U
Meprobamate	tranquilizer	67%	**190**		6.9	U
Sulfadimethoxine	antibiotic	7%	**2.0**		0.6	U
Sulfamethoxazole	antibiotic	80%	**497**	**J**	1.2	U
Sulfanilamide	antibiotic	47%	**118**		27	UJ

WA study of PPCPs in GW from reclaimed waters – only a few compounds above 10 ppt

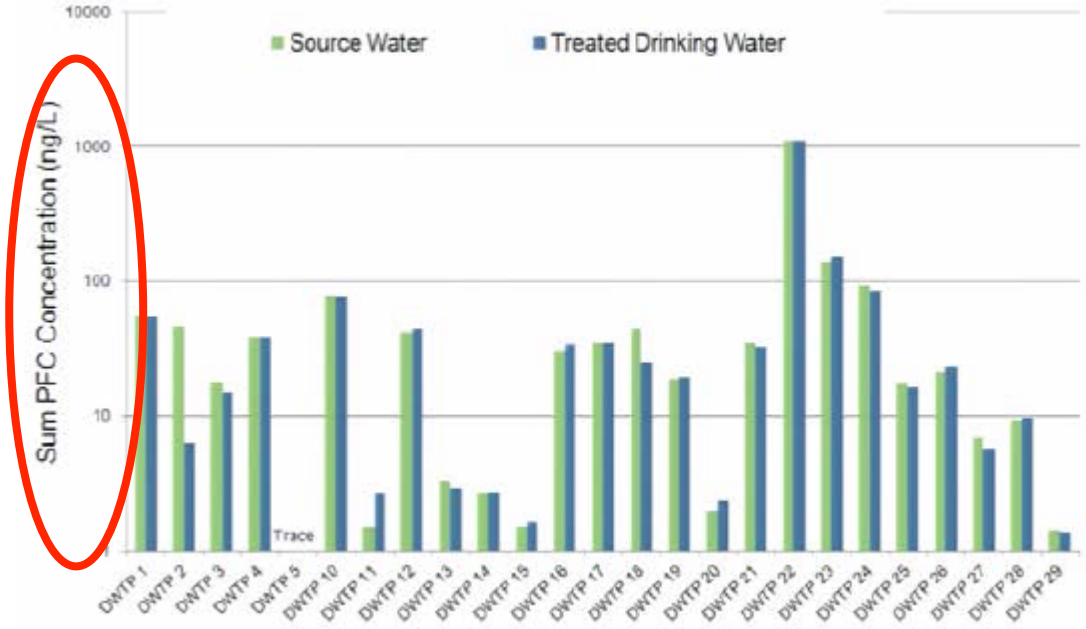
Our work, but also looking for artificial sweeteners and primidone

compound	units	Field Bla	MW	MW-	MW-	MW	MW	MW	MW	MW	MW-8DU
Acesulfame-K	ng/L	0	6000	5600	23000	1100	2000	1100	820	14000	16000
Albuterol	ng/L	0	0	0	31	0	0	0	0	16	26
BPA	ng/L	0	0	0	0	0	0	0	0	31	22
Carbamazepine	ng/L	0	54	0	0	20	22	0	8.5	78	59
DACT	ng/L	0	0	0	0	0	0	0	0	6	8.2
Dehydronifedipine	ng/L	0	5.6	0	0	0	0	0	0	22	21
Primidone	ng/L	0	11	28	52	0	0	0	0	12	10
Sucralose	ng/L	0	3000	1000	10000	400	1400	960	270	2400	2300
Sulfamethoxazole	ng/L	0	48	0	64	0	35	0	0	110	97
TCEP	ng/L	0	13	0	0	0	0	0	0	24	36

Detection Depends on Method Sensitivity (Example of PFCs)

Data from Glassmeyer et al (2013)

Detections in all plants, but MRLs at ng/L level



Data from USEPA UCMR3 Third Data Release (April 2014)

Very low frequency of detection but MRLs range from 10-90 ng/L

Contaminant	MRL1	NRL	Total number of PWSs with result	Number of PWSs with results ≥MRL	% of PWSs with results ≥MRL
PFOS	0.04	0.2	1874	34	1.8%
PFOA	0.02	0.4	1874	36	1.9%
PFNA	0.02	NA	1874	4	0.2%
PFHxS	0.03	NA	1874	21	1.1%
PFHpA	0.01	NA	1874	29	1.5%
PFBS	0.09	NA	1874	2	0.1%

Why LC-MS-MS?

- Polar and non-volatile compounds are widely used and could end up in the environment.
- LC-MS-MS instruments continue to improve in sensitivity and are coming down in cost.
- EPA as long ago as 2008 (UCMR2) was promulgating LC-MS-MS based methods
  - If EPA is doing it, it must be a VERY mature technology

The LC-MS-MS (non-TOF/Orbitrap) Conundrum

Pros

- Highly sensitive now, minimizing sample prep
- Instrument software is much more powerful than before, minimizing data interpretation

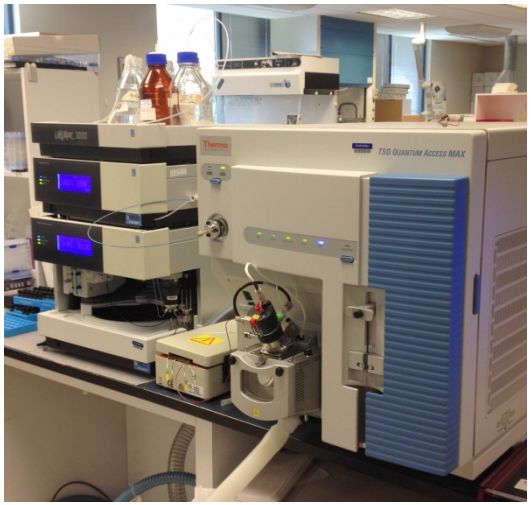
Cons

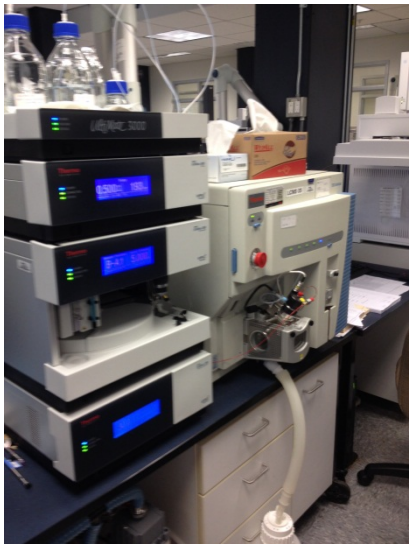
- You can only find what you look for
- Signal suppression or enhancement can be significant

