

LC-MS-MS Analysis of PAH's, Their Derivatives and 3 Oil Dispersants in Sea Water

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Introduction

- Some well known, and many less well known maritime based oil spills.
- Exxon Valdez, Prince William Sound, Alaska March of 1989
 - 10 million gallons
- BP Deepwater Horizon, April, of 2010
 - 4.9 million barrels or 205 million gallons
- June 2011, Conoco-Phillips rig off North Eastern China – Unverifiable amount.
- Unfortunately common events.

Introduction

- PAH's make up 0.2% to 7% of crude oil
- Some of these are toxic (carcinogenic, mutagenic), with the most well known being Benzo(a)pyrene.
- Alkylated PAH's are less well studied and in some cases may be more toxic.
- Oxidation products (OPAH's) are also not well studied, but are more water soluble, and possibly more bioavailable.
- 32 PAH's are classified as Priority Pollutants by the EPA, with 16 being commonly monitored (Method 8270, 1625)

Introduction

- Dispersants often used to “clean up” spills.
- Mixtures of surfactants to break up oil slicks, prevent them from washing onshore.
- Some controversy around use.
- Components can be toxic.
- Two products that were used during Deepwater Horizon made by Nalco –
 - Corexit EC9500A (Propylene Glycol, light petroleum distillates, DOSS)
 - Corexit EC9527A (2-Butoxyethanol, Propylene Glycol, DOSS)
- By some estimates, as much as 1,000,000 gallons of these two products were used.







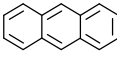
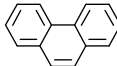
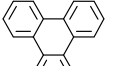
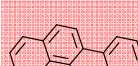
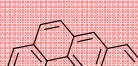
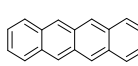



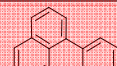
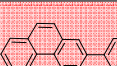
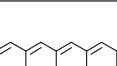





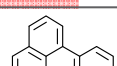
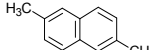
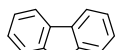
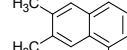
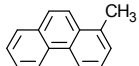
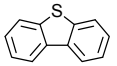
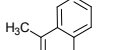
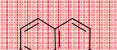
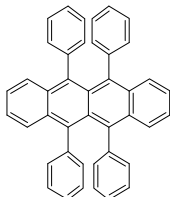
Introduction

- Explored methods for analysis of PAH's and common dispersant components.
- Included analysis of common alkylated PAH's and some oxidized metabolites.
- Based methods on direct injection of sea water
- Compared sensitivity for various ionization techniques.
- Addressed common problems with analysis.

PAH Analysis

PAH's and some common methyl derivatives.

Shaded in red are considered toxic.

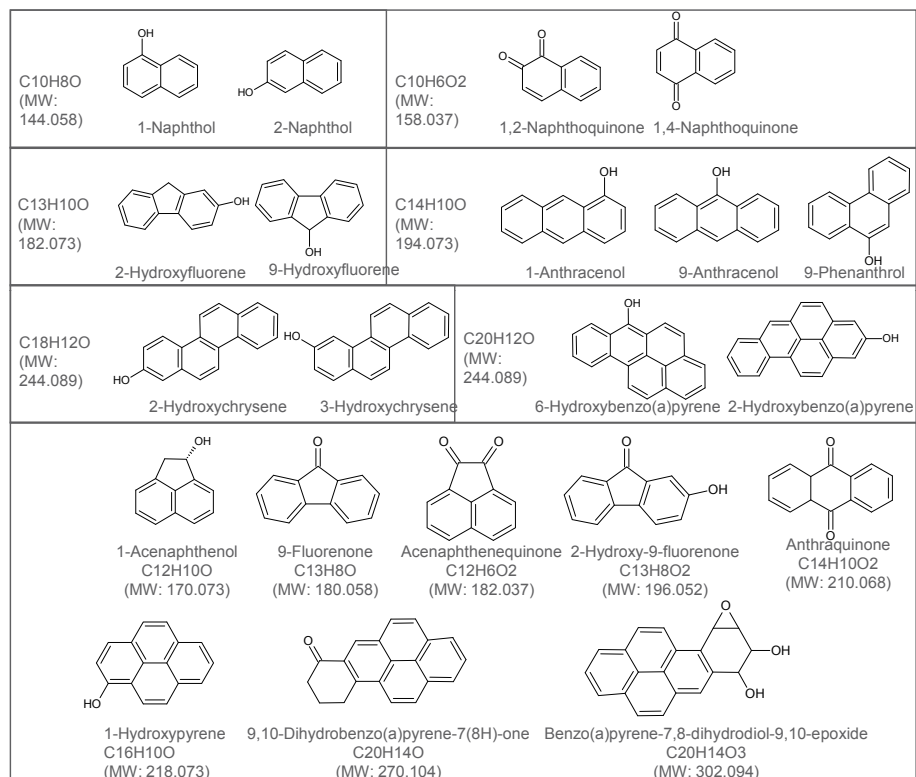
C10H8 (MW: 128.063)	 naphthalene	 azulene	C11H10 (MW: 142.078)	 1-methylnaphthalene	 2-methylnaphthalene	C12H10 (MW: 154.078)	 acenaphthene	 biphenyl
C14H10 (MW: 178.078)	 anthracene	 phenanthrene	C18H12 (MW: 228.094)	 triphenylene	 chrysene	 benzo(a)anthracene	 benzo(b)anthracene	
C16H10 (MW: 202.078)	 fluoranthene	 pyrene	C22H12 (MW: 276.094)	 indeno(1,2,3-cd)pyrene	 benzo(ghi)perylene	C22H14 (MW: 278.110)	 dibenzo(a,h)anthracene	 pentacene
C20H12 (MW: 252.084)	 benzo(j)fluoranthene	 benzo(k)fluoranthene	 benzo(b)fluoranthene	 benzo(e)pyrene	 benzo(a)pyrene	 perylene		
Acenaphthylene C12H8 (MW: 152.062)	 2,6-dimethylnaphthalene C12H12 (MW: 156.094)	 Fluorene C13H10 (MW: 166.078)	 2,3,5-trimethylnaphthalene C13H14 (MW: 170.110)	 1-methylphenanthrene C15H12 (MW: 192.084)	 Dibenzothiophene C12H8S (MW: 184.035)	 7,12-dimethylbenzo(a)anthracene C20H16 (MW: 256.125)	 Coronene C24H12 (MW: 300.094)	 Rubrene C42H28 (MW: 532.219)

PAH Analysis

Oxidative PAH Metabolites described in Literature.

Difficult to impossible to find some of these as analytical standards.

Toxicity unknown for many of these.



PAH Analysis

- Commonly analyzed by GC and GC-MS or LC-Fluorescence.
- For analysis in water:
 - Wanted to develop an LC-MS-MS technique to minimize (or eliminate) sample preparation.
 - Shorter analytical run time.
 - Greater specificity than fluorescence.
- Potentially problematic because they have no obvious, readily ionizable functional group.

