

Multi-residue analysis for Emerging contaminants using on-line LCMS/MS

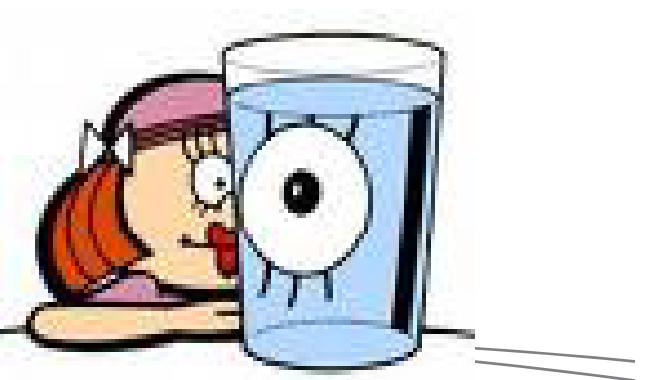
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NEMC
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Why The Interest in EDCs and PPCPs?

- 40 Years of Interest in Fate and Transport of Hormones.
- New analytical technologies make studies possible.
- >3000 drugs and PPCPs being introduced into water.
- Limited knowledge of treatability for all contaminants.
- Very high public visibility.



Issues Requiring Consideration for Emerging Contaminant Analysis Methods

- Selectivity/Specificity
- Sensitivity – MRL determination by LCMRL
- Target Lists
- QA/QC
- Ruggedness/matrix effects
- Type of containers & Holding time
- Inter-lab variability
- Cost

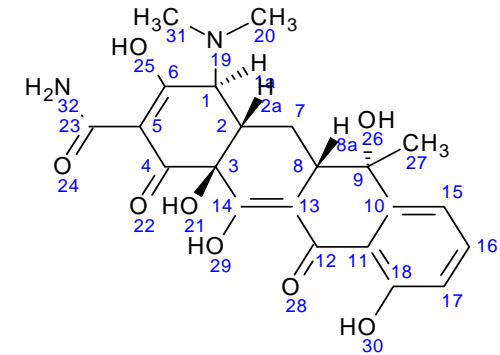
This Led Us to Develop an Innovative LC-MS-MS Approach to PPCP Analysis

- Cost Effective Screen for as Many Analytes as Possible
 - 5 to 10 ng/L reporting level targeted
- Includes common target analytes based on literature and expert panels.
- Accuracy and precision comparable to drinking water methods (e.g. target 70-130% recovery)
- Uses multiple ion transitions (MRM) for each analyte to minimize potential for misidentification
- Minimizing potential for blank contamination

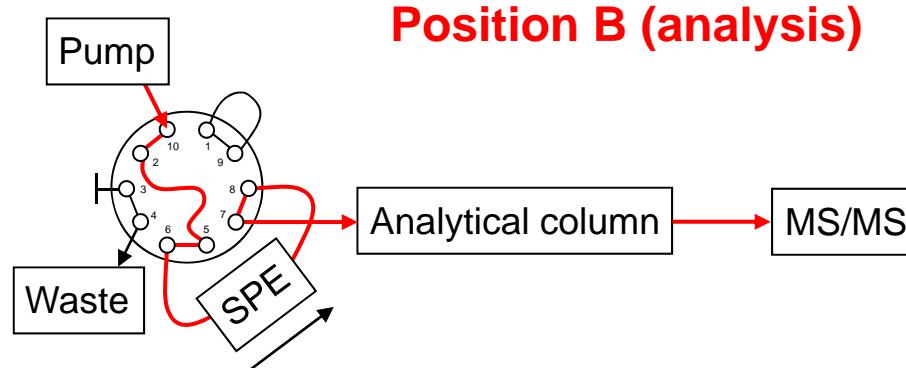
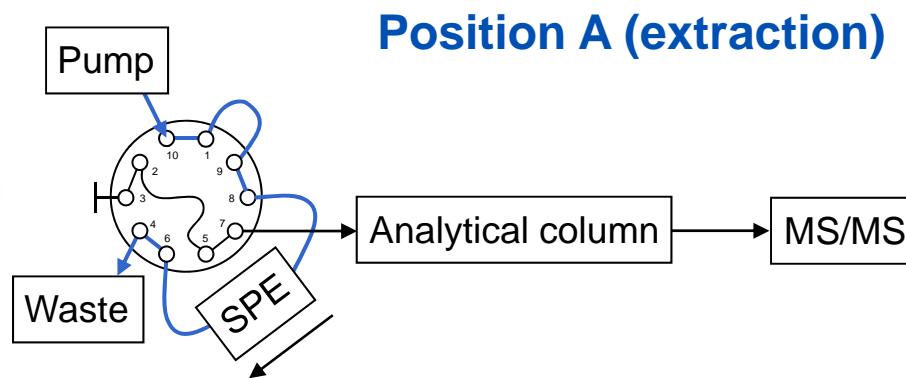
MWH Online Method for Simultaneous Extraction and Analysis of 85+ Analytes

1. Hormones
2. Pharmaceuticals:
 - Antibiotic
 - Anti-inflammatory
 - Lipid regulating agents
 - Triazides
 - Antagonists
3. Personal care products
 - Sunscreen agents
 - Preservatives
4. Pesticide/Herbicides
 - Including EPA 536 compounds -Triazines and their degradates.
5. Flame retardants
6. Stimulants
7. Sugar substitutes (sucralose, acesulfame-k)

This encompasses lots of categories of compounds (not just drugs) and 3 broad classes (**pharma**, **ww indicators**, and **pest/herb**)