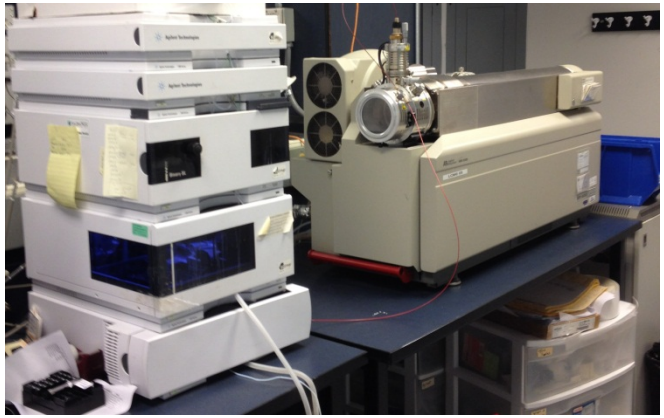
A History of Spending $$$... But Getting Lots of Methods

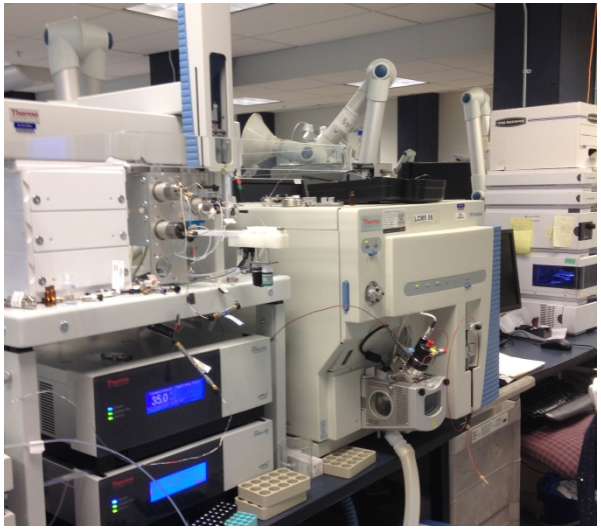
- 2004 – Quattro Ultima – used.. And now obsolete
  - Acrylamide, Oxyhalides, (originally also perchlorate, PPCPs)
- 2007 – API 4000
  - PFCs, PPCPs, Urea Herbicides, Oxyhalides
- 2008 – API 5000
  - PPCPs, PFCs, Perchlorate, Acrylamide, Oxyhalides, Endothall
- 2011 - API 2000; replaced in 2014 by TSQ Quantum Max
  - Perchlorate
- 2012 – TSQ Vantage (2)
  - PPCPs, PFCs, Pesticides, Herbicides, Endothall

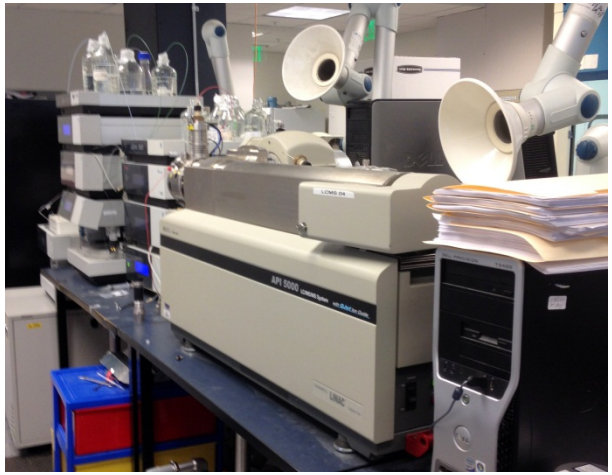
An Equal Opportunity (Almost) Laboratory







