



AXYS Analytical Services Ltd.

**“Analytical Methods to Support a Reconnaissance
of 121 PPCPs, Alkylphenolics and PFCs in San
Francisco Estuary Water, Sediments, and
Mussels”**

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ISO 17025 CERTIFIED



Agenda

□ Purpose of Study

- Reconnaissance in San Francisco Estuary – 107 PPCPs, 4 Alkylphenols, 17 PFCs in marine water and sediment, mussel tissue
- Test new analytical methods, confirm performance in specific matrices

□ Overview of Study, Analytical Methods, and Results

□ Key Focus - Analytical Development / Validation

- PPCP tissue method (s) and validation
- Nonylphenol, Octylphenol, and NPEO1 and NPEO2 tissue method and validation



Analysis - What's Unique in the Study

■ Perfluorinated Compounds

- 2 fluorosulfonamide and 2 fluorosulfonamido ethanol compounds in all matrices

■ PPCPs

- Large PPCP list (107 compounds) in marine waters and sediments
- Method applied to tissue
- 2nd Tissue method confirmation and validated for 121 compounds

■ Alkylphenolics

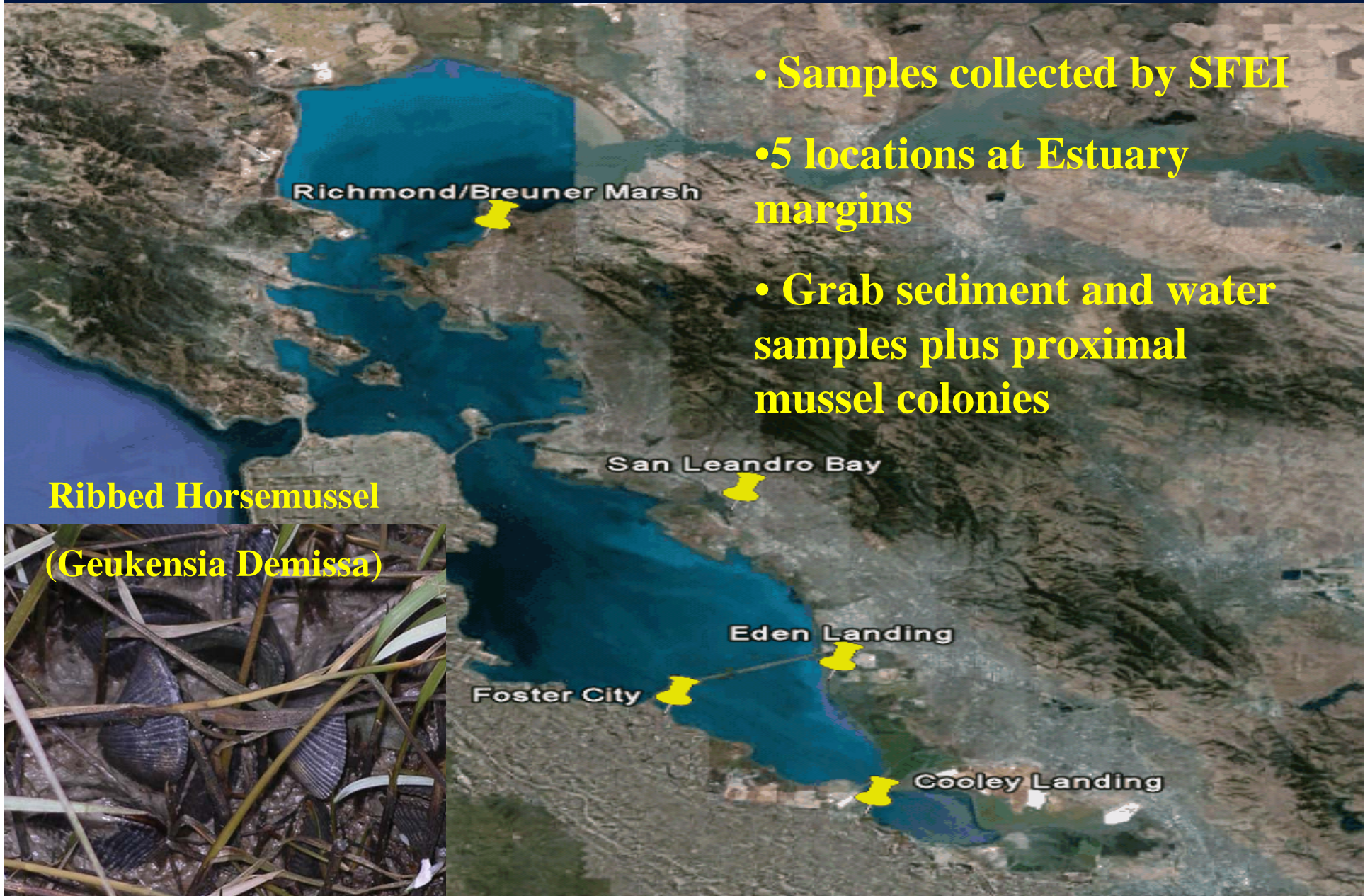
- Low detection limit - whole water and sediment
- Validated tissue method using steam distillation/extraction with LC MS/MS modified (from previous literature)