Online SPE Setup And Procedure



API 5000®
LC/MS/MS system

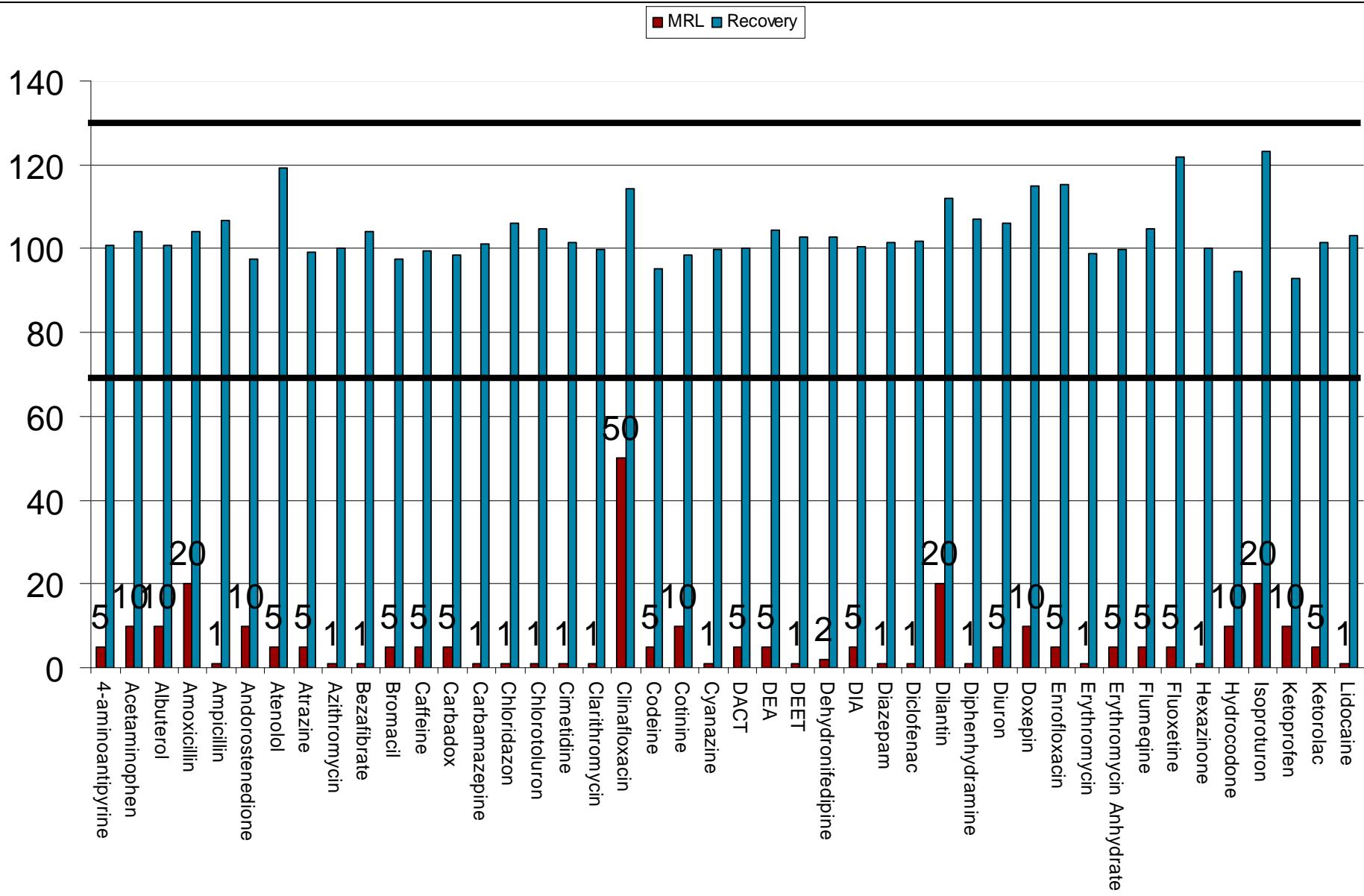
Pros And Cons Of On-line Enrichment Approach Versus Off-Line

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

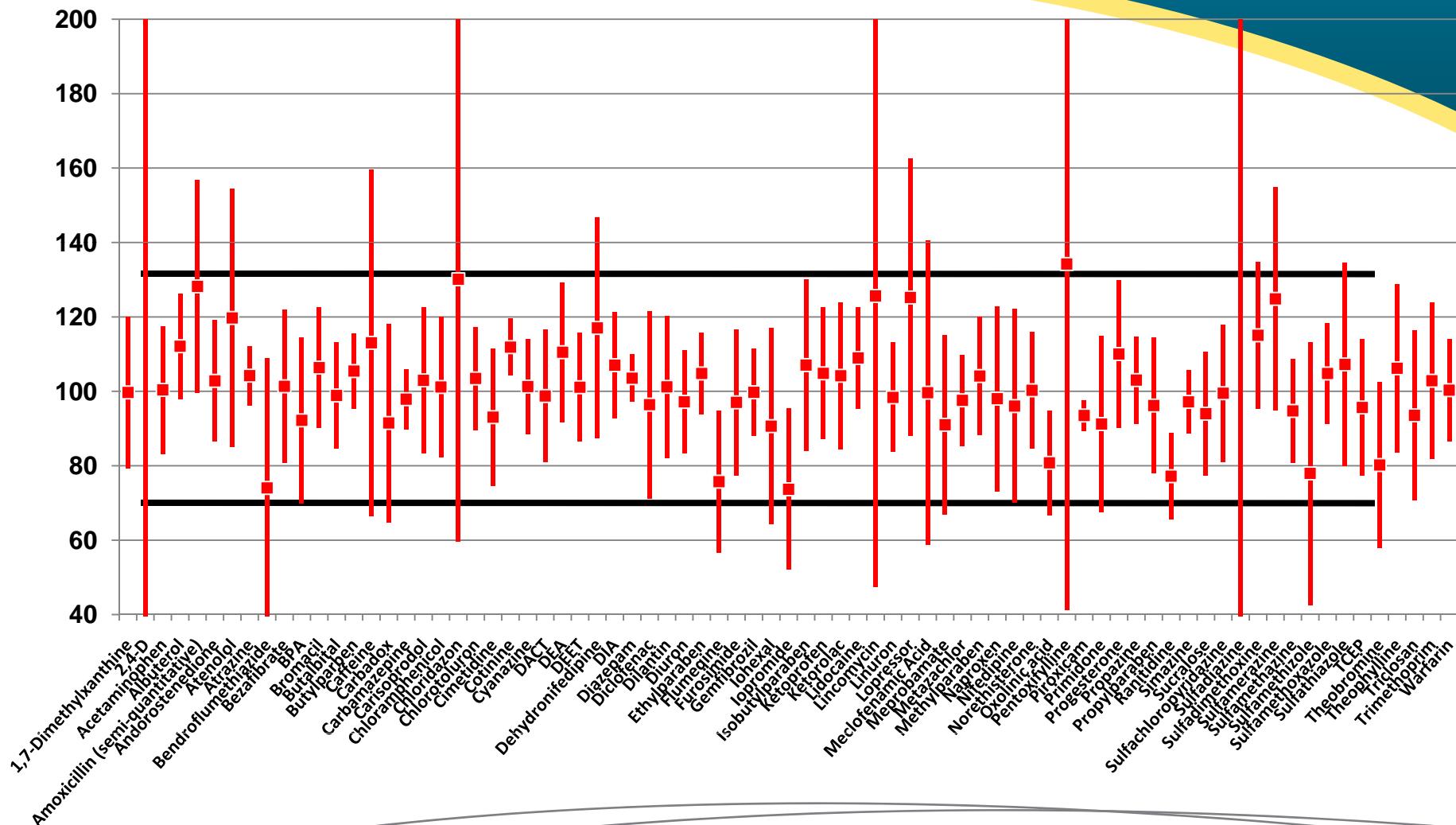
More Pros And Cons Of On-line Enrichment Approach Versus Off-Line