PAH Analysis - Ionization

- Ionization efficiencies investigated using benzo(a)pyrene
- $[M]^+$ radical was observed as charged species for all ionization modes.
- APPI was tested using: no dopant, toluene, anisole chlorobenzene → best, 0.3 ml/min
- APCI was chosen for best combination of sensitivity and robustness

Table 1: Comparison of ionization methods and detector types. MRM $m/z = 252.09 \rightarrow 224.06$ monitored

4000QTRAP™: 5- μ L injections in triplicate per concentration (1 pg/mL to 1,000 ng/mL); unit - unit resolution

Linear regression with "1/x" weighting was used to obtain slope sensitivity, intercept, S/N, etc.; retention time = 5.40 min

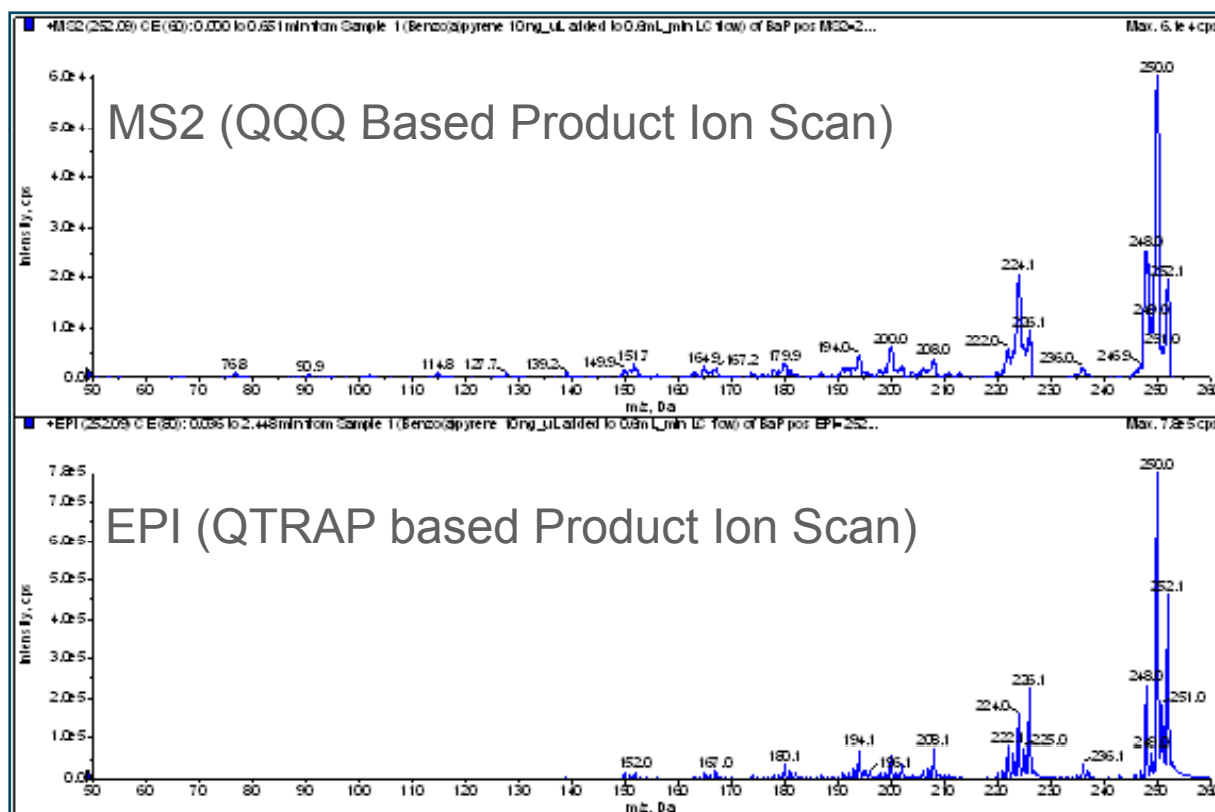
ionization/ detector	Slope cps/(ng/mL)	Intercept cps	Lowest conc. detected(ng/mL)	S/N at LD	Linearity range	Note
APCI	98.4	-21.5	1.00	8.9	1 - 1,000	robust
ESI	2,890.0	68.2	0.10	12.5	0.1 - 100	non-linear above 100 ng/mL
APPI	400.7	40.7	10.00	11.3	10 - 1,000	noisy background
UV (254 nm)	13.8	152.0	10.00	2.9	10 - 1,000	high background
FL(EX=260; EM=460 nm)	841.0	15.2	0.10	15.2	0.1 - 1,000	high carry-over

PAH Analysis

MS/MS of benzo(a)pyrene – from LC-MS-MS run

QTRAP scans much faster than QQQ resulting in higher quality spectra for ID purposes.

Retro Diels-Alders fragments (loss of H₂ and CH₂) are observed for most PAH's.



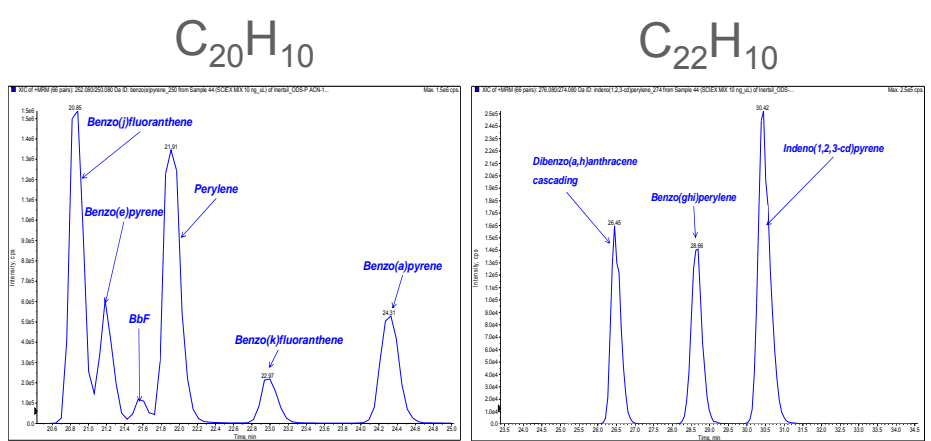
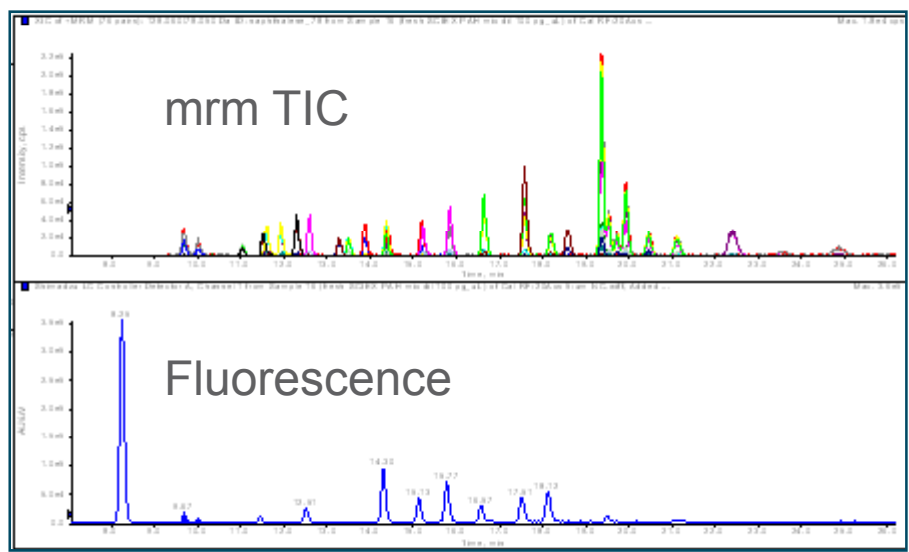
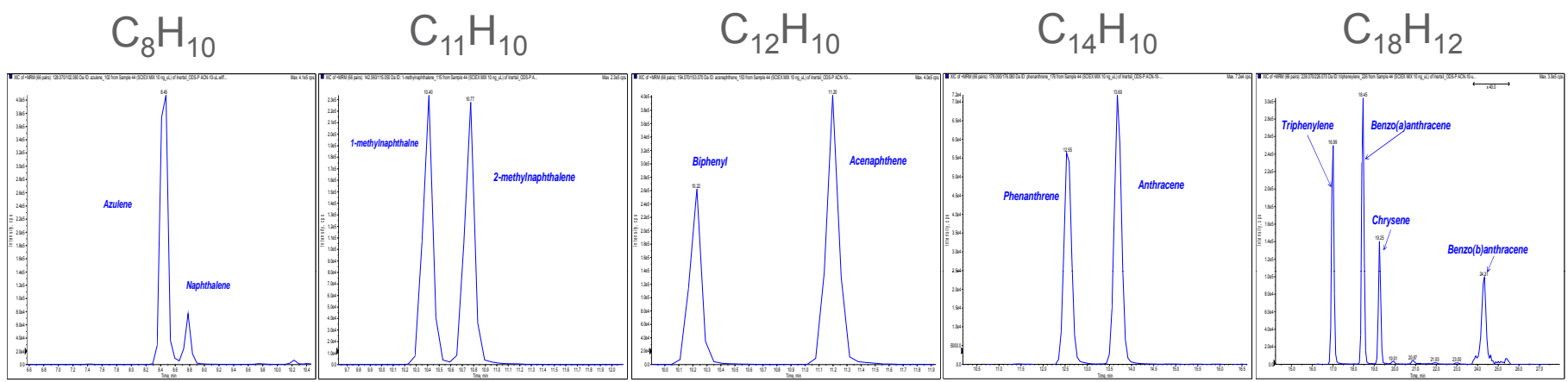
PAH Analysis - Chromatography