Reconnaissance Study – San Francisco Estuary

- Samples collected by SFEI
- 5 locations at Estuary margins
- Grab sediment and water samples plus proximal mussel colonies

Ribbed Horsemussel

(*Geukensia Demissa*)



PFC Results (see www.SFEI.org)

- **Wide variation in PFC Aqueous Results**
 - Low ng/L results in most cases
 - Cooley Landing shows significant detects for C4-8 carboxylic acids (60 – 220 ngL)
- **Less variation in PFC Sediment Results**
 - PFOS and PFOA consistently detected < 5 ng/g
- **Mussel tissue results**
 - Sporadic detects (0.5 to 5 ng/g) for PFOS and PFOA except high EL Site PFOS (75 ng/g), other PFCs not consistently detected
- **ND in all matrices** (1 – 15 ng/L, < 1 ng/g sed., < 1ng/g tissue)
 - N-Me-perfluorooctanesulfonamide (N-Me-FOSA)
 - N-Et-perfluorooctanesulfonamide (N-Et-FOSA)
 - 2-(N-Me-perfluorooctanesulfonamido ethanol) (N-Me-FOSE)
 - 2-(N-Et-perfluorooctanesulfonamido ethanol) (N-Et-FOSE)

AXYS PHASE 3 PPCP METHOD (2009)

Study PPCP Method

EPA Method 1694 Compounds + Extended List

**PPCPs by
LC-MS/MS**
121 Compounds
49 labelled
Surrogates

List 1 PPCPs
Acidic Extract
+ve ESI
49 Compounds
11 Surrogates

List 2 TCY's
Acidic Extract
+ve ESI
14 Compounds
1 Surrogates

List 3 PPCPs
Acidic Extract
-ve ESI
12 Compounds
9 Surrogates

List 4 PPCPs
Basic Extract
+ve ESI
14 Compounds
11 Surrogates

List 5 PPCPs
Acidic Extract
+ve ESI
32 Compounds
17 Surrogates

List 3 – PPCPs by Acidic Extraction / - ESI EPA Method 1694 List 3 plus Additions (yellow)

TARGET	LABELED STADARD
Gemfibrozil	d6 Gemfibrozil
Ibuprofen	13C3 Ibuprofen
Naproxen	13C d3 Naproxen
Triclocarban	13C6 Triclocarban
Triclosan	13C12 Triclosan
Warfarin	d5 Warfarin
Glyburide	d3 Glyburide
Glipizide	d11 Glipizide
Furosemide	13C d3 Naproxen
Hydrochlorothiazide	13C d3 Naproxen
2 – Hydroxy – Ibuprofen	13C3 Ibuprofen
Bisphenol A	d6 Bisphenol A

List 4 PPCPs by Basic Extraction / +ESI
EPA Method 1694 List 4 plus Additions (yellow)

TARGET	LABELLED STANDARD
Albuterol	d3 Albuterol
Cimetidine	d3 Cimetidine
Metformin	d6 Metformin
Ranitidine	d3 Albuterol
Amphetamine	d5 Amphetamine
Atenolol	d7 Atenolol
Atorvastatin	d3 Cimetidine
Clonidine	d4 Clonidine
Codeine (previously APOS, List 1)	d6 Codeine
Cotinine (previously APOS, List 1)	d3 Cotinine
Enalapril	d5 Enalapril
Hydrocodone	d3 Hydrocodone
Oxycodone	d6 Oxycodone
Triamterene	d4 Clonidine