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

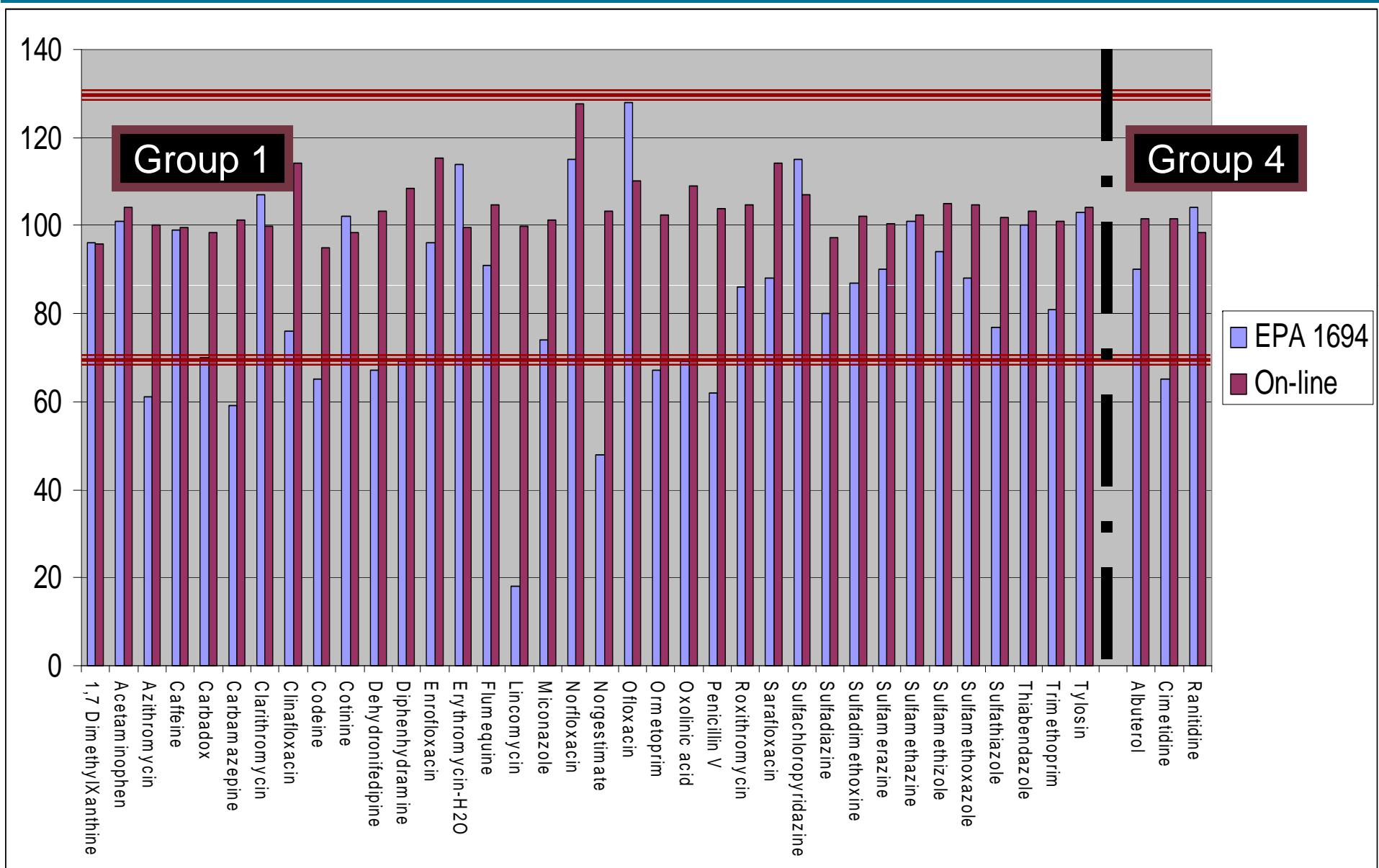
Accuracy for LCS in Synthetic Matrix at 100ppt and Analyte MRLs by ESI-Positive



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



Comparing EPA 1694 (Appendix) Results For Reagent Water Spikes To Online Method



WaterRF 4167

**Evaluation of Analytical Methods
for EDCs and PPCPs via
Interlaboratory Comparison**

WaterRF 4167 – What Was It?