- MPA: H₂O
- MPB: CH₃CN
- GL Sciences Inertsil ODS-P HP 3μm, 2.1x250mm
- Shimadzu Nexera UHPLC system with RF-20Axs fluorescence detector.
- Quantitative work done on API 5000 mass spectrometer

Time(min)	Module	Events	Parameter
0.01	Pumps	Pump B Conc.	40.00
7.00	Pumps	Pump B Conc.	60.00
14.00	Det. A	Emission wavelength	352.00
14.01	Det. A	Emission wavelength	440.00
16.00	Pumps	Pump B Conc.	100.00
18.90	Pumps	Pump B Conc.	100.00
18.90	Pumps	Total Flow	0.50
19.00	Pumps	Total Flow	1.00
23.00	Det. A	Emission wavelength	440.00
23.10	Det. A	Emission wavelength	420.00
25.00	Pumps	Pump B Conc.	100.00
25.00	Pumps	Total Flow	1.00
25.10	Pumps	Total Flow	0.50
25.11	Pumps	Pump B Conc.	40.00
29.00	Det. A	Emission wavelength	420.00
29.10	Det. A	Emission wavelength	352.00
30.00	System Controller	Stop	

Excitation Wavelength=260 nm; Lamp=D2; Gains x4; Sensitivity Low
Response=1.5 sec

PAH Analysis - Chromatography

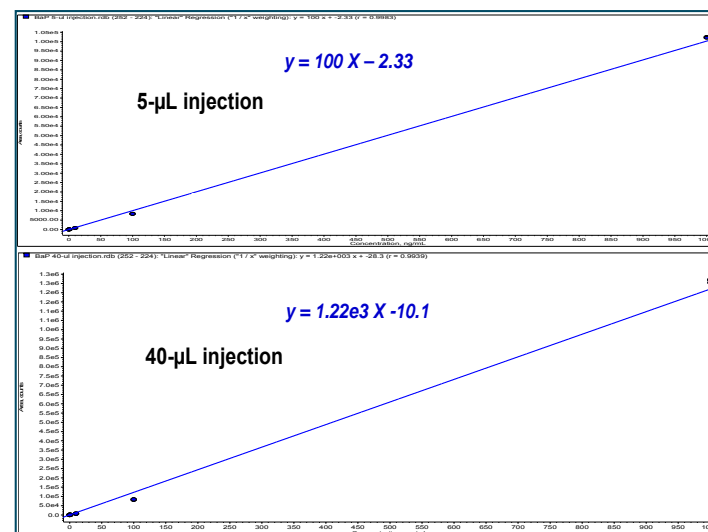


PAH Analysis

- Optimized compounds, developed chromatography, how else can we increase sensitivity without resorting to sample prep?
- For APCI → good correlation with injection volume & s/n

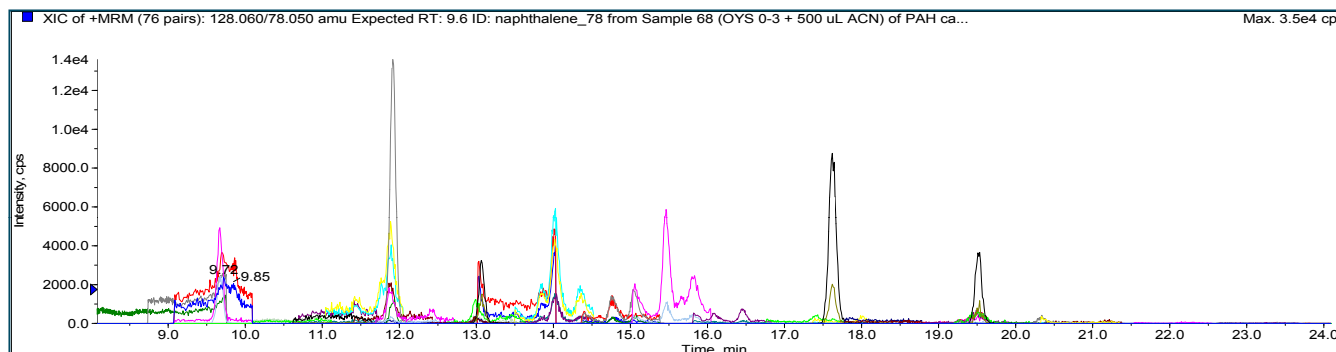
Measurements were in triplicates. S/N, retention time, peak width, theoretical plate are averages of 3 measurements

5- μ L Injections m/z or UV	Slope sensitivity	linear correlation coefficient	Detection limit (ng/mL)	S/N at detection limit	Retention time (min)	peak width base(min)	Theoretical plate
m/z = 252.09 → 224.06	100.0	0.9981	1	8.5	5.64	0.139	479
m/z = 252.09 → 250.09	397.0	0.9986	1	13.8	5.48	0.234	481
UV @ 254 nm	14.5	0.9983	10	7.2	7.19	0.290	446
10- μ L Injections m/z or UV	Slope sensitivity	linear correlation coefficient	Detection limit(ng/mL)	S/N at detection limit	Retention time (min)	peak width base(min)	Theoretical plate
m/z = 252.09 → 224.06	221.0	0.9829	1	17.9	5.32	0.169	453
m/z = 252.09 → 250.09	863.0	0.9830	1	26.6	5.33	0.269	454
UV @ 254 nm	28.6	0.9856	10	21.5	5.28	0.217	447
20- μ L Injections m/z or UV	Slope sensitivity	linear correlation coefficient	Detection limit(ng/mL)	S/N at detection limit	Retention time (min)	peak width base(min)	Theoretical plate
m/z = 252.09 → 224.06	519.0	0.9952	1	33.1	5.43	0.230	472
m/z = 252.09 → 250.09	1980.0	0.9961	0.1	5.8	5.41	0.157	469
UV @ 254 nm	53.8	0.6848	10	15.5	5.25	0.229	442
40- μ L Injections m/z or UV	Slope sensitivity	linear correlation coefficient	Detection limit(ng/mL)	S/N at detection limit	Retention time (min)	Peak width base(min)	Theoretical plate
m/z = 252.09 → 224.06	1220.0	0.9939	0.1	6.5	5.36	0.103	461
m/z = 252.09 → 250.09	4480.0	0.9955	0.1	5.1	5.37	0.171	461
UV @ 254 nm	107.0	0.9996	1	6.2	5.32	0.230	453