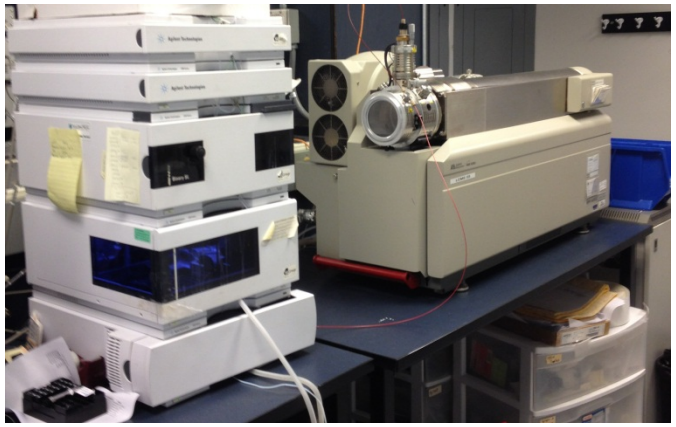




METHOD DEVELOPMENT ACTIVITIES

Early Opportunities to Break New Ground

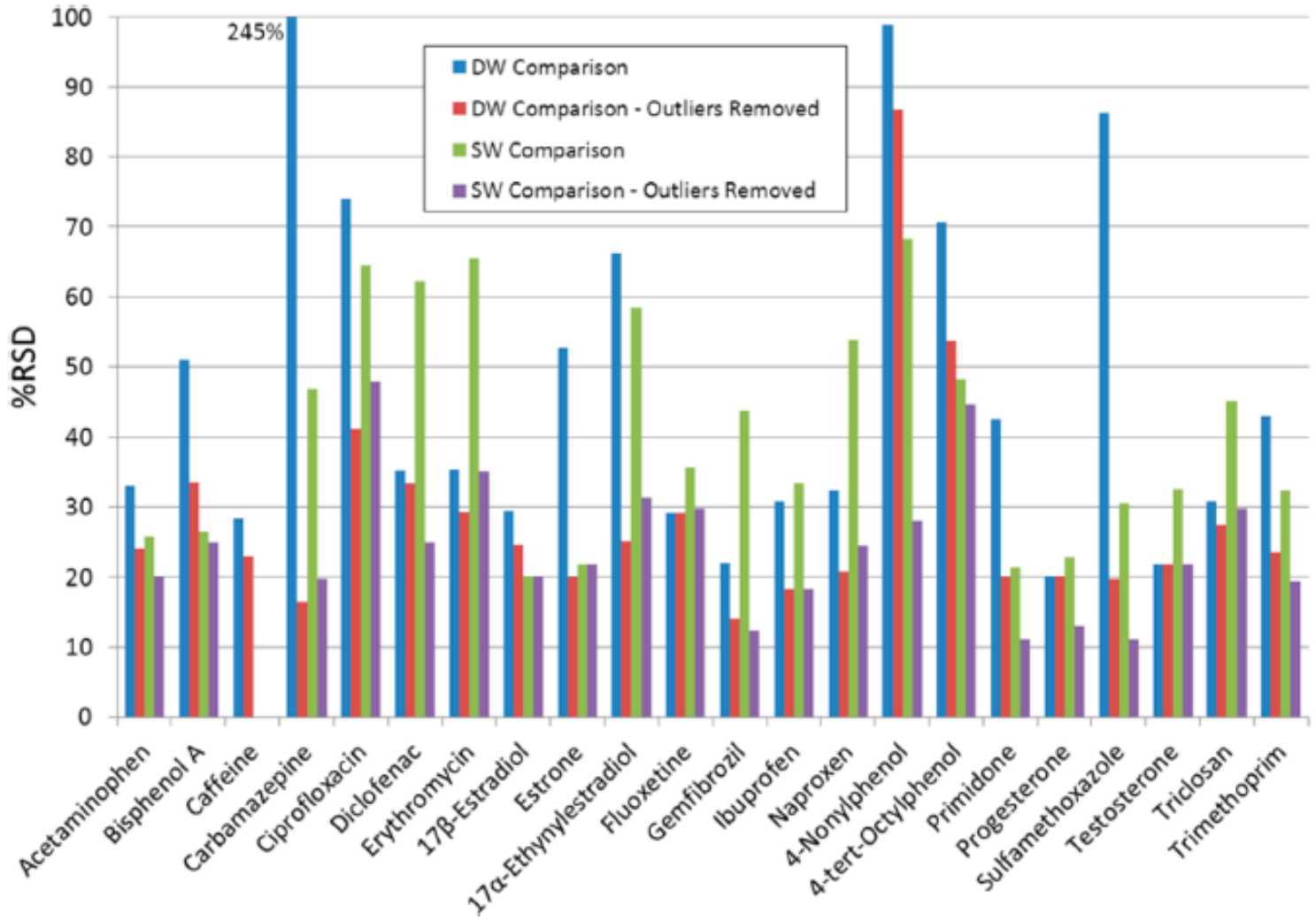
- PPCPs were our first major target
  - Prompted by USGS ES&T publication and subsequently "aided" by the Associated Press
  - No standardized methods
  - No standardized lists
  - How could we improve sensitivity and speed?
  - Quattro instrument not good enough for needed sensitivity.
    - Moved to ABI-4000 and then ABI-5000



Interlaboratory study - WaterRF 4167 – What Was It?

- SNWA was Principle Investigator, along with EEA, MWD, Colorado School of Mines, Shane Snyder and German Institute of Hydrology and ERA
- Purpose was to evaluate all aspects of PPCP analysis
  - Develop reasonable target list
  - Assess precision, accuracy, and sensitivity of methods
  - Select best method(s)
  - Determine best bottle type and preservation and HT
  - Evaluate multi-lab performance (25 labs involved, worldwide)

Not All Methods Are Created Equal – Example of PPCPs



Round robin study of PPCPs and hormones in water, involving up to 25 labs.

There are clearly some bad methods out there, even for simple matrices.

There are ALSO some difficult to measure analytes.

From Vanderford et al (2014)

The Lower We Look the Greater the Potential for False Positives

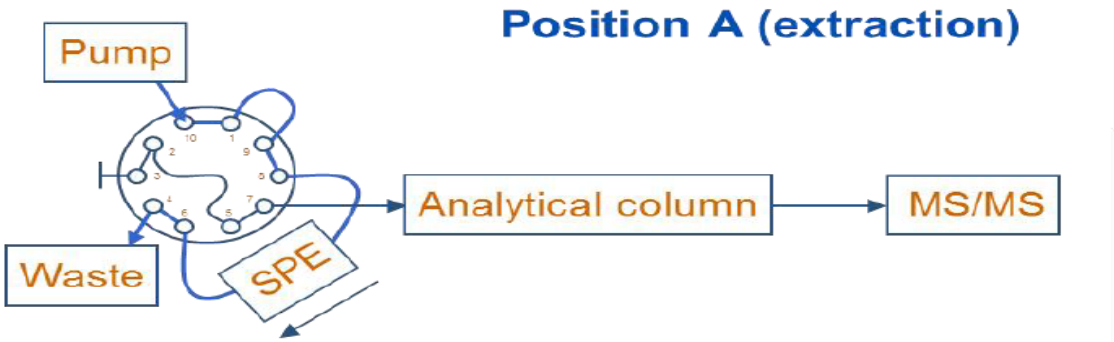
compound	no. false positives	no. of samples	rate (%)	range (ng/L)	no. of false negatives	no. of samples	rate (%)
acetaminophen	1	99	1	1.3	9	221	4
bisphenolA	21	87	24	1.2–46	2	193	1
caffeine	8	50	16	2.93–29.2	N/A	N/A	N/A
carbamazepine	4	82	5	2.01–24.4	5	298	2
ciprofloxacin	9	64	14	12–112	17	144	12
diclofenac	3	98	3	1.4–4.99	0	214	0
erythromycin	1	47	2	26.4	4	171	2
17β-estradiol	2	95	2	2.27–5	22	209	11
estrone	3	93	3	1.55–2.62	15	203	7
17α-ethynylestradiol	5	105	5	2.6–13.8	23	231	10
fluoxetine	1	92	1	1.5	15	204	7
gemfibrozil	0	67	0	N/Aa	5	243	2
ibuprofen	14	126	11	1–33	3	282	1
naproxen	6	114	5	3.2–18.9	5	254	2
4-nonylphenol	22	32	69	12.8–1280	11	116	9
4-tert-octylphenol	9	40	23	1.6–130	3	148	2
primidone	3	45	7	1.43–22.6	1	165	1
progesterone	2	70	3	1.54–1.66	7	154	5
sulfamethoxazole	4	73	5	2.88–5.17	0	265	0
testosterone	0	78	0	N/Aa	15	170	9
triclosan	13	67	19	1.28–350	4	243	2
trimethoprim	0	67	0	N/Aa	4	243	2