- SNWA is Principle Investigator, along with MWH, MWD, Colorado School of Mines, and German Institute of Hydrology and ERA
- Purpose is 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)

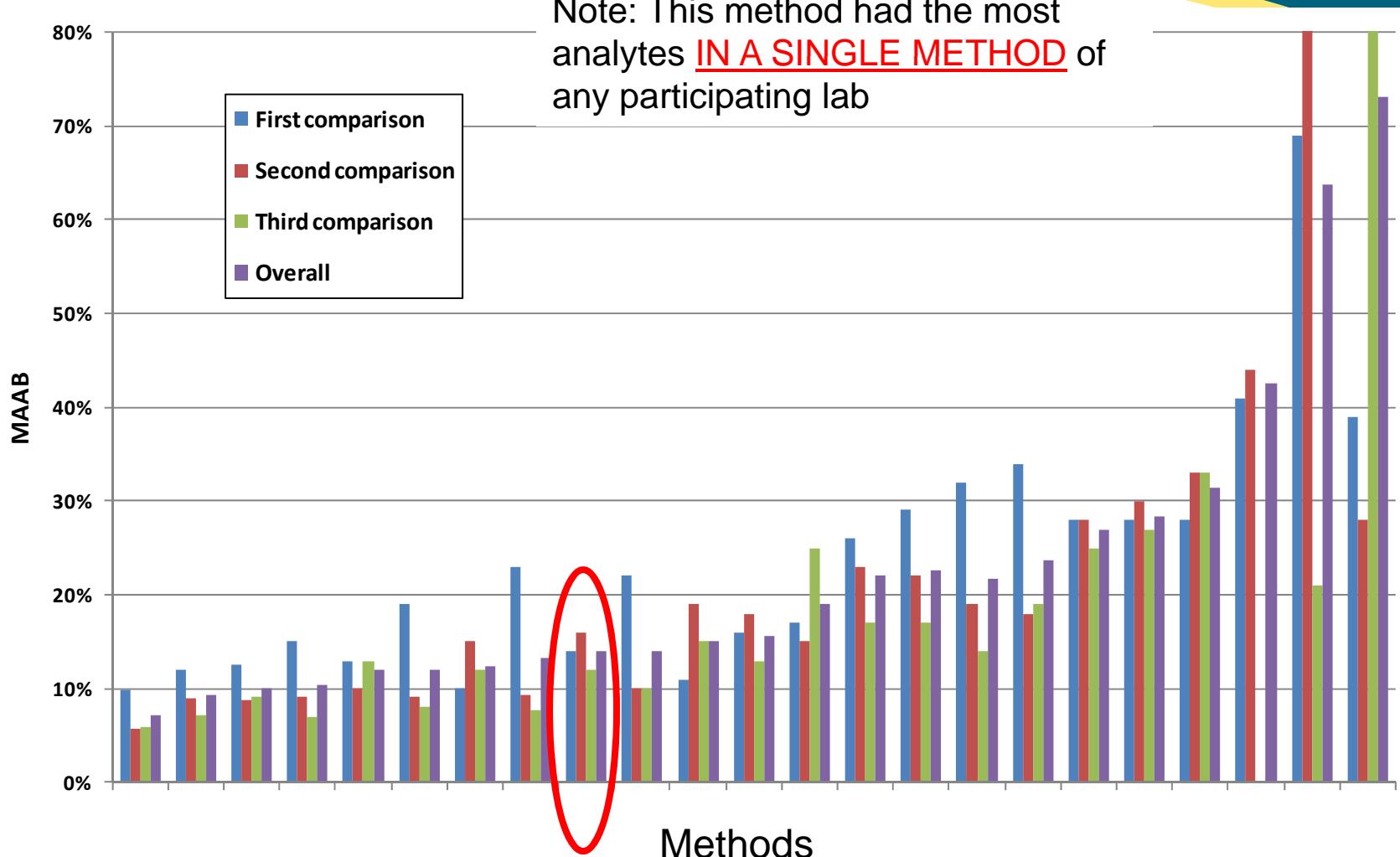
The Compound List for 4167 Based on Literature Review.

PPCPs		Potential EDCs	
Carbamazepine	Erythromycin	17β-Estradiol	Bisphenol A
Sulfamethoxazole	Acetaminophen	17α-Ethynodiolide	Octylphenol
Trimethoprim	Fluoxetine	Estrone	Nonylphenol
Naproxen	Triclosan	Progesterone	
Diclofenac	Primidone	Testosterone	
Ibuprofen	Caffeine		
Gemfibrozil	Ciprofloxacin		