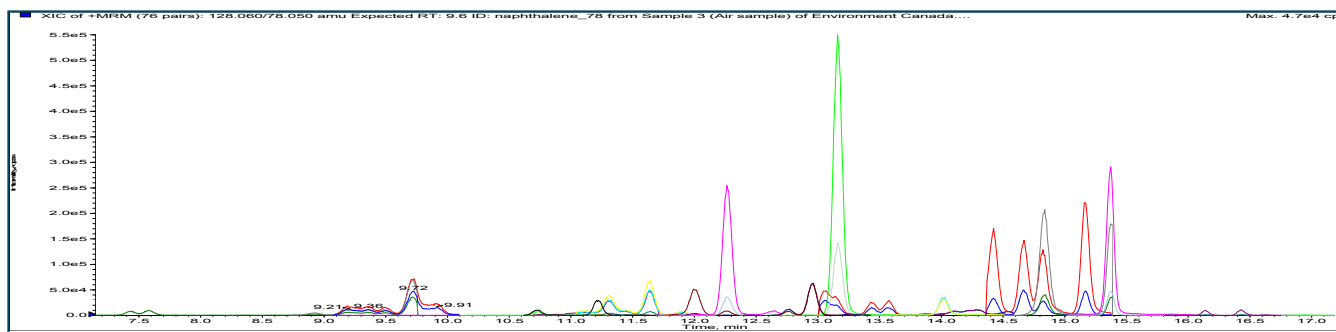


PAH Analysis

Oyster extract prepared using slightly modified NOAA method



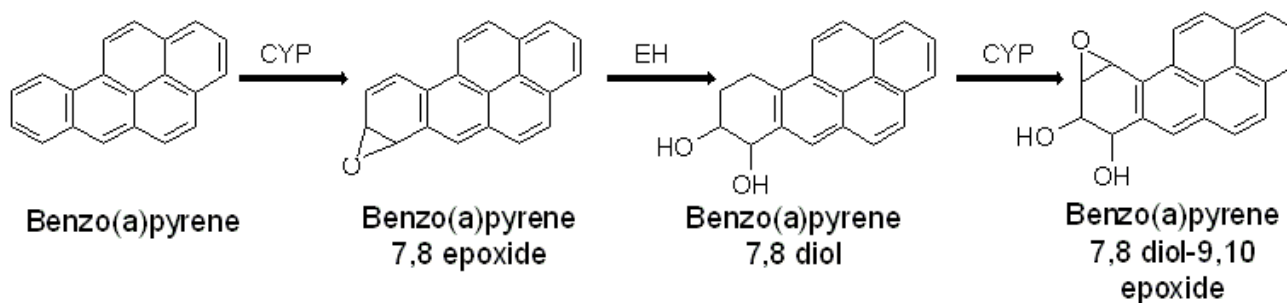
Solvent extracted air sampling disks



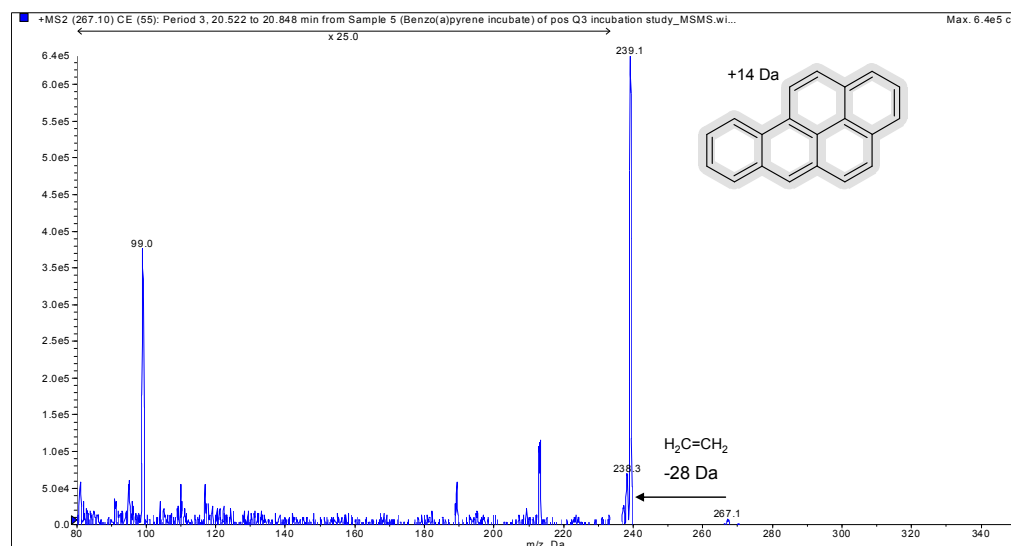
PAH Analysis - Metabolites

Oxidative metabolism of benzo(a)pyrene is well studied.