List 5 PPCPs by Acidic Extraction / + ESI New Targets + Surrogates (* = exact label)

TARGET	TARGET	TARGET	TARGET
Alprazolam*	Hydrocortisone*	Prendinison	Valsartan
Amitriptyline*	10 Hydroxy Amitriptyline	Promethazine*	Verapamil
Amlodipine	Methyl – Prendinisolone*	Propranolol*	DEET*
Benzotropine*	Metoprolol*	Sertraline	Diazepam*
Betamethasone	Norfluoxetine*	Simvastatin	Propoxyphene*
Desmethyl - diltiazem	Norverapamil	Theophylline*	Cocaine*
Fluocinonide	Paroxetine*	Trenbolone	Benzoyllecgonine*
Fluticasone propionate	Prendinisolone	Trenbolone Acetate	Meprobamate

OVERVIEW of PPCP ANALYSIS

Sediments and Aqueous – Extended EPA 1694 List

Sample – 2 X 1 gram wet solids or 2 X 1L
Acidic Fraction to pH 2 + EDTA stabilizer, Basic Fraction to pH10

Respective Fractions – Add labeled Surrogates
Acidic Fraction – 3X ultrasonic extraction with phosphate buffer/ACN
Basic Fraction – 3x ultrasonic extraction with H₂O/NH₄OH

SPE Cartridge Cleanup (1 gram Waters Oasis HLB)
SPE Steps – condition, load, rinse, elute, reduce, transfer to MeOH
Add recovery standards

ANALYSIS by LC/MS/MS (x5)
ACIDIC EXTRACT – Neg. Ionization, Tetracyclines, Pos. Ionization
BASIC EXTRACT – Pos. Ionization

12 PPCPs Detected in All Waters

Compound (extended 1694 compounds in red)	Use	Units = ng/L	
		Max	Mean
Valsartan	antihypertensive	92	45
Sulfamethoxazole	antibiotic	67	26
Carbamazepine	anticonvulsant	44	18
Caffeine	stimulant	41	27
Gemfibrozil	antilipidemic	38	25
Atenolol	beta blocker	37	18
Meprobamate	antianxiety	36	20
Diethyl-3-methyl-benzamide, N,N-(DEET)	insect repellent	21	11
Erythromycin-H2O	erythromycin metabolite	12	4
Triamterine	antihypertensive	10	4
Benzoylecgonine	cocaine metabolite; analgesic	7	5
Diltiazem	antianginal	3	1

19 Other PPCPs Detected in Some Water Samples

Compound (# sites detected) (extended 1694 compounds in red)	Use	Units = ng/L	
		Max	Mean
Ibuprofen (1)	antiinflammatory	38	8
Metoprolol (3)	antianginal	26	6
Cotinine (4)	nicotine metabolite	25	11
Clarithromycin (2)	antibiotic	18	5
Sulphamethizole (1)	antibiotic	16	3
Amphetamine (2)	stimulant	10	4
Naproxen (1)	antiinflammatory	8	2
Hydrocodone (1)	analgesic	7	1
Trimethoprim (2)	antibiotic	4	1
Thiabendazole (1)	fungicide	3	0.5
Cocaine (4)	stimulant	2	1
Diphenhydramine (4)	antihistamine	2	1
Desmethyldiltiazem (2)	diltiazem metabolite	2	0.4
Dehydronifedipine (4)	antianginal	1	0.7
Albuterol (1)	antiasthmatic	1	0.2
Propoxyphene (2)	analgesic	0.7	0.2
Amitriptyline (2)	antidepressant	0.6	0.2
Diazepam (1)	antianxiety	0.5	0.1
10-hydroxy-amitriptyline (2)	amitriptyline metabolite	0.3	0.1

