Electronic Supplementary Material (ESI) for Analytical Methods.

SUPLEMENTARY INFORMATION

Method Performance for Ultra-Trace Level Naphthenic Acids

1. ***System Suitability***: The suitability of the instrument system was assessed at the start and end of each analytical sequence by replicate injections of a solution of internal standard. The internal standard employed was decanoic-d3 acid at a final concentration of 0.03 µg/mL in 0.1% v/v concentrated (≥ 28% ammonia) ammonium hydroxide solution.

Table 1: Example System Suitability

Sample ID	ISTD Response	Retention Time (min)
System Suitability	11417554	7.276
	11304680	7.281
	11277335	7.278
	11370944	7.277
	11521031	7.277
	11476377	7.277
Mean	11394654	7.278
Std Dev	95494	0.002
%RSD	0.8	0.024

2. ***Regression:*** Duplicate calibration standards in the concentration range 0.011 to 0.905 µg/mL, were analyzed at the beginning and end of each analytical sequence. Standards were prepared from acid extractable organics (AEOs, derived from fresh OSPW) as reference material in 0.1% v/v concentrated (≥ 28% ammonia) ammonium hydroxide solution and spiked with internal standards prior to analysis. Method blanks of 0.1% v/v ammonium hydroxide were analyzed before each calibration curve. For calculation of sample concentrations the method employed weighted 1/x linear regression using Graph Pad Prism software followed by data reduction in Microsoft Excel. Example results for the standards and regression are provided in **Table 2**.

Table 2: Example Weighted 1/x Linear Regression

Sample ID	Nominal Conc. (µg/mL)	ISTD Response	Pooled Ion Response	Response Ratio	Measured Conc. (µg/mL)	% Recovery
Curve 1	0.011	11592558	7671790	0.661786	0.012	104
	0.023	11396177	14700024	1.289908	0.023	103
	0.068	10975323	42495888	3.871949	0.071	104
	0.226	10520386	136634462	12.987590	0.239	106
	0.452	10031740	263052210	26.221992	0.483	107
	0.905	9423950	474303977	50.329637	0.927	102
Curve 2	0.011	11930620	7284313	0.610556	0.011	96
	0.023	11718018	13877257	1.184267	0.021	95
	0.068	11502654	40300973	3.503624	0.064	94
	0.226	11260146	132762166	11.790448	0.217	96
	0.452	10690948	254958361	23.848059	0.439	97
	0.905	10143237	476998464	47.026255	0.866	96
				Mean	**%Recovery**	100

3. ***Method Detection Limit**:* The Method Detection Limit (MDL) was set at 99% confidence level above zero. Standard limit of detection (LOD) replicates prepared at 0.002 µg/mL and 0.005 µg/mL both showed a calculated MDL of 0.0003 µg/mL. Results for LOD samples are provided in **Table 3**.

Table 3: LOD Replicate Results

Nominal Conc. (µg/mL)	ISTD Response	Pooled Ion Response	Response Ratio	Measured Conc. (µg/mL)	
0.0023	11226498	1401569	0.124845	0.0018	BQL
0.0023	11734502	1530414	0.130420	0.0019	BQL
0.0023	11100041	1492707	0.134478	0.0020	BQL
0.0023	11622153	1542185	0.132694	0.0020	BQL
0.0023	10837031	1463851	0.135079	0.0020	BQL
0.0023	11932868	1546055	0.129563	0.0019	BQL
0.0023	11631061	1602647	0.137790	0.0021	BQL
0.0023	11230360	1618444	0.144113	0.0022	BQL
0.0023	11469794	1558289	0.135860	0.0020	BQL
0.0023	11024504	1404196	0.127370	0.0019	BQL
			Mean	0.0020	
			Std Dev	0.0001	
			%RSD	5.2	
			MDL	0.0003	
0.0045	10914611	2824065	0.258742	0.0043	BQL
0.0045	10815706	2782113	0.257229	0.0043	BQL
0.0045	11030049	2903949	0.263276	0.0044	BQL
0.0045	11013920	2973305	0.269959	0.0045	BQL
0.0045	11008329	3002527	0.272751	0.0045	BQL
0.0045	10847109	2932962	0.270391	0.0045	BQL
0.0045	10945576	2866581	0.261894	0.0043	BQL
0.0045	11087291	2996880	0.270299	0.0045	BQL
0.0045	11082262	3034047	0.273775	0.0046	BQL
0.0045	11204192	2946500	0.262982	0.0044	BQL
			Mean	0.0044	
			Std Dev	0.0001	
			%RSD	2.5	
			MDL	0.0003	

4. ***Method Accuracy and Robustness****:* Quality control samples, prepared from a secondary stock reference solution, were included in each analytical run in order to assess the accuracy of the analytical procedure. In addition, quality assurance standard solutions were prepared by a secondary analyst and this solution was employed to prepare QA samples at the nominal QC concentration for confirmation of both method robustness and working standard solution 5 month stability. Results for LOD samples are provided in **Table 4**.