CYP450 substrate



RLM incubation yielded expoxide



PAH Analysis

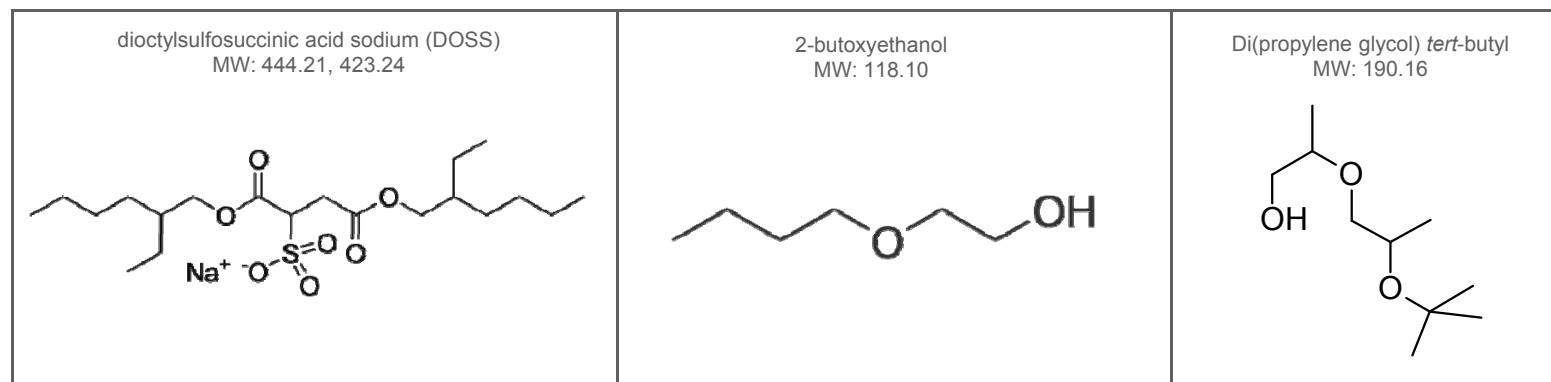
M1	M3	ID	DP	EP	CE	CXP	M1	M3	ID	DP	EP	CE	CXP
128.06	78.05	naphthalene 1	214	10.0	42	5.0	134.07	151.04	3-phenanthrol 1	97	10.0	29	9.8
128.06	102.05	naphthalene 2	214	10.0	39	5.0	134.07	166.05	3-phenanthrol 2	184	10.0	23	12.0
128.07	78.06	azulene 1	222	10.0	42	4.2	202.07	150.05	fluoranthene 1	110	10.0	81	8.1
128.07	102.06	azulene 2	222	10.0	40	6.0	202.07	200.07	fluoranthene 2	110	10.0	60	12.5
136.11	84.08	naphthalene-d8 1	222	10.0	42	5.0	202.08	150.06	pyrene 1	181	10.0	85	8.9
136.11	108.08	naphthalene-d8 2	222	10.0	39	5.0	202.08	200.08	pyrene 2	181	10.0	64	11.5
142.06	89.05	1-methylnaphthalene 1	239	10.0	65	5.0	219.07	190.05	1-hydroxypyrene 1	263	10.0	49	13.3
142.06	115.05	1-methylnaphthalene 2	239	10.0	46	5.0	219.07	200.70	1-hydroxypyrene 2	169	10.0	17	15.0
142.07	115.06	2-methylnaphthalene 1	248	10.5	47	6.5	228.07	200.07	triphenylene 1	120	10.9	77	10.6
142.07	141.07	2-methylnaphthalene 2	248	10.5	30	7.2	228.07	226.07	triphenylene 2	120	10.9	55	12.1
145.05	63.00	1-naphthol 1	40	10.0	37	14.0	228.08	200.09	chrysene 1	110	10.9	83	11.2
145.05	122.04	1-naphthol 2	40	10.0	18	17.1	228.08	226.08	chrysene 2	110	10.9	58	12.5
145.06	63.01	2-naphthol 1	85	10.0	38	11.0	228.09	150.08	benzo(a)anthracene 1	175	11.0	105	9.3
145.06	122.05	2-naphthol 2	85	10.0	17	19.7	228.09	226.09	benzo(a)anthracene 2	175	11.0	58	13.1
152.06	126.06	acenaphthylene 1	248	10.6	47	6.7	228.10	202.08	benzo(b)anthracene 1	100	10.7	71	13.5
152.06	151.06	acenaphthylene 2	249	10.6	43	9.3	229.09	226.08	benzo(b)anthracene 2	100	10.7	69	14.0
154.07	126.05	acenaphthene 1	260	10.7	67	7.4	240.17	208.11	chrysene-d12 1	110	10.9	83	11.2
154.07	153.07	acenaphthene 2	260	10.7	32	8.5	240.17	236.14	chrysene-d12 2	110	10.9	58	12.5
154.08	126.06	biphenyl 1	272	10.8	55	7.1	252.05	224.06	benzo(j)fluoranthene 1	190	10.8	86	12.8
154.08	153.08	biphenyl 2	272	10.8	32	8.9	252.05	250.05	benzo(j)fluoranthene 2	190	10.8	64	13.6
156.09	115.05	2,6-dimethylnaphthalene 1	272	10.9	49	7.0	252.06	224.07	benzo(k)fluoranthene 1	192	10.7	87	13.7
156.09	141.06	2,6-dimethylnaphthalene 2	272	10.9	29	7.8	252.06	250.06	benzo(k)fluoranthene 2	192	10.7	65	15.3
159.03	103.04	1,2-naphthoquinone 1	125	10.0	34	16.2	252.07	224.08	benzo(l)fluoranthene 1	120	10.7	85	12.7
159.03	131.04	1,2-naphthoquinone 2	125	10.0	24	20.3	252.07	250.07	benzo(l)fluoranthene 2	120	10.7	64	16.1
159.04	103.05	1,4-naphthoquinone 1	125	10.0	33	16.1	252.08	222.09	benzo(e)pyrene 1	190	10.7	116	12.5
159.04	131.05	1,4-naphthoquinone 2	125	10.0	24	20.3	252.08	250.08	benzo(e)pyrene 2	190	10.7	64	14.8
164.14	132.08	acenaphthene-d10 1	272	10.7	67	7.4	252.09	224.09	benzo(a)pyrene 1	193	10.6	90	12.3
164.14	162.13	acenaphthene-d10 2	272	10.7	32	8.5	252.09	250.09	benzo(a)pyrene 2	193	10.6	66	15.0
166.08	115.05	fluorene 1	272	10.5	61	7.0	252.10	224.10	perylene 1	190	10.6	90	13.6
166.08	165.08	fluorene 2	272	10.5	37	9.0	252.10	250.10	perylene 2	190	10.6	67	14.7
170.11	128.06	2,3,5-trimethylnaphthalene 1	277	10.9	57	7.5	257.12	226.10	7,12-dimethylbenzo(a)anthracene 1	97	10.5	71	13.2
170.11	153.08	2,3,5-trimethylnaphthalene 2	277	10.9	45	8.8	257.12	242.10	7,12-dimethylbenzo(a)anthracene 2	83	10.5	33	13.2
171.07	46.00	1-acenaphthenol 1	95	10.0	24	5.7	264.17	232.11	perylene-d12 1	190	10.6	90	13.6
171.07	72.00	1-acenaphthenol 2	95	10.0	30	11.0	264.17	260.14	perylene-d12 2	190	10.6	67	14.7
178.08	152.08	anthracene 1	291	10.5	47	9.0	271.10	215.07	9,10-dihydrobenzo(a)pyrene-7,(8H)	245	10.0	45	16.2
178.08	176.07	anthracene 2	291	10.5	55	10.3	271.10	253.09	9,10-dihydrobenzo(a)pyrene-7,(8H)	245	10.0	29	20.5
178.09	151.05	phenanthrene 1	296	10.3	58	8.2	276.08	248.09	indeno(1,2,3-cd)pyrene 1	210	10.5	94	13.0
178.09	176.08	phenanthrene 2	296	10.3	49	9.9	276.08	274.08	indeno(1,2,3-cd)pyrene 2	210	10.5	73	15.6
181.06	127.03	3-fluorenone 1	150	10.0	51	20.7	276.09	246.09	benzo(ghi)perylene 1	205	10.2	127	17.3
181.06	152.05	3-fluorenone 2	150	10.0	45	23.4	276.09	274.09	benzo(ghi)perylene 2	205	10.2	77	15.0
182.07	165.05	3-hydroxyfluorene 1	58	10.0	13	10.1	278.11	248.09	dibenzo(a,h)anthracene 1	188	10.5	113	16.0
183.07	95.00	3-hydroxyfluorene 2	121	10.0	29	15.1	278.11	276.09	dibenzo(a,h)anthracene 2	188	10.5	64	17.0
184.03	139.03	dibenzothiophene 1	298	10.0	55	8.2	278.12	250.10	pentacene 1	200	10.0	84	6.1
184.03	152.03	dibenzothiophene 2	298	10.0	45	9.5	278.12	276.10	pentacene 2	200	10.0	67	15.4
188.14	158.10	phenanthrene-d10 1	320	10.3	58	8.2	300.09	296.10	coronene 1	213	10.0	115	7.8
188.14	184.11	phenanthrene-d10 2	320	10.3	49	9.9	300.09	298.10	coronene 2	213	10.0	84	7.9
192.09	165.08	1-methylphenanthrene 1	553	10.5	54	9.6	533.22	377.13	rubrene 1	78	10.3	75	22.0
192.09	191.09	1-methylphenanthrene 2	553	10.5	34.6	10.3	533.22	455.18	rubrene 2	78	10.3	36	6.9

PAH Analysis

- Developed a method for:
 - 26 PAH's
 - 6 Alkylated (methyl) species
 - 11 Oxidized degradants
 - 5 labeled internal standards
- Chromatographically separates isobaric compounds
- Sub ppb detection limit in water – other matrices will be based on sample prep techniques.