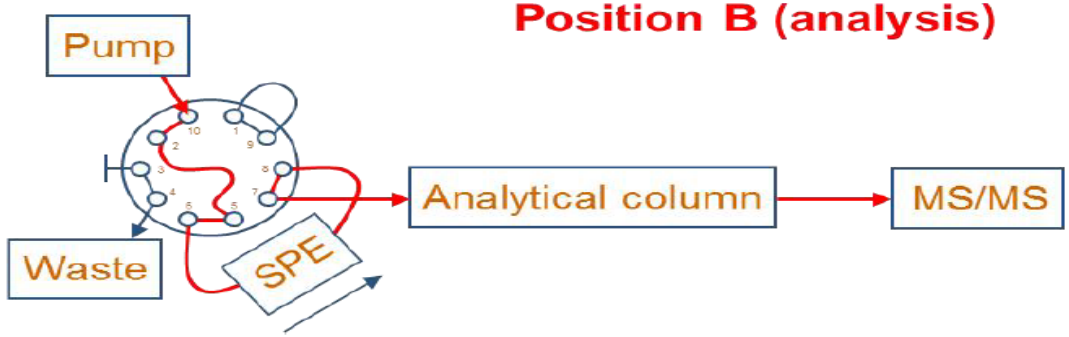
False positives are of much greater concern analytically than false negatives

Vanderford et al (2014)

PPCPs - Analytical Methodology

We Developed a Cost Effective Precise and Accurate Online Method for Simultaneous Extraction and Analysis of 90+ Analytes





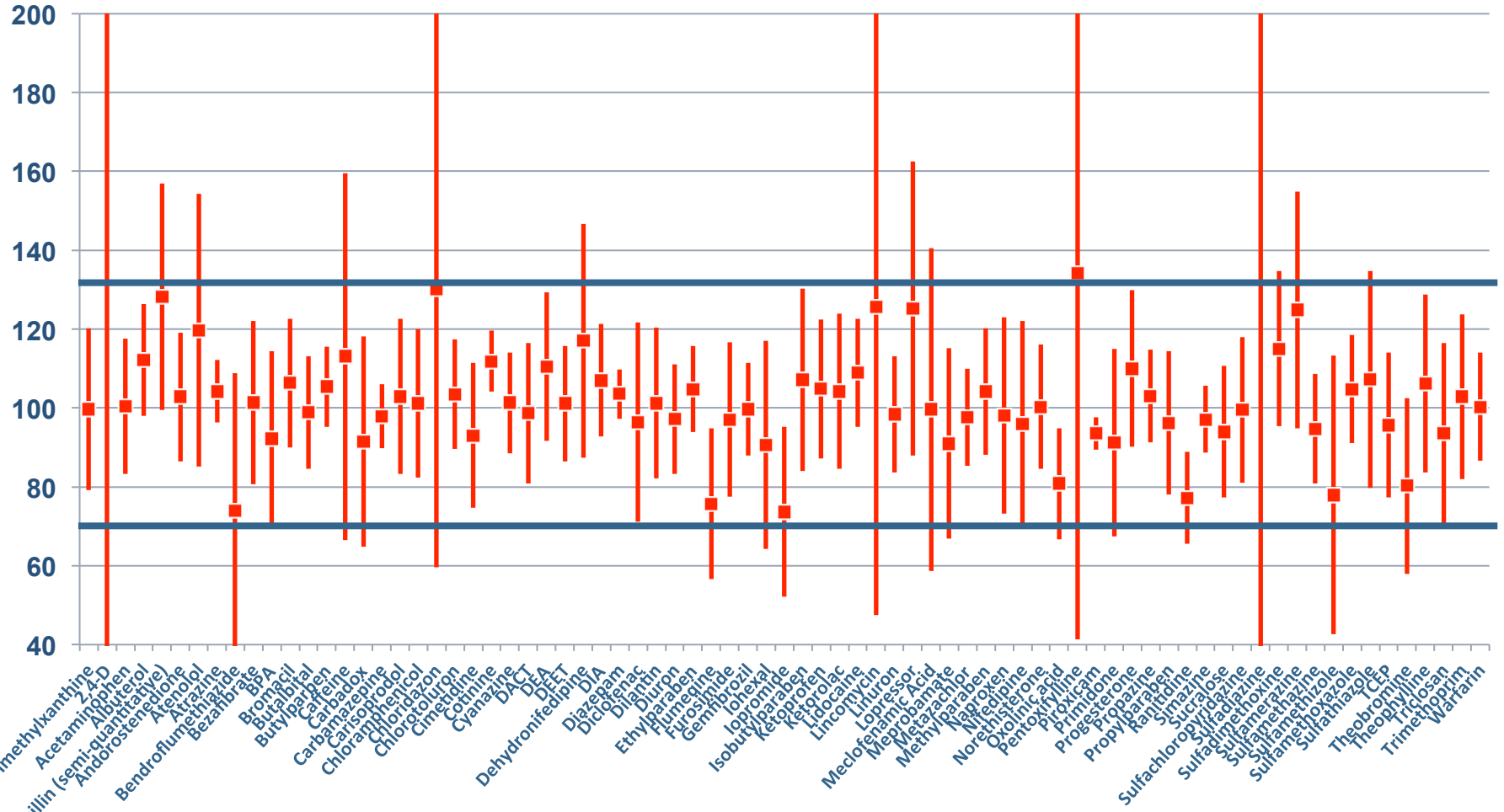
Pros And Cons Of On-line Enrichment Approach Versus Off-Line

On-line	Off-line
Small sample volume -2.5mls	Large sample – 500-4000 mls
Sample prep time – 5 min/sample	Extraction time 6-8 hours for 20 samples
Less Solvent and waste generated	Larger amount of solvent and waste is generated 3-10X
No evaporation step – better recovery for some compounds	Concentration step needed – during evaporation close to dryness, some compounds lost
No reconstitution of volume is needed	Volume needs to be reconstituted – compounds are lost to the walls
Less human error	More chances of human error

More Pros And Cons Of On-line Enrichment Approach Versus Off-Line

On-line	Off-line
Better sensitivity: entire injected sample is analyzed – 2.5 mls	A fraction of concentrate gets injected- 2-100 ul (from 1 ml extract)
Higher sample throughput	Extraction and analysis are separate – more complex scheduling
Less matrix effect	Interferences can be concentrated along with the sample
Less prone to lab contamination – less handling of sample	More prone to lab contamination
Data processing is bottle neck	Slower throughput
More cost effective	More costly