Table 4: QC and QA Replicate Results

Nominal Conc. (µg/mL)	ISTD Response	Pooled Ion Response	Response Ratio	Measured Conc. (µg/mL)	% Recovery
0.113	11283190	69973363	6.201558	0.1138	101
0.113	10688513	66881171	6.257294	0.1148	102
0.113	10702885	65751646	6.143357	0.1127	100
0.113	11539904	68447939	5.931413	0.1088	96
0.113	10988492	70207933	6.389224	0.1172	104
0.113	12273675	76321770	6.218331	0.1141	101
0.113	12535975	74862853	5.971841	0.1096	97
			Mean	0.1130	100
			Std Dev	0.0030	
			%RSD	2.6	

5. ***Method Precision and Accuracy in Pre-Concentrated Sample Matrix****:* Spiked field blank matrix samples were prepared at three concentration levels, including the lower limit of quantitation and upper limit of quantitation. These spiked samples were employed to assess the precision and accuracy of the sample pre-concentration procedure.

   A surface water sample collected in the field was randomly selected and employed as matrix. The matrix was made alkaline to pH>10 using concentrated (≥ 28% ammonia) ammonium hydroxide to a final concentration of approximately 0.1% volume per volume. A volume of 35 mL of this matrix was spiked with AEOs reference material and internal standard prior to dry-down under centrifugal evaporation to dryness. The samples were re-suspended in 0.1% v/v ammonium hydroxide solution to a final volume of 1.4 mL by multi-vortex for 20 to 30 min. The nominal final concentrations of naphthenic acids in the x25 pre-concentrated samples was 0.011, 0.034, and 0.905 µg/mL (corresponding to original concentrations of 0.452, 1.357, and 36.19 µg/L in sample matrix). Results for spiked matrix samples are provided in **Tables 5**.

Table 5a: Spiked Field Sample Matrix Results

Original Sample Nominal Spike Conc. (µg/L)	Final Nominal Spike Conc. (µg/mL)	ISTD Response	Pooled Ion Response	Response Ratio	Measured Conc. (µg/mL)		%Spike Recovery
0	0	11495810	249197	0.021677	0.0000	BQL	na
0	0	11232211	390840	0.034796	0.0006	BQL	na
0	0	11583765	233265	0.020137	0.0000	BQL	na
0	0	11155747	459746	0.041212	0.0008	BQL	na
0	0	10928279	300950	0.027539	0.0003	BQL	na
0	0	10311237	389626	0.037787	0.0007	BQL	na
				Mean	0.0004		
				Std Dev	0.0004		
0.452	0.011	11233076	7735780	0.688661	0.0122		108
0.452	0.011	11340382	7899978	0.696624	0.0124		109
0.452	0.011	11280815	7944766	0.704272	0.0125		111
0.452	0.011	11593475	7753317	0.668766	0.0118		105
0.452	0.011	11297837	7889380	0.698309	0.0124		110
0.452	0.011	10630158	7244864	0.681539	0.0121		107
				Mean	**0.012**		108
				Std Dev	**0.0002**		
				%RSD	**2.0**		
1.357	0.034	11144592	22314892	2.002307	0.0364		107
1.357	0.034	11172378	22375258	2.002730	0.0364		107
1.357	0.034	11031239	21673226	1.964714	0.0357		105
1.357	0.034	11052807	21397024	1.935891	0.0352		104
1.357	0.034	10699288	21193001	1.980786	0.0360		106
1.357	0.034	11164551	22327188	1.999829	0.0364		107
				Mean	**0.0360**		**106**
				Std Dev	**0.0005**		
				%RSD	**1.4**		
36.185	0.905	9544436	494833862	51.845269	0.9548		106
36.185	0.905	9957313	508428951	51.060856	0.9403		104
36.185	0.905	9807501	493442834	50.312802	0.9265		102
36.185	0.905	9842008	498582861	50.658654	0.9329		103
36.185	0.905	9683988	490221696	50.621882	0.9322		103
36.185	0.905	10052371	503941583	50.131614	0.9232		102
				Mean	**0.9350**		**103**
				Std Dev	**0.0113**		
				%RSD	**1.2**		