11 PPCPs Detected in Sediment

Compound (# sites detected) (extended 1694 compounds in red)	Use	ng/g dry wt	
		Max	Mean
Ciprofloxacin (2)	antibiotic	680	400
Caffeine (3)	stimulant	38	18
Triclocarban (3)	antimicrobial	33	8
Trimethoprim (1)	antibiotic	18	3
Triamterene (5)	antihypertensive	11	3
Thiabendazole (2)	fungicide	9	2
Diethyl-3-methyl-benzamide, N,N- (DEET) (2)	insect repellent	3	1
Erythromycin-H2O (1)	erythromycin metabolite	3	1
Amphetamine (2)	stimulant	3	1
Sulphamethoxazole (1)	antibiotic	1	0.1
Cocaine (1)	stimulant	0.2	0.1

Not quantified (NQ) compounds occur in silicate (sand) and aluminosilicate (clay) matrices. This is a systemic interaction between the matrix and specific targets in list 1 and 5. Surrogates and natives are not recovered. Fluoroquinolone compounds are examples.

17 PPCPs Detected in Mussels

Compound (# sites detected) (extended EPA 1694 compounds in red)	Use	ng/g wet wt	
		Max	Mean
Diethyl-3-methyl-benzamide, N,N- (DEET) (5)	insect repellent	14	7
Digoxigenin (3)	cardiac drug metabolite	10	5
Carbamazepine (5)	anticonvulsant	5	3
Amphetamine (3)	stimulant	4	1
Triclocarban (2)	antimicrobial	2	0.5
Sertraline (5)	antidepressant	1	0.5
Dehydronifedipine (5)	antianginal	0.7	0.4
Triamterine (3)	antihypertensive	0.6	0.2
Ranitidine (3)	antacid	0.4	0.2
Diphenhydramine (3)	antihistamine	0.3	0.2
Atenolol (1)	beta blocker	0.3	0.1
Cocaine (2)	stimulant	0.3	0.1
Amitryptiline (2)	antidepressant	0.2	0.1
Sulphamethiazole (1)	antibiotic	0.2	0.04
Erythromycin-H2O (4)	erythromycin metabolite	0.2	0.1
Enalapril (2)	antihypertensive	0.1	0.04
Diltiazem (2)	antianginal	0.1	0.04

Alkylphenol Results

ND Results for OP all matrices, NP1EO + NP2EO in water

Matrix / Target	Cooley Landing	Eden Landing	Foster City	Richmond	San Leandro
Sediment NP	86.3	50.9	34.7	21.5	30.8
Sediment NP1EO	39.8	17.1	13.5	4.1	8.7
Sediment NP2EO	18.7	11.1	8.4	ND	8.6
Water NP	ND	72.9	ND	42.7	34.7
Tissue NP	ND	90.4	ND	ND	94.5
Tissue NP1EO	ND	ND	ND	ND	41.3
Tissue NP2EO	ND	ND	ND	ND	19.2

Units – 1) Sediments = ng/g dry wt. 2) Water = ng/L 3) Tissue = ng/g wet weight

OVERVIEW of Sediment and Aqueous Method by Acetylation + GC/MS SIM (NP, OP, NPEO 1 and 2 targets)

Sample Size– 5 gram dry wt. solid or 1L water
Solid - Homogenize or sub-sample as required for moisture and weight
Add surrogates

Solids – Base Digestion KOH, Solvent Extraction, Acetylation
Aqueous – Aqueous Acetylation, solvent exchange to hexane, acetylation
pH control at all steps vital, reduce extract