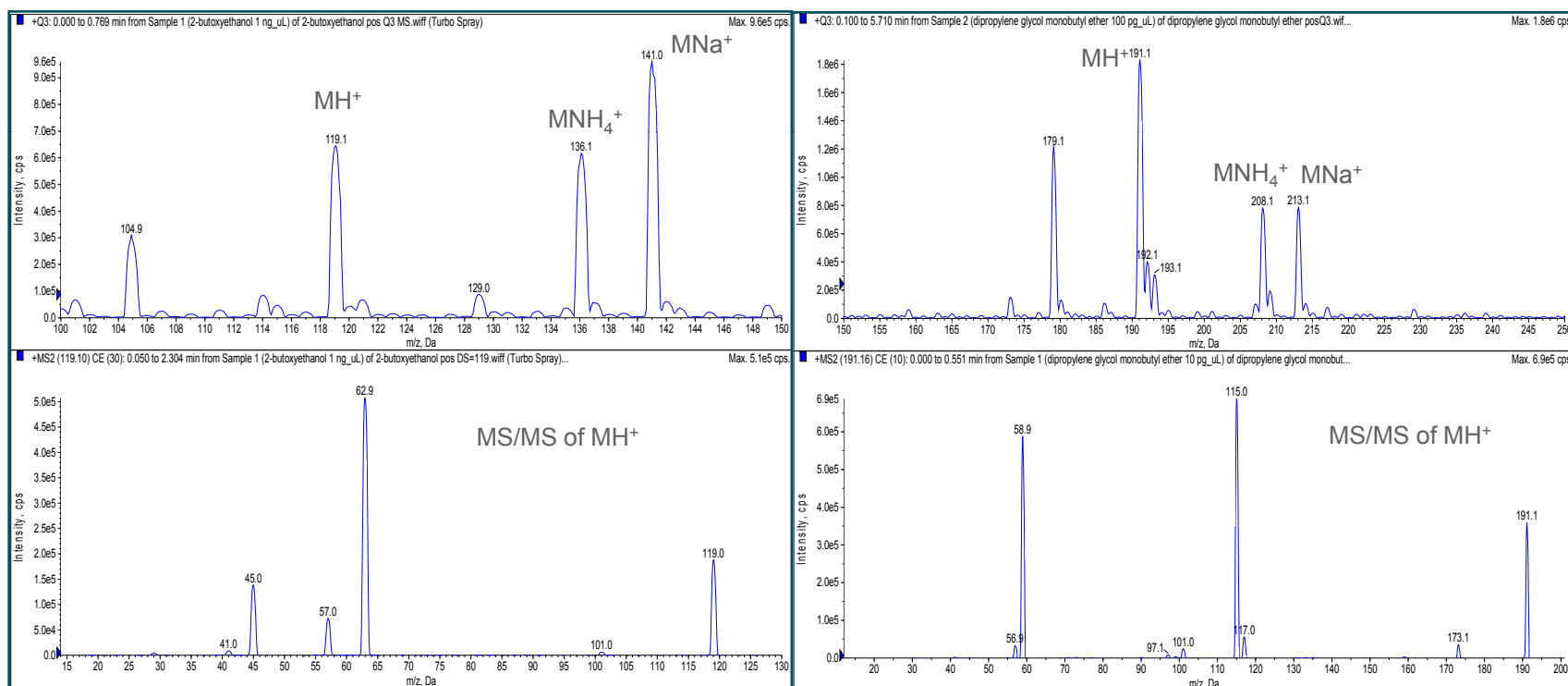
Dispersant Analysis

- Developed methods for:
 - DOSS (dioctylsulfosuccinic acid)
 - 2-Butoxyethanol
 - Di(propylene glycol) *tert*-butyl ether
- 2-butoxyethanol is present in Corexit EC9527A



Dispersant Analysis

2-Butoxyethanol & D(ipropylene glycol) *tert*-butyl ether, no strong ionizable groups. Do associate well with NH_4^+ in ESI.



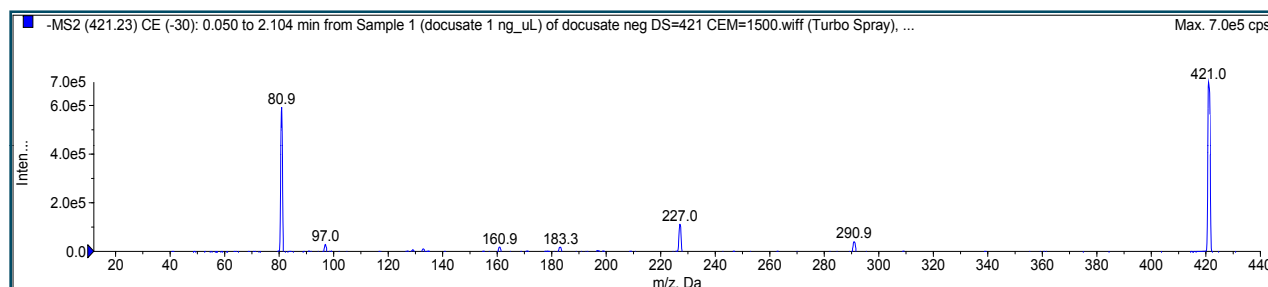
2-butoxyethanol

Dipropylene glycol *t*-butyl ether

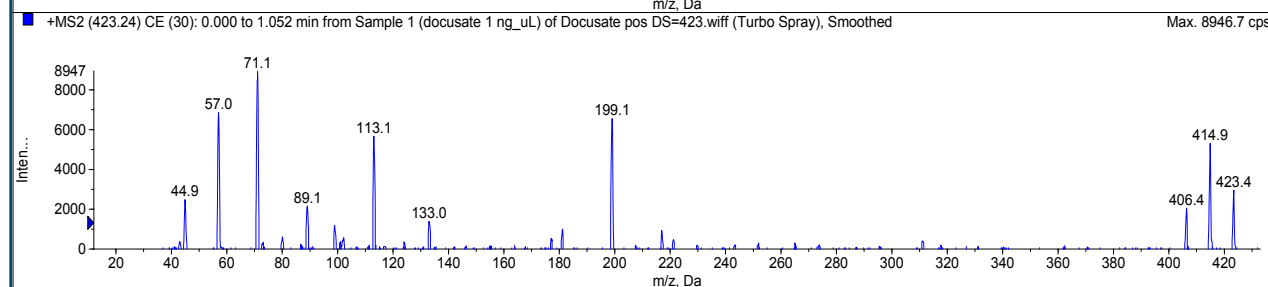
Dispersant Analysis

DOSS ionizes in both negative mode and positive mode. Positive mode also works best as an NH_4^+ adduct.

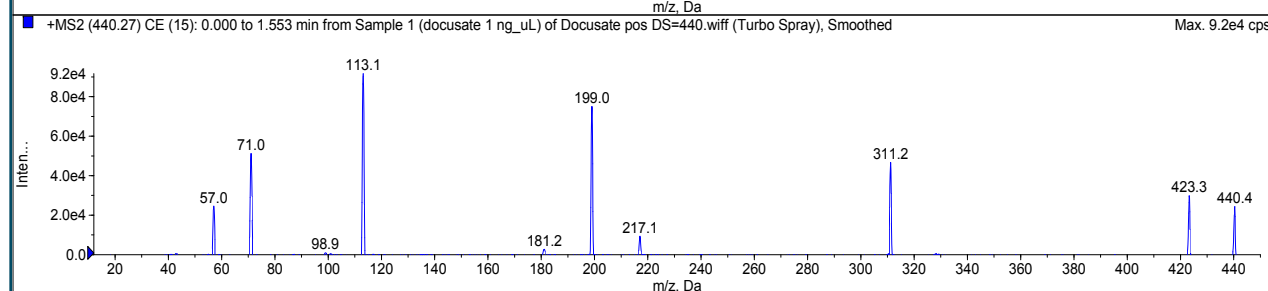
Negative mode
MS/MS $[\text{M}-\text{H}]^-$



Positive mode
MS/MS $[\text{M}+\text{H}]^+$



Positive mode
MS/MS $[\text{M}+\text{NH}_4]^+$



Dispersant Analysis

Standard compound optimization techniques.

Work done on an API 5000 triple quadrupole mass spectrometer.

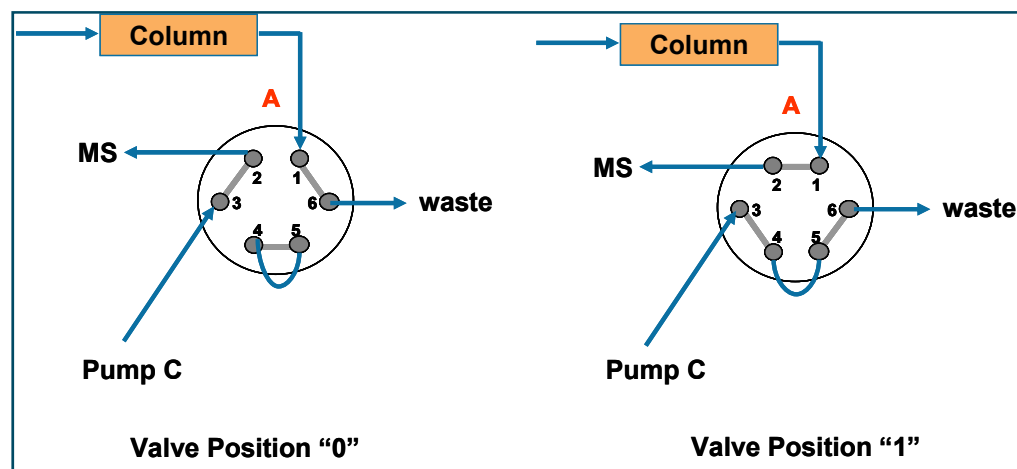
Negative ion mode MRM Table							
M1	M3	Dwell Time	ID	DP	EP	CE	CXP
421.24	80.90	50	dioctylsulfosuccinate_81	-210	-10	-45	-34.0
421.24	183.10	50	dioctylsulfosuccinate_183	-210	-10	-45	-24.0
421.24	227.10	50	dioctylsulfosuccinate_227	-210	-10	-34	-28.0
421.24	290.90	50	dioctylsulfosuccinate_291	-210	-10	-33	-37.0