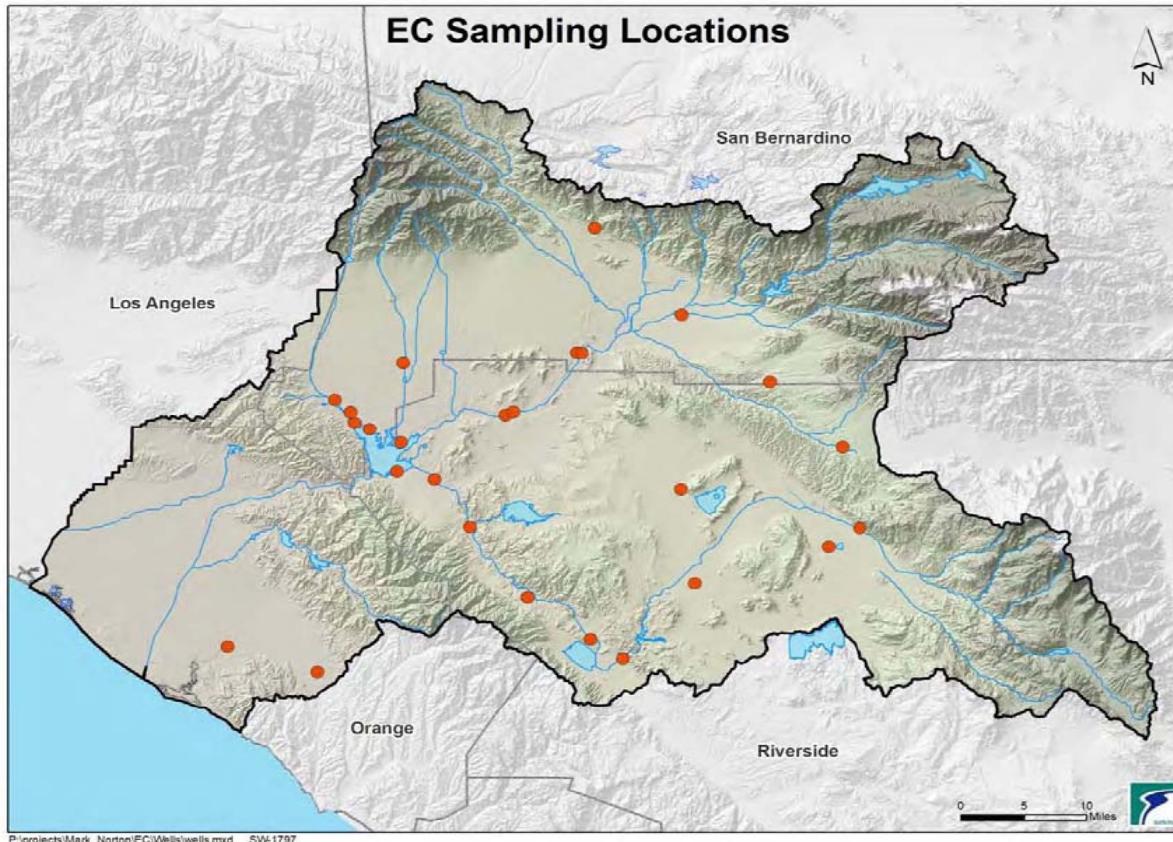
Compounds in red are also on other “indicator lists (SAWPA 2010)

Ones in green or red bolded are also on the Blue Ribbon Panel List (4)

The Online Method Stacks Up Well in Interlab Comparisons (WaterRF 4167)



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



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

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

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

Analyte	% RSD	Assigned Value	Mean Recovery	Median Recovery	Lab 1	Lab 2	Lab 3	MWH-online
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	-	-	-	-	-	-
Ethyneestradiol	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

Analyte	% RSD	Assigned Value	Mean Recovery	Median Recovery	Lab 1	Lab 2	Lab 3	MWH-online
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
Ethyneestradiol	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
Ethynodiol	-		-	-	<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

Holding Time And Preservation Studies

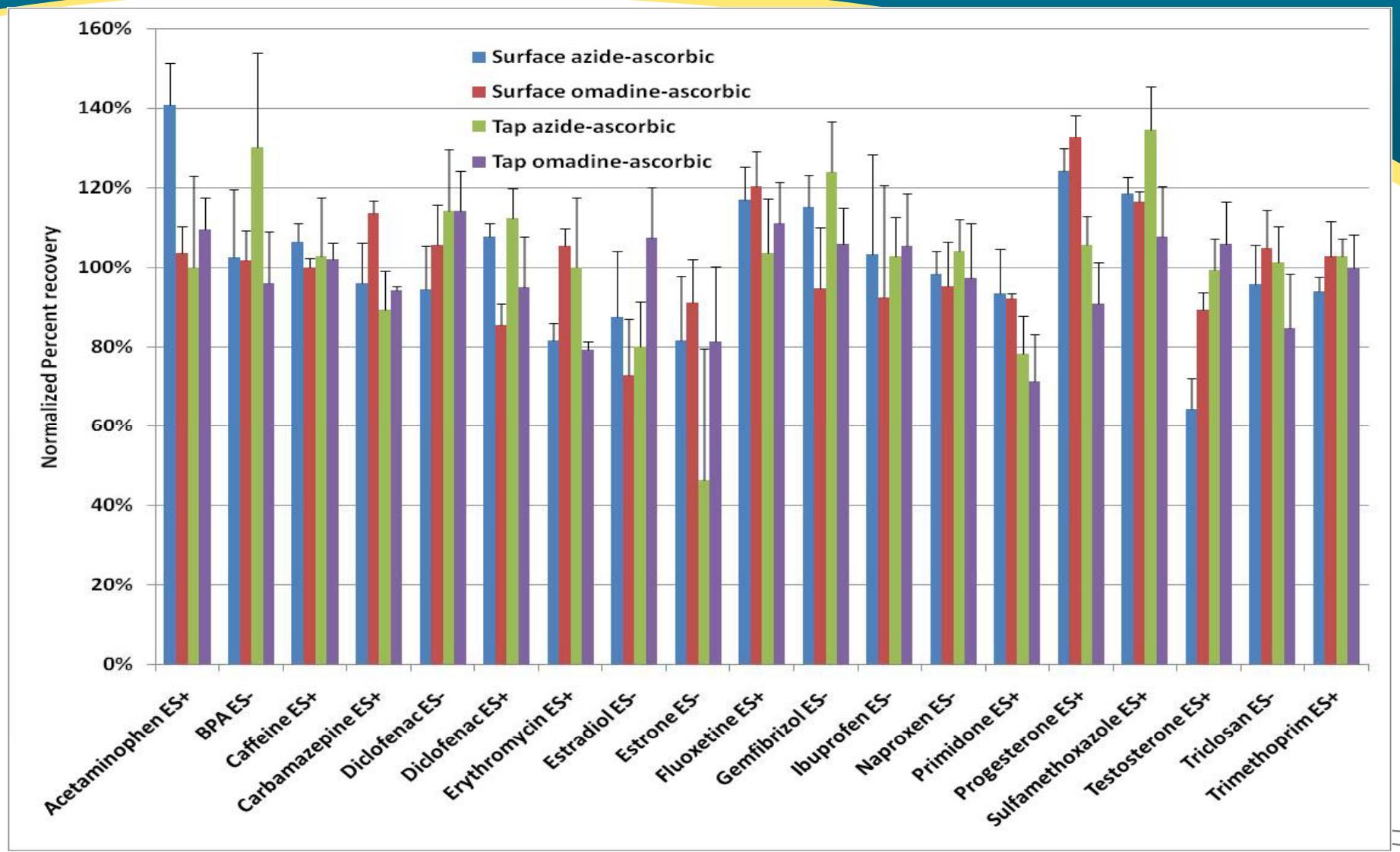
- Substitution of sodium azide with sodium omadine
- Stability of preserved compounds
- Stability of compounds at room temperature - transit
- Stability of preserved compounds in plastic or glass containers