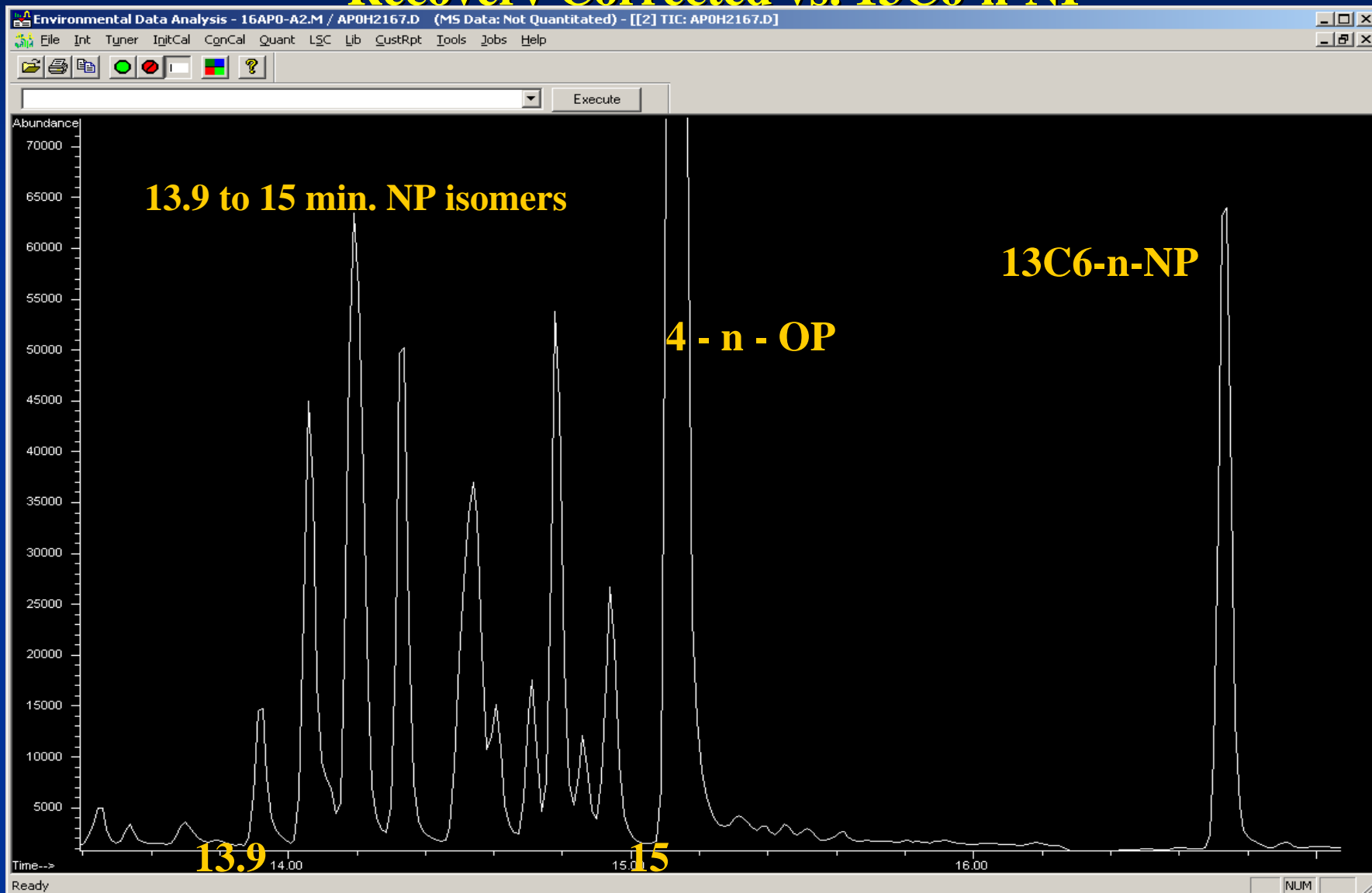
Clean-Up – 5% silica column, optional 25% basic silica column
Load, elute with 25 ml hexane, discard (F1 fraction)
Elute with 50 mL 1:9 ethyl acetate (F2 fraction), rotovap, add d10 pyrene recovery std.

ANALYSIS by GC MS EI with Multiple Ion Detection
Quantification – Sum of isomers recovery corrected from 13C6 – p - nonylphenol
Minimum 2 ions per isomer, calibration from technical standard

AP Standard GC-TIC View – What are we measuring ?

Summed Response of NP Isomers in Technical Mix (EQ Labs)

Recovery Corrected vs. 13C6-n-NP



OVERVIEW Tissue Method by LC/MS/MS (NP, OP, NPEO 1 and 2)

Sample – 2 gram wet tissue
Virtis homogenization (create slurry, tissue disruption)

Disperse slurry in Water
Addition of C labeled Surrogates
Steam distillation / hexane extraction