Positive ion mode MRM Table							
M1	M3	Dwell Time	ID	DP	EP	CE	CXP
119.11	45.05	50	2-butoxyethanol 119_45	52	10	15	6.7
119.11	63.08	50	2-butoxyethanol 119_63	52	10	9	9.8
136.11	45.05	50	2-butoxyethanol 136_45	7	10	21	5.5
136.11	63.08	50	2-butoxyethanol 136_63	7	10	13	6.5
191.16	59.07	50	di(propylene glycol) <i>t</i> -butyl ether_191_59	72	10	17	8.8
191.16	115.09	50	di(propylene glycol) <i>t</i> -butyl ether_191_115	72	10	10	12.3
208.19	59.07	50	di(propylene glycol) <i>t</i> -butyl ether_208_59	6.5	10	23	8.9
208.19	115.09	50	di(propylene glycol) <i>t</i> -butyl ether_208_115	6.5	10	16	13.0
423.24	113.10	50	dioctylsulfosuccinic acid_423_113	197	10	13.4	13.0
423.24	199.10	50	dioctylsulfosuccinic acid_423_199	197	10	15	15.7
440.27	113.10	50	dioctylsulfosuccinic acid_440_113	152	10	18	18.3
440.27	199.10	50	dioctylsulfosuccinic acid_440_199	152	10	20	20.5

Dispersant Analysis

- MPA: H₂O + 5 mM ammonium acetate
- MPB: CH₃CN + 5 mM ammonium acetate
- Total gradient flow of 0.5 ml/min
- Zorbax RRHD SB-C18 column, 50 mm X 2.1 mm id, 1.8 μm HPLC column

Time (min)	%B	Valve Position
0	5	1
3	5	0
8	85	1
9	85	
9.1	5	
11	5	



Dispersant Analysis

These compounds – particularly DOSS - are very “sticky”, and thus highly prone to carryover.

Used the Shimadzu NEXERA’s autosampler rinse program to dramatically reduce injection to injection carryover.

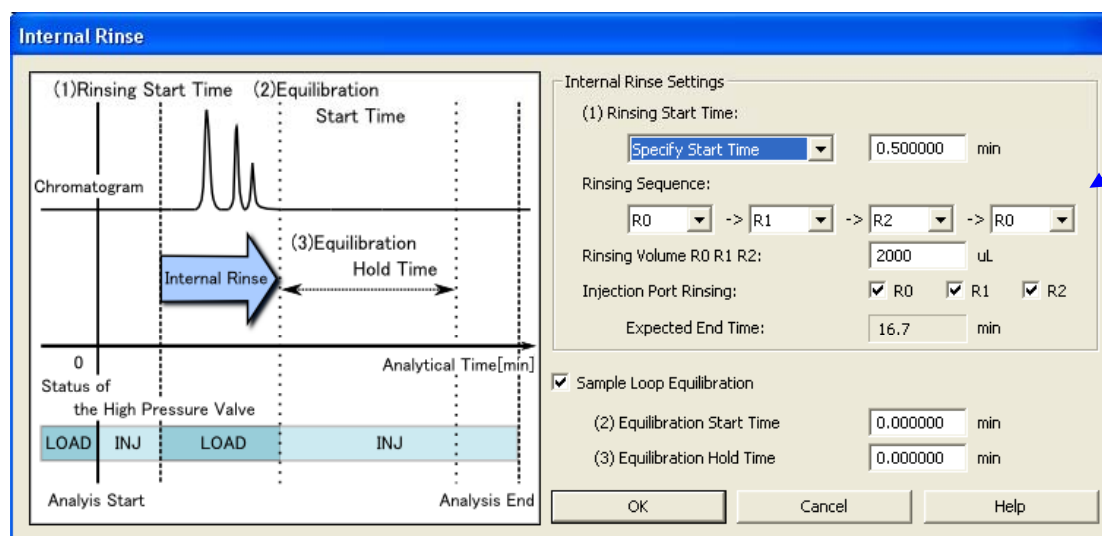
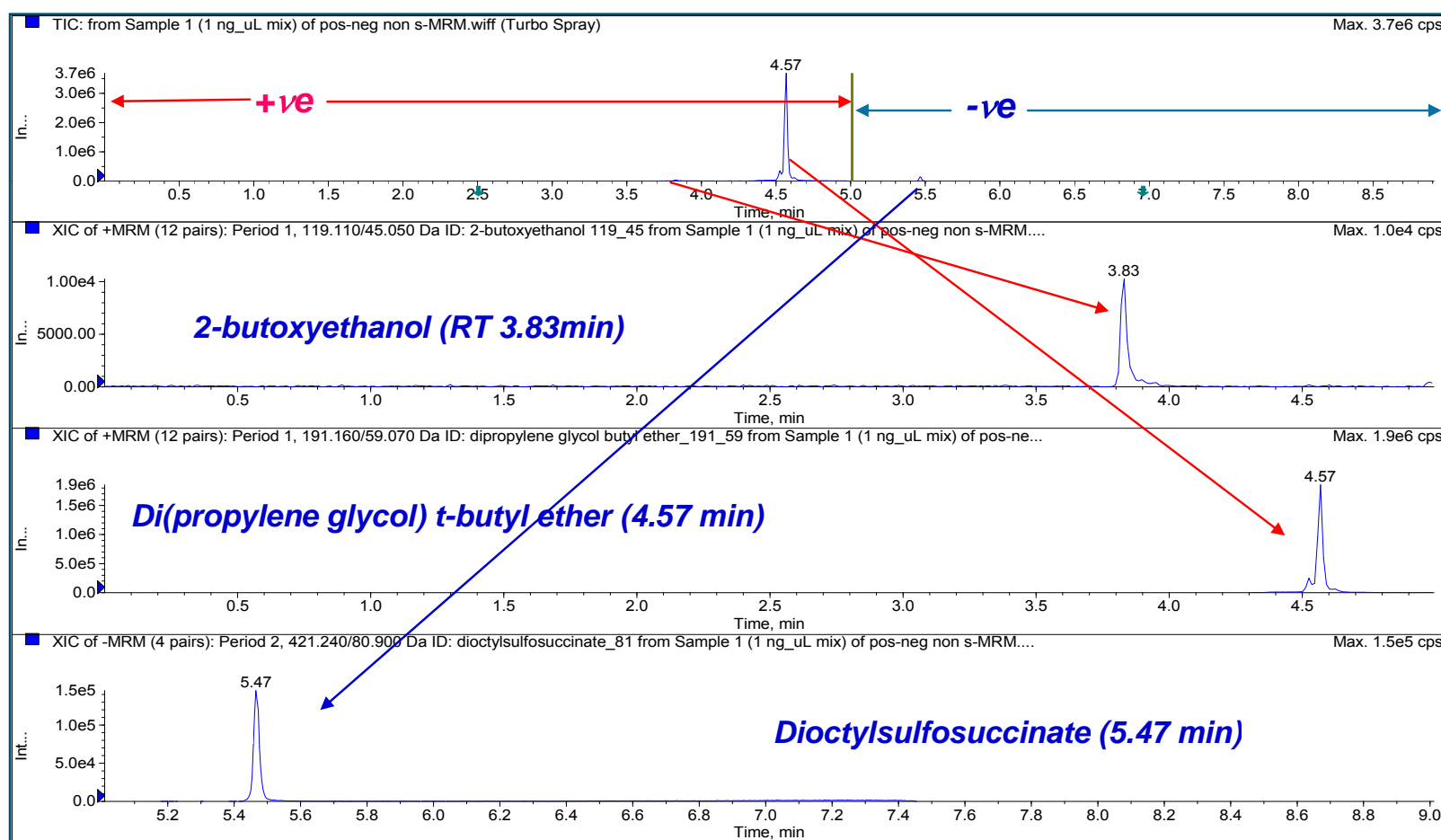


Fig. 13. Internal/External Rinse steps used
 R0 = reagent alcohol;
 R1 = acetonitrile;
 R2 = 50% water + 50% acetonitrile