After 4 Weeks Refrigerated – 3 Replicates

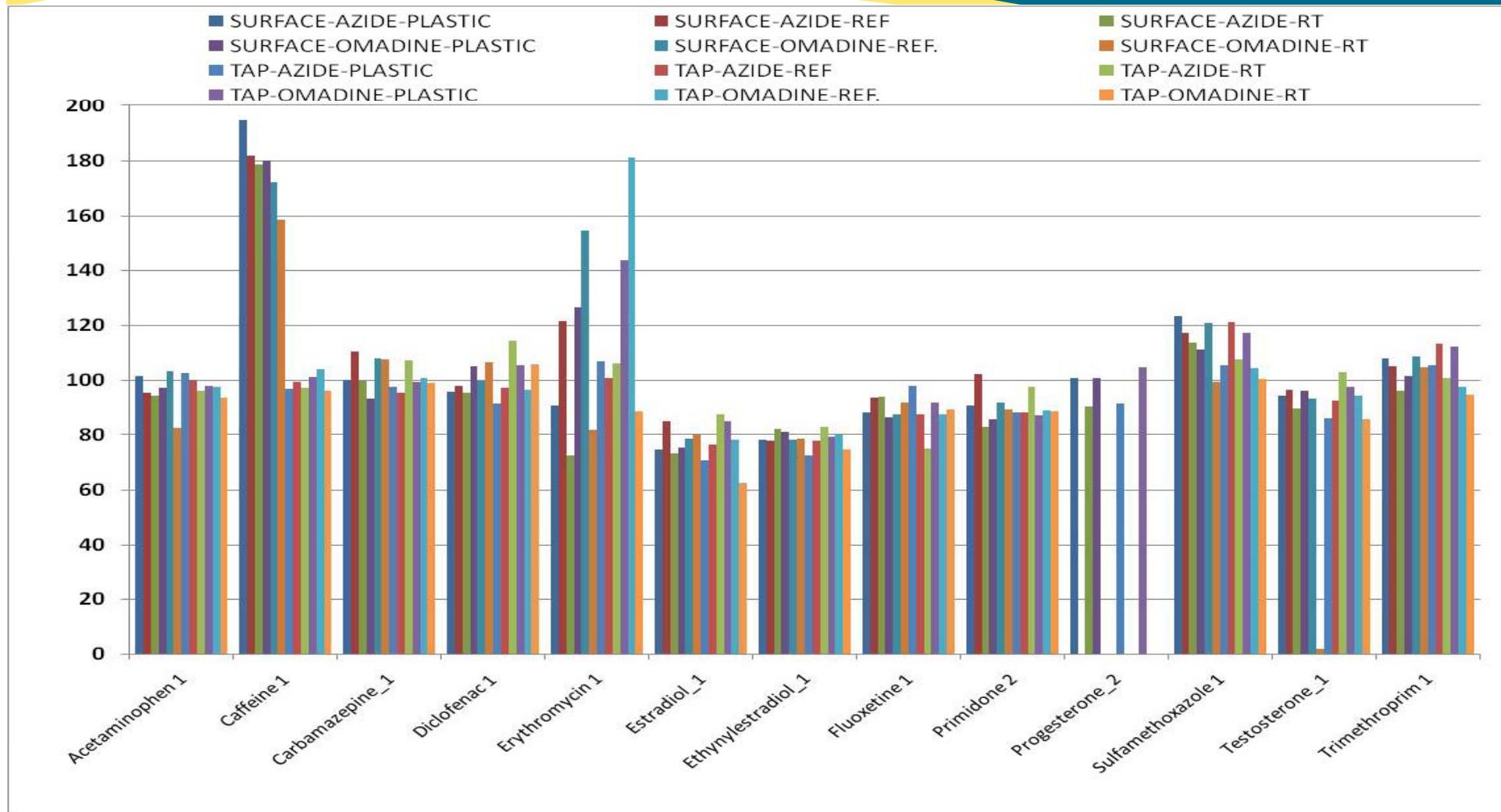
SW provided by SNWA

		average	average	average	average	RSD	RSD	RSD	RSD
		Surface azide-ascorbic	Surface omadin e-ascorbic	Tap azide-ascorbic	Tap omadin e-ascorbic	Surface azide-ascorbic	Surface omadin e-ascorbic	Tap azide-ascorbic	Tap omadin e-ascorbic
ES+	Acetaminophen ES+	141%	104%	100%	110%	11%	7%	23%	8%
ES-	BPA ES-	103%	102%	130%	96%	17%	8%	24%	13%
ES+	Caffeine ES+	107%	100%	103%	102%	4%	2%	14%	4%
ES+	Carbamazepine ES+	96%	114%	89%	94%	10%	3%	10%	1%
ES-	Diclofenac ES-	95%	106%	114%	114%	11%	10%	16%	10%
ES+	Diclofenac ES+	108%	86%	112%	95%	3%	5%	7%	13%
ES+	Erythromycin ES+	82%	105%	100%	79%	5%	4%	17%	2%
ES-	Estradiol ES-	88%	73%	80%	107%	17%	14%	11%	13%
ES-	Estrone ES-	82%	91%	46%	81%	16%	11%	33%	19%
ES+	Fluoxetine ES+	117%	120%	104%	111%	8%	9%	14%	10%
ES-	Gemfibrizol ES-	115%	95%	124%	106%	8%	15%	12%	9%
ES-	Ibuprofen ES-	103%	92%	103%	106%	25%	28%	10%	13%
ES-	Naproxen ES-	98%	95%	104%	97%	6%	11%	8%	14%
ES+	Primidone ES+	94%	92%	78%	71%	11%	1%	10%	12%
ES+	Progesterone ES+	124%	133%	106%	91%	6%	5%	7%	11%
ES+	Sulfamethoxazole ES+	119%	116%	135%	108%	4%	3%	11%	13%
ES+	Testosterone ES+	64%	89%	99%	106%	8%	4%	8%	10%
ES-	Triclosan ES-	96%	105%	102%	85%	10%	10%	9%	13%
ES+	Trimethoprim ES+	94%	103%	103%	100%	4%	9%	4%	8%