Table 5b: Precision Analysis for Spiked Field Sample Matrix

Original Sample Nominal Spike Conc. (µg/L)	Final Nominal Conc. (µg/mL)	Result #1 (x1)	Duplicate of Result #1 (x2)	Difference x2-x1	Average (x2+x1)/2	Difference/ Average
0.452	0.011	0.0122	0.0118	0.000	0.012	-0.030
0.452	0.011	0.0124	0.0124	0.000	0.012	0.003
0.452	0.011	0.0125	0.0121	0.000	0.012	-0.034
1.357	0.034	0.0364	0.0352	-0.001	0.036	-0.034
1.357	0.034	0.0364	0.0360	0.000	0.036	-0.011
1.357	0.034	0.0357	0.0364	0.001	0.036	0.018
36.185	0.905	0.9548	0.9329	-0.022	0.944	-0.023
36.185	0.905	0.9403	0.9322	-0.008	0.936	-0.009
36.185	0.905	0.9265	0.9232	-0.003	0.925	-0.004
					%RSD	1.3
					% MU	2.6

Table 6: Effect of Acidic Cold Storage and pH Adjustment on O4 Acid Species Measured in AEOs.

Nominal Spiking Conc. for O4 NA Species from AEOs (mg/L)	Measured O4 NA Species Conc. (mg/L) in Original Alkaline Solutions	Post Refrigerated Storage at Acid pH3		Post Refrigerated Storage at Acidic pH3 and Readjusted to Alkaline pH≥10 in Original Container	
		Measured O4 NA Species Conc. (mg/L)	% Recovery from Original Measured O4 Conc.	Measured O4 NA Species Conc. (mg/L)	% Recovery from Original Measured O4 Conc.
0.45	0.46	0.19	41	0.42	93
0.45	0.44	0.23	52	0.44	97
0.91	0.83	0.40	47	0.84	99
0.91	0.87	0.45	53	0.85	99
2.26	2.10	1.14	55	2.01	97
2.26	2.06	1.18	57	2.04	98
4.53	4.31	2.19	51	4.05	94
4.53	4.34	2.01	46	4.14	96
9.05	9.15	4.41	50	8.33	94
9.05	8.65	3.80	43	8.86	100
Mean % Recovery from Original			49		97

Table 7: Comparison of the current method with other published methods

	Current method (Brunswick et *al.*)	Brunswick et *al.*, 2015	Zhang et *al.*, 2014	Woudneh et *al.*, 2013	Ross et *al.*, 2012	Bataineh et *al.* 2006
Standard matrix	Acid extracted organics (AEOs) from fresh oil sands process water Note: method can accommodate any NA standard.	Merichem standard	Merichem standard	Derivatized 1-Pyrenebutyric acid in combination with a calculated "factor" of 0.38 for ratio into Merichem equivalents.	Merichem standard	Merichem and AEOs
Naphthenic acid species application	CnH2n+z O2 and O4	CnH2n+z O2 and O4	CnH2n+z O2 only	CnH2n+z O2 only	Non routine analysis for fingerprinting of NA profiles	CnH2n+z O2 and O4
Instrument mass accuracy	±5ppm high resolution QToF	±5ppm high resolution QToF	±5ppm high resolution Orbitrap	Low resolution MS/MS	High resolution Orbitrap (mass resolution reported as ~30,000 at m/z 250)	<10ppm high resolution QToF
MS mode	ESI -ve	ESI -ve	ESI -ve	ESI +ve (derivatized ions to single MRM at 129 m/z). No confirmation of % derivatization of homologs	ESI -ve	ESI -ve
Sample storage	Samples stored	Samples stored	No sample	Samples stored	Sample	Samples stored