Mean and Std Deviation of MS/MSD Recoveries over 6 months



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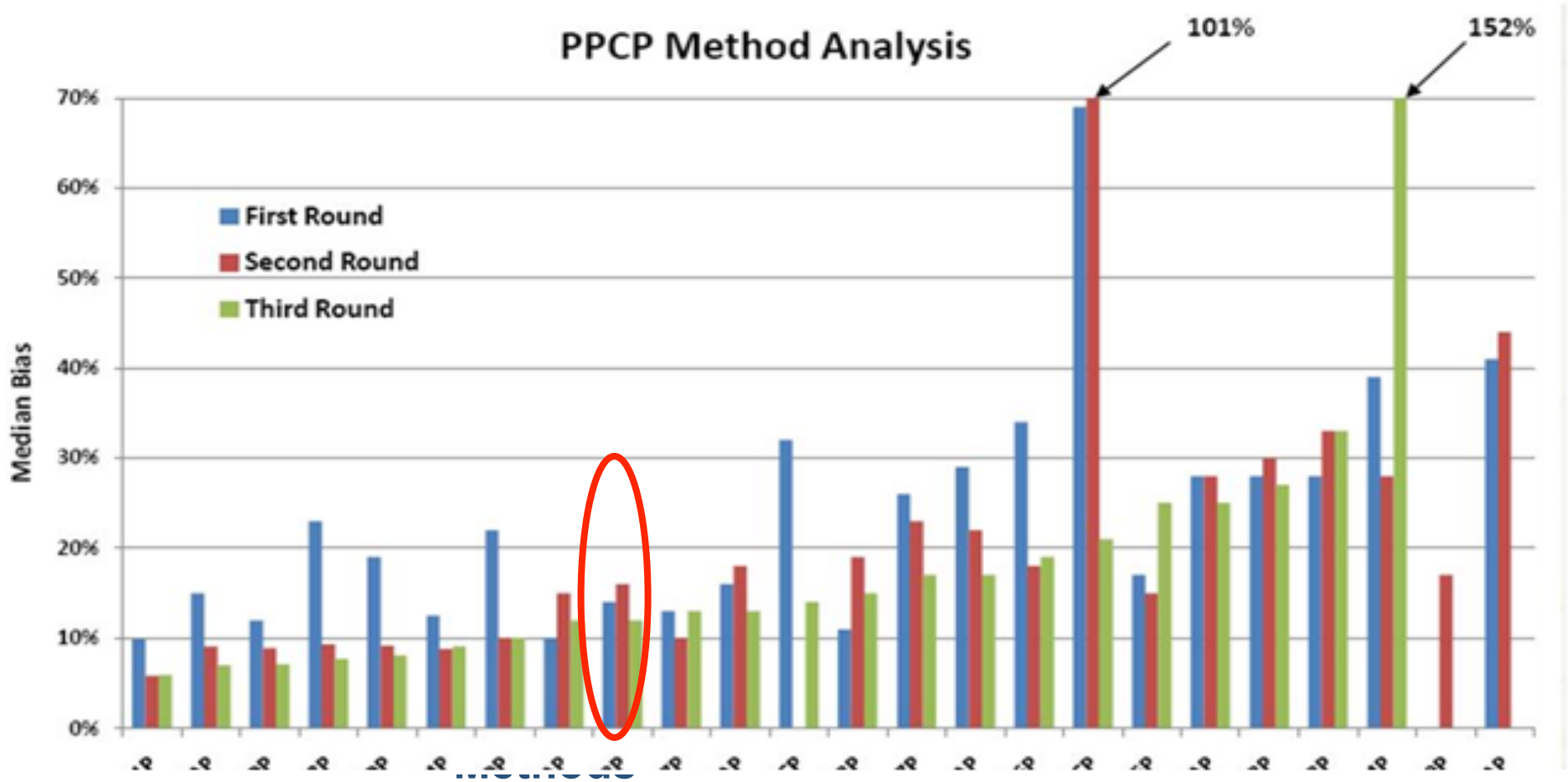
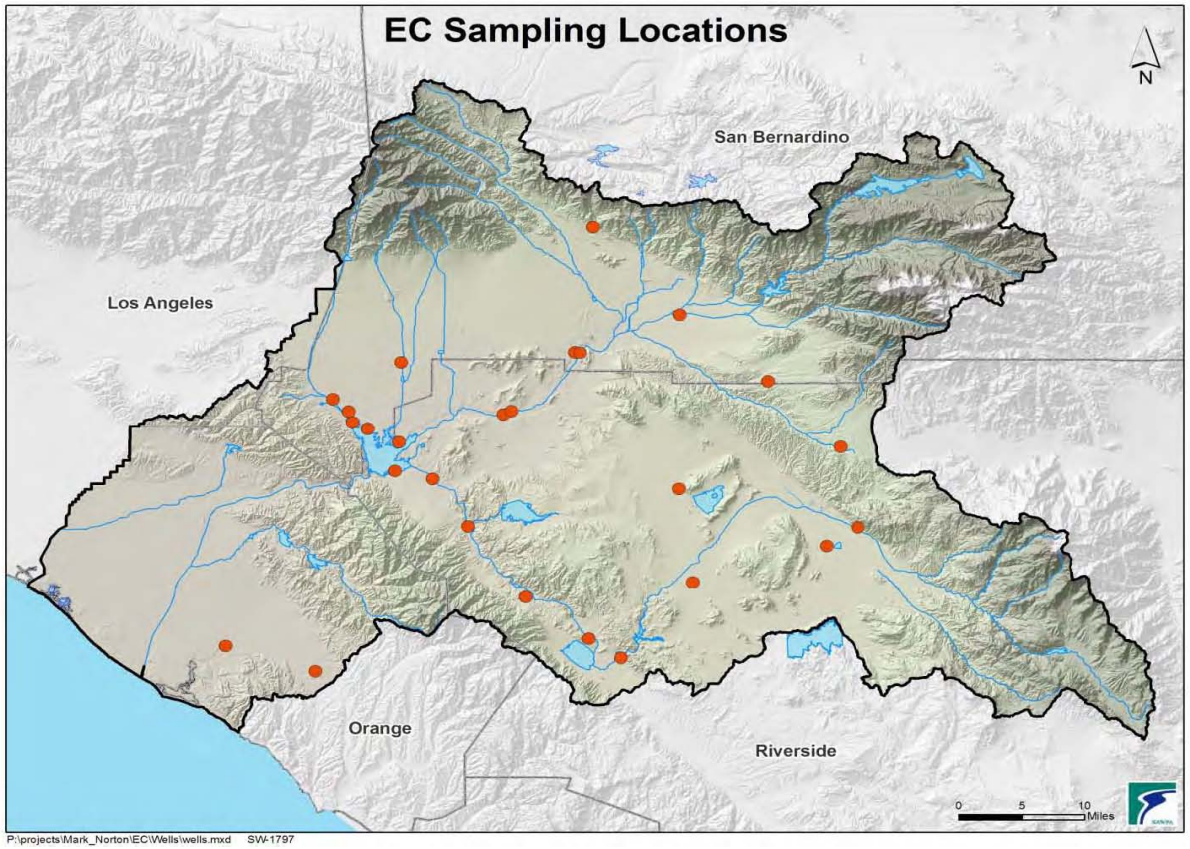
The Method Performed Well in the Interlab Comparisons (WaterRF 4167)

Note: This method had the most analytes IN A SINGLE METHOD of any participating lab



The Next Interlab Study was Performed for SAWPA– Samples from 23 SoCal WW Effluents



Sites	SAWPA List only	EEA Long List
Number of Sites	4	13
Percent of Sites	24%	76%

Overall there were 23 Discharge Sites, of which EEA tested 17.