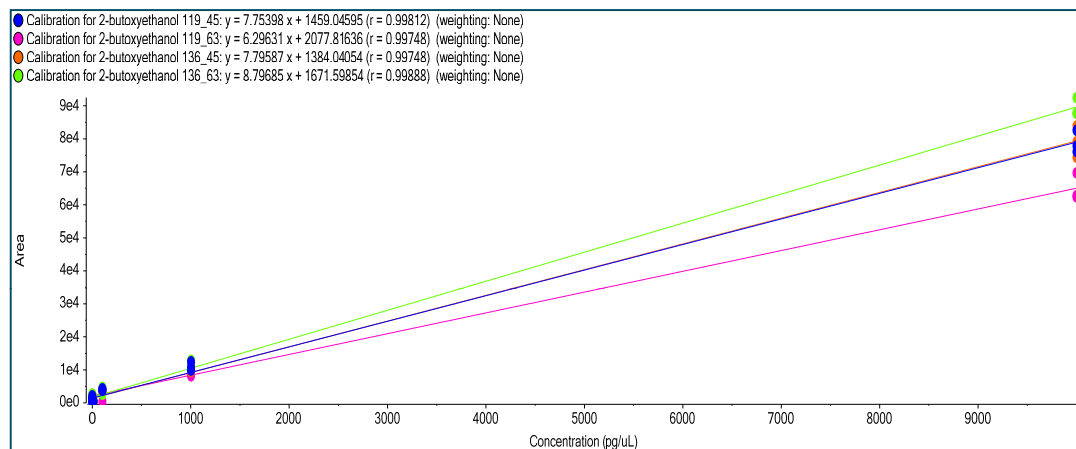
Dispersant Analysis

Because DOSS is more sensitive in negative mode and the compounds are chromatographically resolved, a 2 period experiment was used.

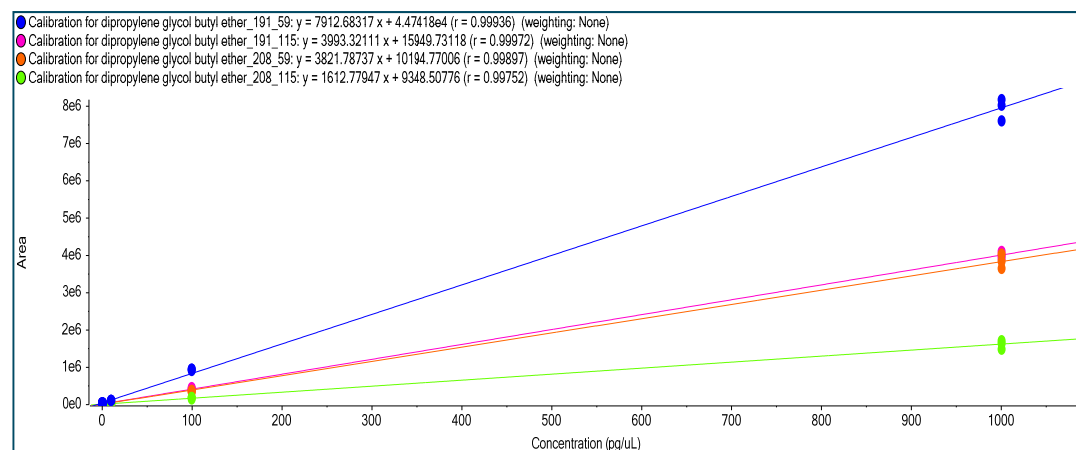


Dispersant Analysis

2-butoxyethanol 136.1 → 63.1
LOD 18ng/ml

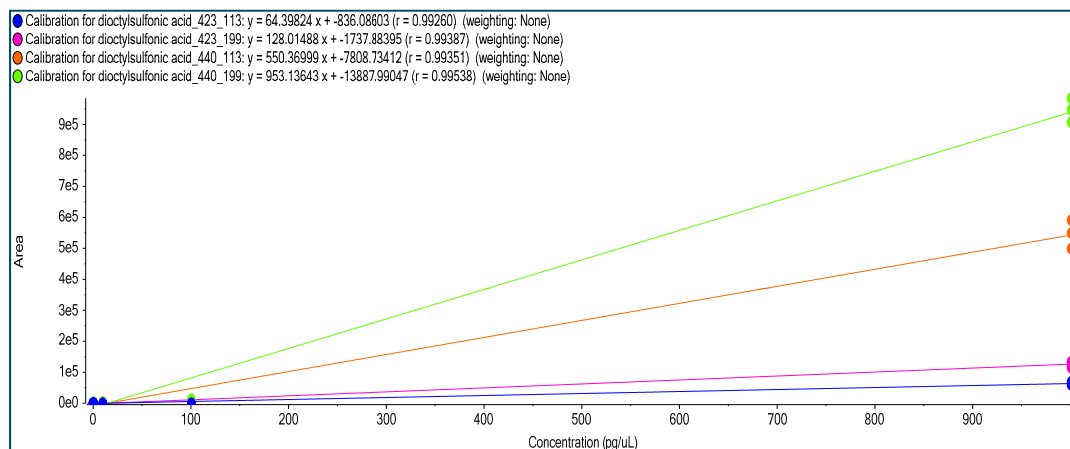


Dipropylene glycol *t*-butyl
ether 191 → 59 LOD 0.5 ng/ml

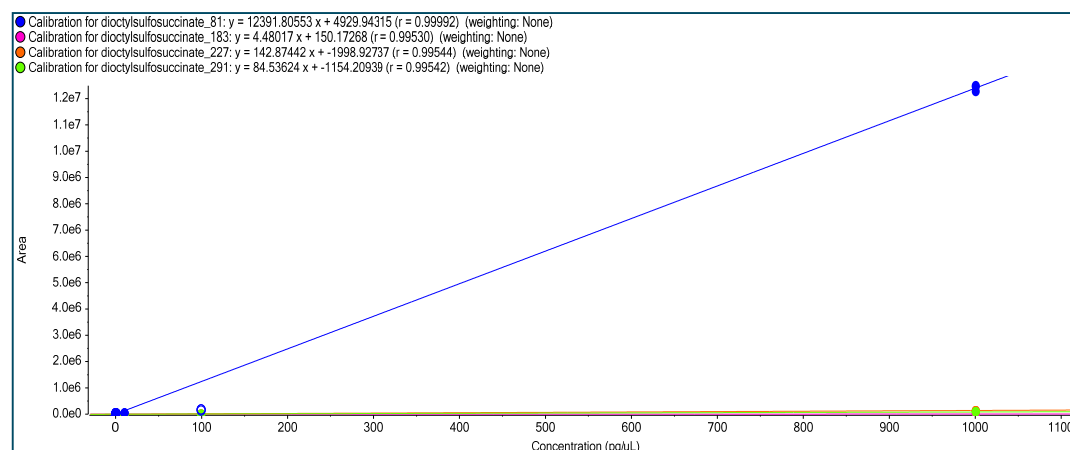


Dispersant Analysis

DOSS Pos mode
440 → 199.1, 0.9 ng/mL



DOSS Neg mode
421.2 → 80.9, 0.8 ng/mL



Neg mode is actually ~13x more sensitive than Pos mode – carryover still a problem!

Conclusions & Further Work

- Developed reliable, sensitive methods that should be fairly easy for most labs to implement.
 - Sub ppb LLOQ's for PAH's & related compounds
 - Low to sub ppb LOD's for dispersant compounds
- Relatively short run times compared to traditional, GC based approaches.
- No sample prep required for water samples.

Conclusions & Further Work

- Challenges for PAH analysis
 - Lack of standards for alkylated and oxidized PAH's
 - Lack of action limits/toxicity data for oxidized PAH's
 - Need to investigate whether additional PAH metabolites/degradants are present in the maritime spill environment.
- Challenges for dispersant analysis
 - Even though reduced, carryover is still a problem.

Conclusions & Further Work

Questions?

With Thanks to:

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Questions and Answers



Some References

- OSU Superfund PAH & OPAH Monitoring
<http://oregonstate.edu/superfund/oilspill>
- BaP Metabolism
<http://herkules.oulu.fi/isbn9514270398/html/x203.html>