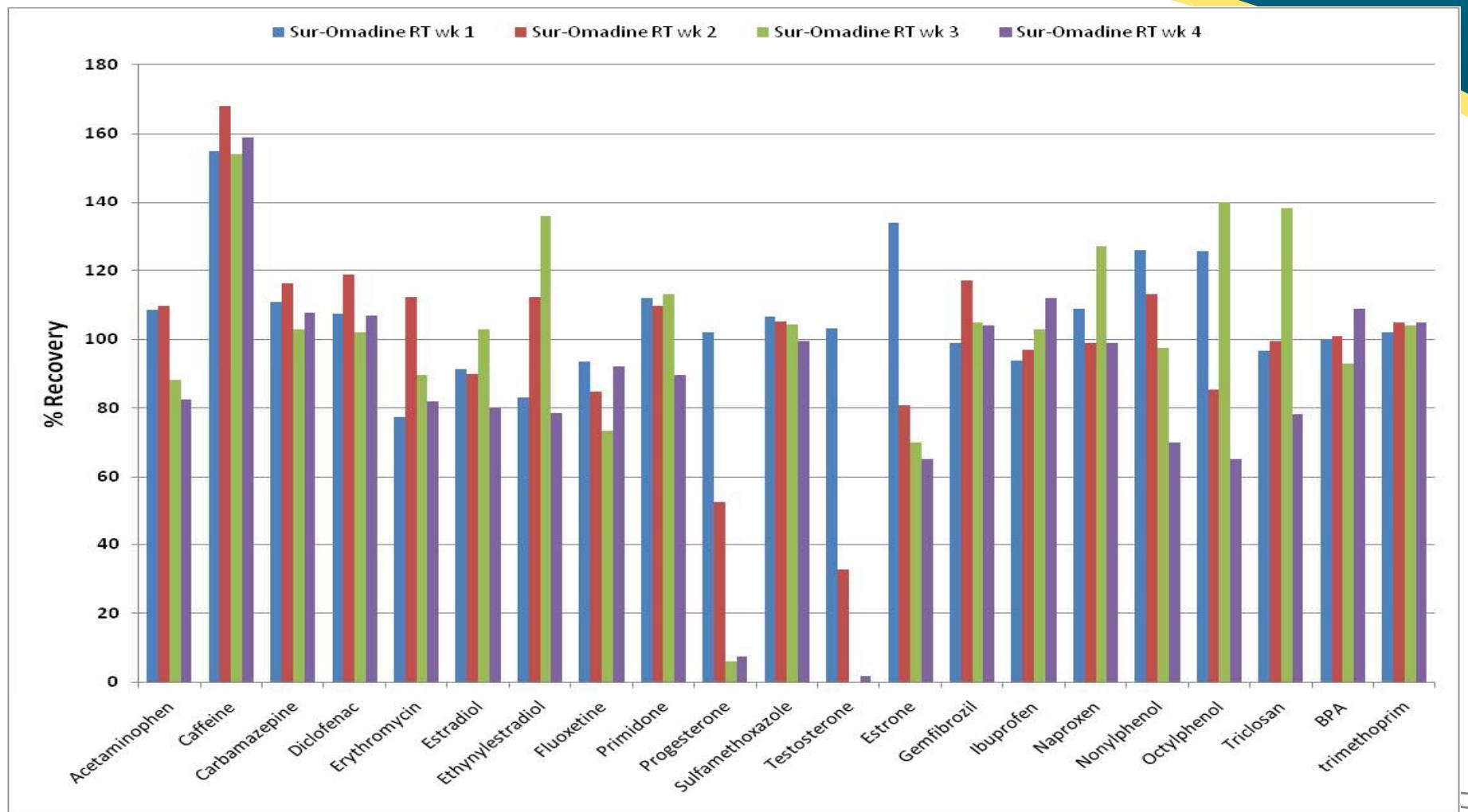
Sodium azide vs sodium omadine – Refrigerated for 4 Weeks



Not Normalized – SW Contained 18 ppb Caffeine



Room Temperature - Most Compounds Are Stable For >1 Week Except the Hormones.