	refrigerated until extraction.	refrigerated until extraction.	storage details.	at -4 °C prior to extraction.	transferred to polypropylene with 10mL methanol rinse and frozen prior to extraction.	refrigerated until extraction.
Sample aliquot and processing	Sample made alkaline to >pH10 prior to aliquot. Spiking with internal standard and pre-concentration by rotary evaporation. Note: minimal processing achieves improved sensitivity without issues relating to extraction solvent, acid pH precipitation, and derivatization selectivity. Acidic pH avoided due to known NA precipitation and losses reported in current paper.	Sample only indicated as adjusted to >pH10 after aliquot.	liquid/liquid, dry down, SPE silica, elute DCM, dry down and reconstitute	pH 5-7, SPE silica, elute methanol, dry-down, redry-down 40°C, derivatize 20min at 60°C plus 15min at 60°C	pH to acid using H2SO4, spiked with internal standard, liquid/liquid in DCM, dry down and reconstitute	pH to 11 with NaOH, centrifuge, pH to <2 with H2SO4 liquid/liquid in acidic ethyl acetate, wash with NaCl, dry over Na2SO4, dry down and reconstitute ethyl acetate, dry down, SPE at pH3, final residue in 60% meOH with 0.1% formic acid. (final x50 preconcentration)
Surrogate standard	Single internal standard, decanoic-d3 acid added pre sample processing.	Single internal standard, decanoic-d3 acid added pre sample processing.	Octanoic-d15 acid (C8 deuterated caprylic saturated fatty acid)	Decanoic-2H19 acid (C10 tritiated capric saturated fatty acid) Hexadecanoic-2H31 acid	Tetradecanoic-^{13}C acid	Tetradecanoic-^{13}C acid

			Hexadecanoic -d2 acid (C16 deuterated palmitic saturated fatty acid)	(C16 tritiated palmitic saturated fatty acid)		
Internal standard	Single internal standard, decanoic-d3 acid added pre sample processing. Note: reduced sample preparation. All published methods use internal standards that are linear and unrelated to the complex NA structures. No amount of internal standards can compensate for all of the potential NA compounds present.	Single internal standard, decanoic-d3 acid	Myristic-d27 acid (C14 deuterated saturated fatty acid) (C16 deuterated palmitic saturated fatty acid)	Atrazine-13C3 (herbicide, aromatic amine with N ring and chlorine) Reported that when suppression IS observed the extract diluted and reanalyzed to bring the recovery values for atrazine-13C3 within 50–150%.	None listed	None listed but model compounds used to assess matrix versus matrix free OSPW.
Spiked samples	Surface water samples 0.452 µg/L AEOs (or 0.00452 mg/L) Accuracy 108 ±3% recovery Precision 2.0%RSD Note: significant improvement of sensitivity using the current method together with good	Surface water samples Merichem 0.05 mg/L (50 µg/L) accuracy 90-110% recovery	Surface water samples Merichem accuracy recovery 40-110% for D15-C8:0 80-110% for D2-C16:0	Surface water samples Merichem 7.72 µg/500mL (0.0154 mg/L) accuracy 40 130% 38.6 µg/500mL (0.0772mg/L) accuracy 47 150%	Not reported, fingerprinting analysis only.	Lab water samples with model compounds accuracy 60-84% recovery and 89-126% when corrected using surrogate. Spiked OSPW at 2.5 ppm (mg/L) accuracy was

	precision and accuracy.					79-108% recovery of model compounds but suppression and 67% accuracy for C8 compound.
Method LOD/LOQ (excludes sample processing pre-concentration)	Method for AEOs LOD 0.0003 µg/mL LOQ 0.011 µg/mL	Method for Merichem NA LOD 0.0004 µg/mL LOQ 0.02 µg/mL	Method for saturated straight chain fatty acid apparent (SSFAs) LOD 0.007 to 0.017 µg/mL LOQ 0.02 to 0.05 µg/mL	Method for 1-Pyrenebutyric acid MDL 0.0025 µg/mL (adjusted for sample size and extract volume-detection limit varies as a result of chromatographic noise in samples)	Method for Merichem NA LOQ 0.0002 µg/mL (no validation data provided)	Method for Merichem NA LOQ 100 µg/mL LOD on-column 24 to 528 pg model compounds
Interferences	The environmental estrogen pollutants estrone, E2, EE2 were demonstrated to show no interference at the retention times for the NA, although the potential for interference in low resolution MS is noted. Similarly, fatty acids and humic acids were	No reported	Not reported	N-acyl urea reaction by product shown to be constant. Humic acids used for relative retention time. Due to surface water contamination with fatty acids, straight chain	Potential for distinguishing bitumen derived NA from biologically derived fatty acids noted by non-routine fingerprinting	None reported

	identified by the method procedure for inclusion or rejection. Note: while two other publications have reported potential for humic and fatty acid interference, they did not provide any evidence. The current work provides chromatographic and high resolution qualitative identification evidence of the potential. It additionally includes review of interference from prevalent estrogen environmental pollutants.			isomer peaks were consistently excluded from the data (peak identity confirmed by spiking study for selected saturated fatty acids).		