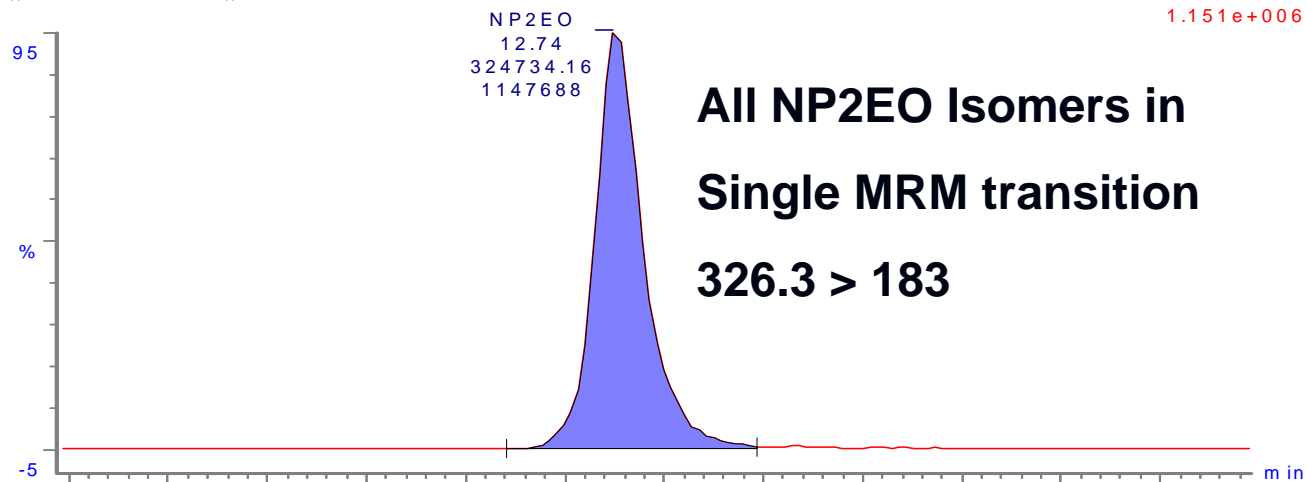
SPE Cartridge Cleanup (1 gram aminopropyl)
MeOH Eluent, reduce to 1 mL
Add recovery standard (d6 –BPA)

ANALYSIS by LC/MS/MS (x2)
NP and OP in ESI negative
NP1EO and NP2EO in ESI positive

NP2EO Native and Surrogate Response – Single MRM

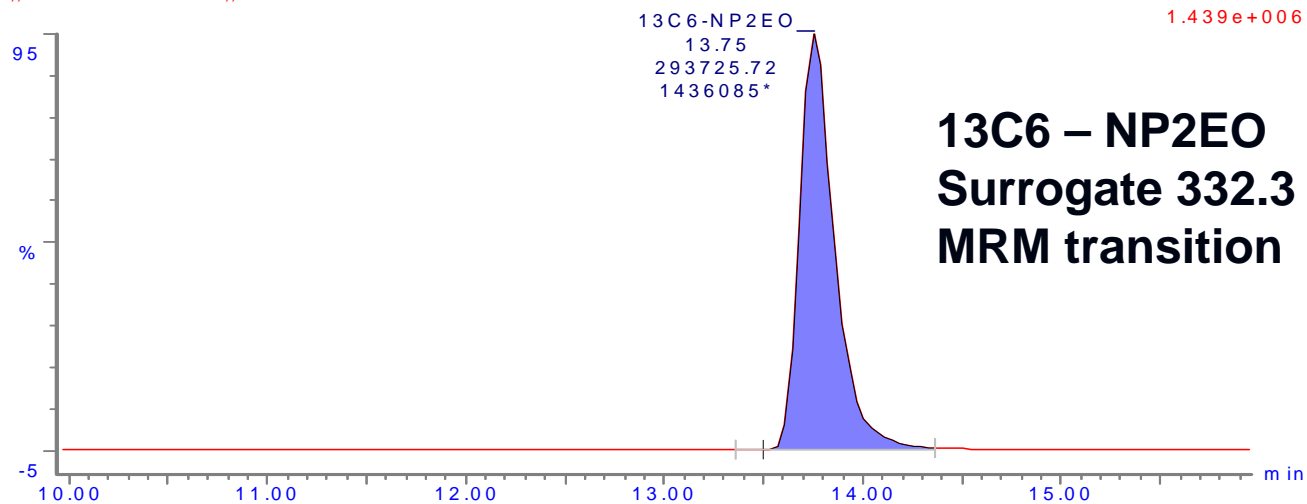
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1,,15ul AP021D-Cal,,/01