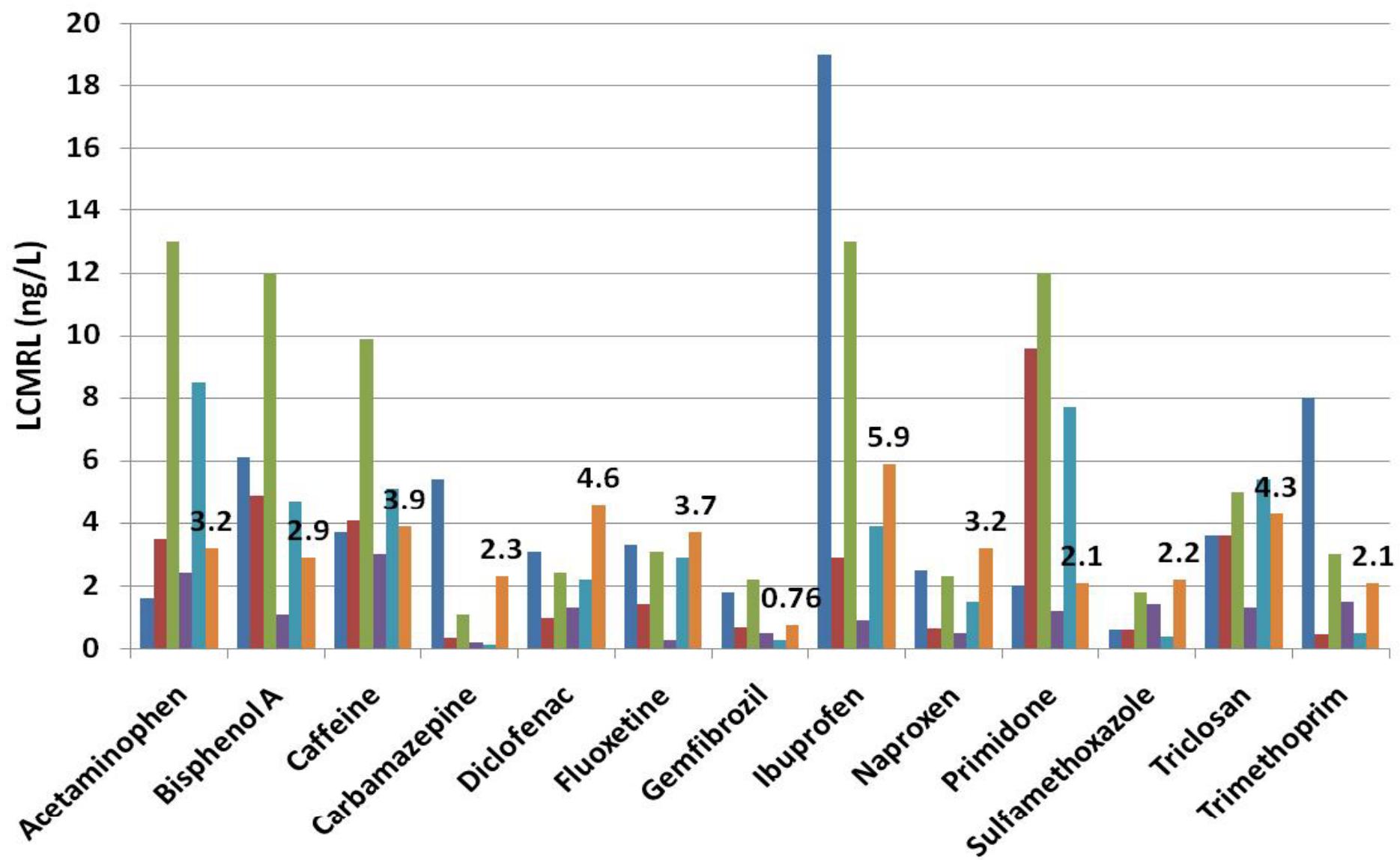


LCMRL Determinations – 4167 PPCP Compounds (no hormone data)

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	ONLINE	Min.	Max.
Acetaminophen	1.6	3.5	13	2.4	8.5	3.2	1.6	13
Bisphenol A	6.1	4.9	12	1.1	4.7	2.9	1.1	12
Caffeine	3.7	4.1	9.9	3	5.1	3.9	3	9.9
Carbamazepine	5.4	0.33	1.1	0.2	0.13	2.3	0.13	5.4
Diclofenac	3.1	0.96	2.4	1.3	2.2	4.6	0.96	3.1
Fluoxetine	3.3	1.4	3.1	0.27	2.9	3.7	0.27	3.3
Gemfibrozil	1.8	0.69	2.2	0.49	0.28	0.76	0.28	1.8
Ibuprofen	19	2.9	13	0.91	3.9	5.9	0.91	19
Naproxen	2.5	0.65	2.3	0.5	1.5	3.2	0.5	2.5
Primidone	2	9.6	12	1.2	7.7	2.1	1.2	12
Sulfamethoxazole	0.6	0.6	1.8	1.4	0.37	2.2	0.37	1.8
Triclosan	3.6	3.6	5	1.3	5.4	4.3	1.3	5.4
Trimethoprim	8	0.46	3	1.5	0.48	2.1	0.46	8

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	ONLINE		
Extraction factor	500	500	500	500	500	NA		
Injection	2 uL (ESI +)	20 uL (ESI +)	2500 uL (ESI +)					
Volume (uL)	3 uL (ESI -)	20 uL (ESI -)	2500 uL (ESI -)					
Instrument	API 5000	API 4000	API 4000	API 4000	API 3200	API 5000		

■ Lab 1 ■ Lab 2 ■ Lab 3 ■ Lab 4 ■ Lab 5 ■ ONLINE



Even the Confirmation MRM's LCMRL is Close to the Primary MRM's

	Lab 4	Conf. MRM	ONLINE	Conf. MRM
Acetaminophen	2.4	0.51	3.2	2.5
Bisphenol A	1.1	1.3	2.9	2
Caffeine	3	5.3	3.9	4.1
Carbamazepine	0.2	0.65	2.3	4.2
Diclofenac	1.3	0.97	4.6	4.5
Fluoxetine	0.27	0.37	3.7	4.1
Gemfibrozil	0.49	0.63	0.76	0.57
Ibuprofen	0.91	0.38	5.9	2.1
Naproxen	0.5	2.1	3.2	5.5
Primidone	1.2	0.79	2.1	1.6
Sulfamethoxazole	1.4	0.8	2.2	1.8
Triclosan	1.3	NA	4.3	NA
Trimethoprim	1.5	0.77	2.1	2.3

Lab 4	ONLINE
500	NA
2 uL (ESI +)	2500 uL (ESI +)
3 uL (ESI -)	2500 uL (ESI -)
API 4000	API 5000

Summary

- The On-Line Method for a broad group of Pharmaceuticals and Personal Care Products is a rugged, sensitive method that provides comparable results to conventional methods.
- Sodium omadine is a reliable substitute for sodium azide
- Multiple MRMs assure higher confidence in identification
- The LCMRL achieved by both transitions show no hindrance in MRL.

Thank You For Your Attention

Any questions?

Feel free to contact us:

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