ERA PT Samples for Low Level – Project MRL Set at 10ppt

					Lab 1	Lab 2	Lab 3	MWH-online
Analyte	%RSD	Assigned Value	Mean Recovery	Median Recovery	% Rec.	% Rec.	% Rec.	% Rec.
Acetaminophen	18	14	103	106	121	100	78	111
Bisphenol A	9.9	10.4	97	97	NR	NR	104	90
Caffeine	22	11	123	116	115	118	160	97
Carbamazepine	2.8	11	101	101	103	100	105	98
DEET	6.6	13.8	116	116	124	109	121	110
Diuron	-	ND	-	-	-	-	-	-
Ethynylestradiol	6.4	12.5	89	90	96	88	91	82
Gemfibrozil	-	ND	-	-	-	-	-	-
Ibuprofen	24	12	100	102	112	92	125	70
Sulfamethoxazole	8.1	11.5	106	105	104	96	117	106
TCEP	-	ND	-	-	-	-	-	-

ERA PT Samples Spiked at Medium Level

					Lab 1	Lab 2	Lab 3	MWH-online
Analyte	%RSD	Assigned Value	Mean Recovery	Median Recovery	% Rec.	% Rec.	% Rec.	% Rec.
Acetaminophen	13	150	108	108	123	91	115	101
Bisphenol A	5.1	102	85	84	NR	90	82	84
Caffeine	14	85	89	90	104	93	87	74
Carbamazepine	5.8	34.9	96	94	105	95	94	92
DEET	13	105	106	104	125	92	107	101
Diuron	13	134	108	106	103	93	109	127
Ethynylestradiol	18	145	85	81	102	81	80	75
Gemfibrozil	10	27	106	108	91	115	103	113
Ibuprofen	14	33	107	103	108	94	98	127
Sulfamethoxazole	4.2	77.5	104	105	106	98	103	108
TCEP	22	195	84	86	92	103	81	60

Split unknown samples – Discharge

					Lab 1	Lab 2	Lab 3	MWH-online
Analyte	%RSD		Mean Result	Median Result	Result (ng/L)	Result (ng/L)	Result (ng/L)	Result (ng/L)
Acetaminophen	98		41	22	14.8	<5	87.8	21.5
Bisphenol A	-		-	-	NR	<30	ND	<10 (9.7)
Caffeine	7.7		32	32	28.2	32	34.0	32.3
Carbamazepine	4.1		104	104	105	109	98.8	103
DEET	12		88	85	103	89	81.0	79.3
Diuron	71		111	78	74.1	60	230	81.5
Ethynylestradiol	-		-	-	<2	<10	ND	<5
Gemfibrozil	29		10	9	8.15	9	13.7	7.54
Ibuprofen	-		-	-	<1	<10	48.6	<10 (4.2)
Sulfamethoxazole	11.7		59	57	56.6	58	52.2	68.5
TCEP	35		207	227	239	215	271	104

Perfluorinated Compounds- 2007

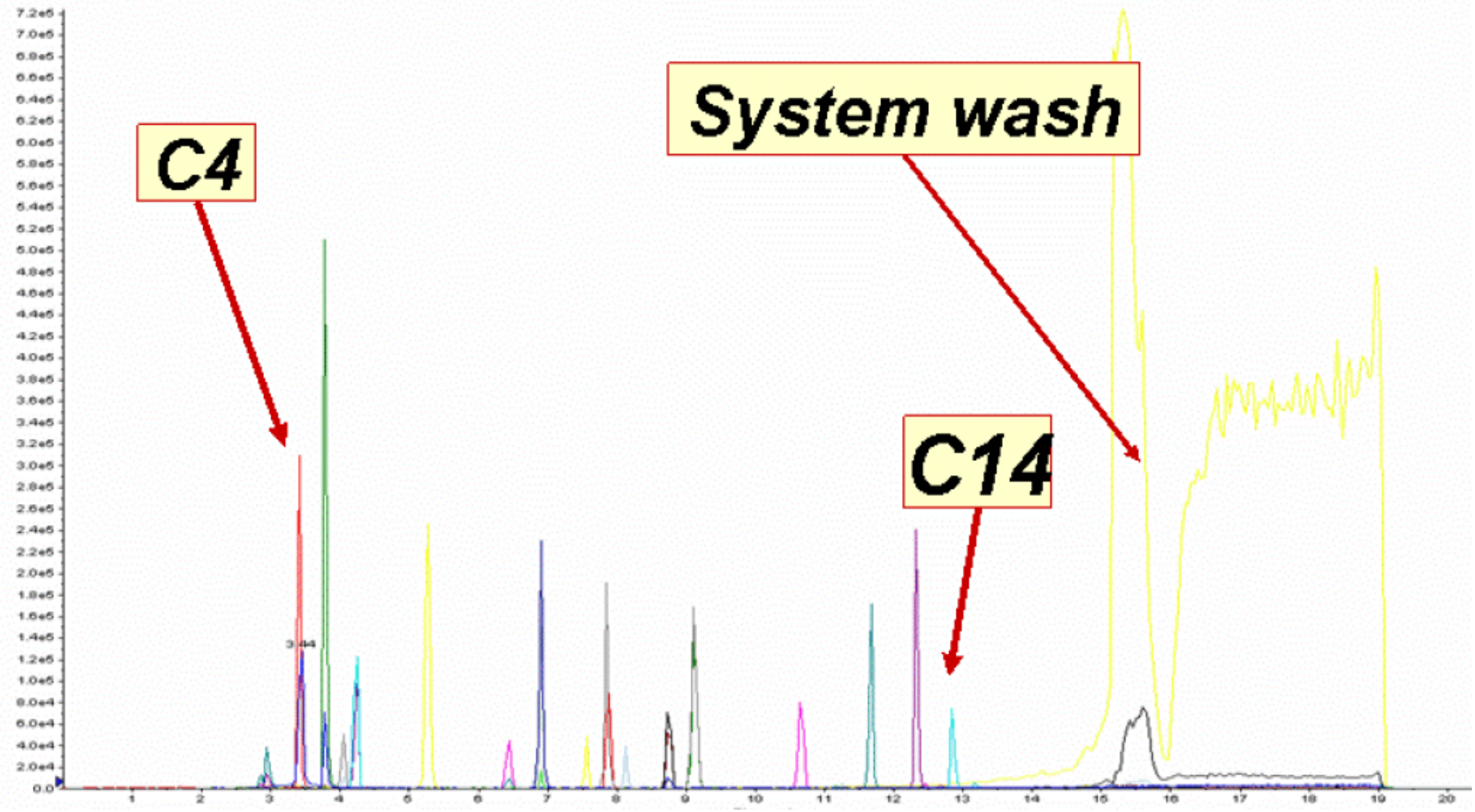
- With the prevalence of opportunities for artifacts with PFC analysis (teflon, etc) we looked for ways to simplify the analysis and minimize handling.