MRM of 16 channels,ES+
326.3>183
1.151e+006



AP0J_195S025 Smooth(Mn,1x1)
1,,15ul AP021D-Cal,,/01

MRM of 16 channels,ES+
332.3>127
1.439e+006



Key Notes on Alkylphenol Analysis

- **GC/MS Method (Water and Sediment)**
 - Water matrices - whole water method, includes AP adsorbed to solids (vs. ASTM D-7065)
 - Acetylation increases volatility for GC, improved response - greatly improved chromatography
 - Recovery correction allows aggressive clean-up
 - Detection limits in 20-40 ng/L , 1- 7 ng/g sediment
- **LC MS/MS Analysis**
 - Steam distillation of extract leaves interfering matrix compounds behind, key limit of other approaches
 - Recovery correction enables steam distillation approach
 - LC MS/MS analysis simplifies quantification as all isomers of same target represented by single peak MRM
 - Detection limit of analysis blank limited at 20-40 ng per target.

Modification of PPCP ANALYSIS

Tissue Matrix – Extended EPA 1694 List

Sample – 2 X 5 gram wet wt. tissue

Acidic Fraction – Virtis with 15 mL phosphate buffer (pH 2)

Basic Fraction – Virtis with reagent water plus NaOH (pH 10)

Respective Fractions – Add labeled Surrogates + Virtis

Acidic Extraction – 2X ultrasonic with 1) phosphate buffer 2) ACN

Basic Fraction – 2x ultrasonic with 1) H₂O/NH₄OH 2) ACN

Rotovap + SPE Cartridge Cleanup of Supernatant (1 g Waters Oasis HLB)

SPE Steps – condition, load, rinse, elute, reduce, transfer to MeOH

Add recovery standards

ANALYSIS by LC/MS/MS (Lists 1,3,4,5 – List 2 Tetracyclines not run)

ACIDIC EXTRACT – Neg. Ionization, 2 X Pos. Ionization,

BASIC EXTRACT – Pos. Ionization

Confirmation of Modified PPCP Extraction Tissue Matrix – Extended EPA 1694 List

Sample – 2 x 1-5 gram wet wt. tissue
2 x tissue + surrogates + 8 mL ACN + sonication
Centrifuge, collect supernatant for each fraction

Acidic Fractions – (2X) Phosphate buffer extraction (pH 2), centrifuge
Basic Fraction – (2X) H₂O/NaOH extraction (pH 10), centrifuge
Add supernatants to ACN extract, rotovap for ACN removal

SPE Cartridge Cleanup of supernatant (1 g Waters Oasis HLB)
SPE Steps – condition, load, rinse, elute, reduce, transfer to MeOH
Add recovery standards

ANALYSIS by LC/MS/MS (5X – all lists)
ACIDIC EXTRACT – Neg. Ionization, Tetracyclines, 2X Pos. Ionization
BASIC EXTRACT – Pos. Ionization

PPCP Method QC Notes

- **Reference Matrix Selection (IPRs, MS/MSD)**
 - 2 mussel species – variation in matrix may limit sample size (1 – 5 grams wet)
 - 2 “clean” fish tissues from AXYS (ling cod, salmon)
 - Best match per batch
- **Alignment with EPA 1694 QC Specs.**
 - Most 1694 QC specs. applicable
 - Exception – recovery criteria – tighter, low RPD, but may be marginally outside of EPA 1694 recovery ranges
- **DL, and % RPD Notes**
 - 90% of compounds 0.1 to 10 ng/g range
 - 10% of compounds 10 – 40 ng/g range
 - < 20% RPD between method variations, MS/MSDs, duplicates, dilutions

PPCP Tissue Method Conclusions

- From reconnaissance and validation work 120 of 121 PPCP compounds studied appear to work in tissue method(s)
 - Casualties – Atorvastatin (cimitedine marginal)
- Both extraction methods viable, initial extraction by ACN is favored – better theoretical extraction
- ACN “first” approach may elevate detection limits somewhat
- QA criteria largely unchanged from extended EPA 1694 method
- Intend to apply bio-solids QC criteria until completion of upcoming projects in marine and freshwater tissue
- Calculate new QC specs. after significant # of batches run



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- From SFEI
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- From AXYS
 - AXYS operations staff
 - AXYS method development and QA staff