- Online enrichment methods offered improvement.

2005 PERFORCE 1st Worldwide Inter-laboratory Study On Perfluorinated Compounds In Environmental and Human Samples (38 Labs, 13 countries)

Water Results	PFOA	PFOS
Spiked concentration (ng/L)	19.4	19.5
Analytical results (ng/L)		
Minimum concentration	3.4	4.7(6.6)
Median concentration	23	25
Maximum concentration	190	112
Evaluation of results (16 Labs)		
%Satisfactory	31	22
%Questionable	13	6
%Unsatisfactory	56	72

PFCs by online SPE-LCMS/MS



Comparison to 537

537

- Better for High MW PFCs (> C10).
- 250 ml sample.
- Time consuming off-line extraction

Online method

- Better for low MW PFCs (C4).
- Only need a few mls.
- < 5 min prep time

Results of Blind Sample Analysis Demonstrate Comparability

QuiK™ Response Final Report
Project Number: 012110K

MWH Laboratories
750 Royal Oaks Drive
Suite 100
Monrovia, CA 91016

ERA Customer Number:
EPA Lab ID:

Results Reported By: Nilda Cox
Title: QA Officer
Phone # 626-386-1100
Fax # 626-386-1139

Study Open Date: 1/21/2010
Study Close Date: 2/1/2010
Report Issue Date: 2/2/2010

Perfluorinated Carboxylic Acids & Sulfonates (Cat# 093)						
Analyte	Units	Reported Value	Assigned Value	Acceptance Limits	Performance Evaluation	Method Description
Perfluoro-n-butanoic acid (PFBA)	ng/L		700	451 - 742		
Perfluoro-n-octanoic acid (PFOA)	ng/L	1380	1600	782 - 2080	Acceptable	EPA 537
Perfluoro-n-octane sulfonate (PFOS)	ng/L	1070	1000	484 - 1900	Acceptable	EPA 537

Perfluorinated Carboxylic Acids & Sulfonates (Cat# 093)						
Analyte	Units	Reported Value	Assigned Value	Acceptance Limits	Performance Evaluation	Method Description
Perfluoro-n-butanoic acid (PFBA)	ng/L	676	700	451 - 742	Acceptable	MWH SOP-HPLC 12
Perfluoro-n-octanoic acid (PFOA)	ng/L	1450	1600	782 - 2080	Acceptable	MWH SOP-HPLC 12
Perfluoro-n-octane sulfonate (PFOS)	ng/L	1090	1000	484 - 1900	Acceptable	MWH SOP-HPLC 12

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Method comparison in real water matrix (AFFF)

		Number	low pbp	high ppb	AVG ppb	Avg diff
Perfluorohexanoic acid - PFHxA	EPA 537	n=9	0.015	200	57.0	
	@PFC_EXTRA				50.3	13%
Perfluorohexanesulfonic acid - PFHxS	EPA 537	n=9	0.063	220	66.5	
	@PFC_EXTRA				53.1	25%
Perfluorooctanesulfonic acid - PFOS	EPA 537	n=9	0.053	28	15.5	
	@PFC_EXTRA				17.0	8%
Perfluorooctanoic acid - PFOA	EPA 537	n=9	0.0058	75	19.4	
	@PFC_EXTRA				16.4	18%
Perfluorobutanesulfonic acid - PFBS	EPA 537	n=9	0.006	31	11.7	
	@PFC_EXTRA				10.7	9%
Perfluoroheptanoic acid - PFhPA	EPA 537	n=9	0.0051	20	6.57	
	@PFC_EXTRA				5.04	30%
Perfluorononanoic acid - PFNA	EPA 537	n=9	<0.005	0.75	0.236	
	@PFC_EXTRA				0.214	10%
Perfluorodecanoic acid - PFDA	EPA 537	n=9	<0.005	0.031	0.018	
	@PFC_EXTRA				0.02	5%
Perfluoro butanoic acid - PFBA	@PFC_EXTRA	n=9	<0.005	28	7.44	N/A
Perfluoropentanoic acid - PFPA	@PFC_EXTRA	n=9	0.0051	62	15.4	N/A
Perfluoroundecanoic acid	EPA 537	n=9	<0.005	0.028	0.0083	N/A
Perfluorododecanoic acid	EPA 537	n=9	<0.005	<0.005	<0.005	N/A
Perfluorotetradecanoic acid	EPA 537	n=9	<0.005	<0.005	<0.005	N/A
Perfluorotridecanoic acid	EPA 537	n=9	<0.005	<0.005	<0.005	N/A

LATEST METHOD DEVELOPMENT ACTIVITIES

Pesticides – Potential Importance of Metabolites

RESTEK 204 Pesticide Mix

Goal – Measure as Many as Possible at the EU Limit (0.1 ppb) With A Direct Injection LC-MS-MS method

204

Initial List

Sensitivity **~190**

Did MDL/MRL determinations on all compounds using known transitions

Measured stability for 4 weeks under various preservation conditions with a 1 ppb spike level in multiple matrices.

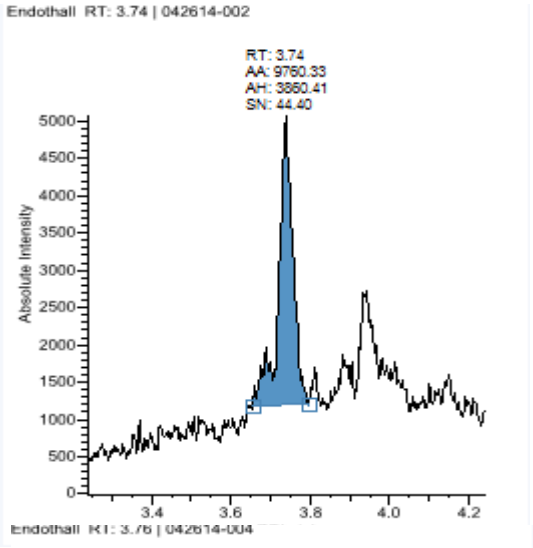
Stability **~150**

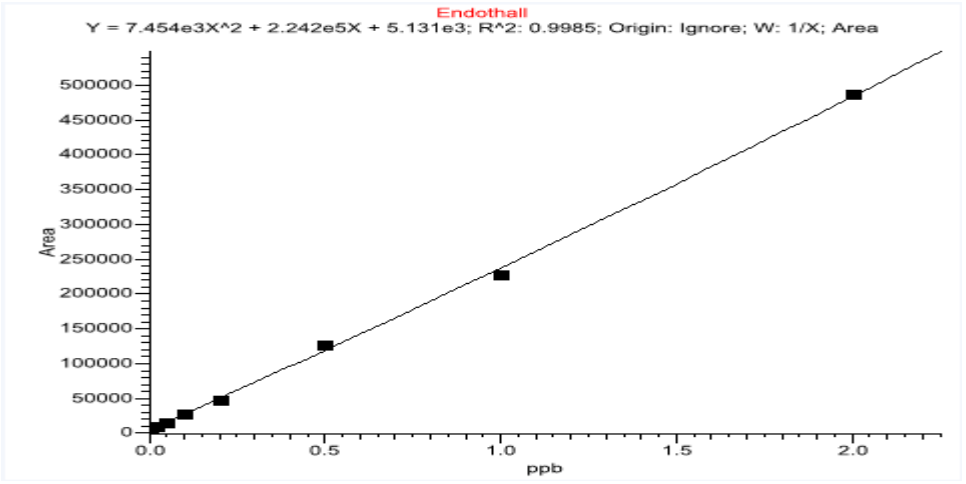
We Did An Extensive Holding Time and Preservation Study

chil	RT r			GW-1	GW-1	GW-1	GW-1	GW-1	GW-2
		preserv?	Row Labels	1101	1104	1110	1117	1130	1101
0.82	0.82	good	(Monceren) Pencycuron.1	0.95	1.09	1.10	1.08	1.20	0.96
0.52	0.00	chill	3-Hydroxycarbofuran.1	0.95	1.17	0.89	0.81	0.94	0.96
0.63	0.28	2 wks	Acephate.2	0.92	1.22	0.96	1.04	1.49	0.93
0.75	0.71	good	Acetamiprid.1	0.96	1.10	1.12	1.04	1.31	0.96
0.00	-0.09	bad	Acibenzolar-S-methyl.1	1.04	1.11	0.66	0.25		0.90
0.45	0.31	2 wks	Alanycarb.2	1.01	1.33	1.02	0.90	1.07	0.99
0.84	0.12	chill or 1 v	Aldicarb sulfone.1	0.98	1.13	1.06	1.12	1.38	0.92
0.62	0.12	chill or 1 v	Aldicarb sulfoxide.1	0.93	1.12	0.99	1.10	1.34	0.96
0.71	0.86	good	Ametryn.1	0.96	1.12	1.12	1.09	1.36	0.97

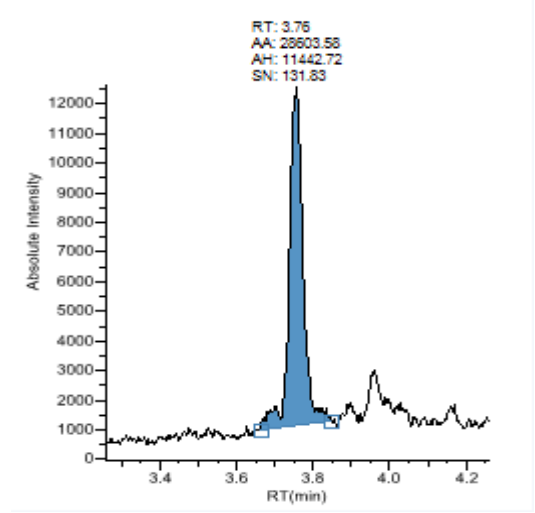
- 4 weeks
- Multiple matrices (GW, SW, Tap)
- Multiple preservatives (none, ascorbic, ammonium chloride, ammonium acetate, thiosulfate)
- Room Temperature vs Refrigeration

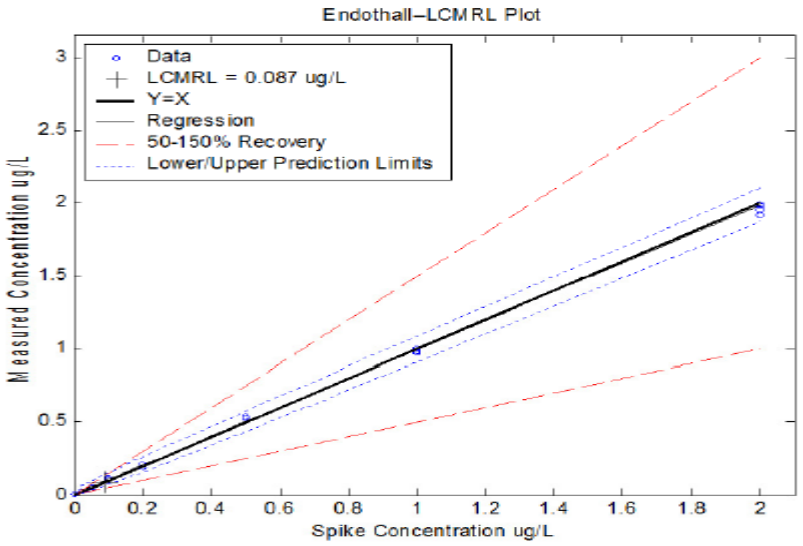
Endothall Ultra Low Level





Graph





Next Generation Method Development Issues

- Orbitrap technology
  - Best of both worlds – accurate mass for unknowns and can be used as an MS-MS-MS system for good quantitation.
- Direct injection techniques thanks to sensitivity
  - Addresses the issue of sample preparation
- Columns and Eluents
  - You still need to get good chromatography
- Data reduction time.
  - The elephant in the room.

Conclusions

- Increased sensitivity of LC-MS-MS instruments and decreased costs of instruments have led to lots of opportunities for labs to “push the envelope”.
- Stable isotopes are critical for the most accurate results, regardless of preparation techniques.
- Preservation and holding time studies are critical.
- Direct injection/Online preconcentration techniques have lots of advantages.

Any Questions?

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