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Determination and Characterization of Oxy-Naphthenic Acids in Oilfield Wastewater

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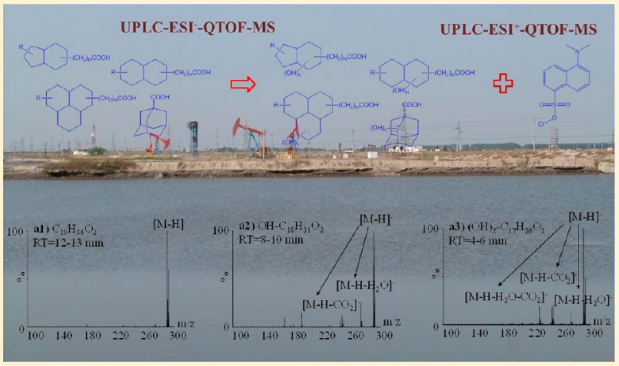
Supporting Information

ABSTRACT: Oxy-naphthenic acids (oxy-NAs) are one of the major components of NA mixtures in wastewaters from petroleum industries. The limited available data indicated that oxy-NAs were considered as a potential marker for the degradation of NAs, and some oxy-NAs exhibited endocrine disrupting activities. However, the lack of information on the structures and occurrences of oxy-NAs in oilfield wastewaters limited the interpretations of the biotransformation pathways of NAs and structure-specific toxicity. A sensitive method for simultaneous determination of oxy-NAs together with NAs was developed by combining MAX extraction column and UPLC-ESI$^-$-QTOF-MS. The 2000-fold SPE preconcentration step was highly specific for acids and the prewash solvent greatly reduced matrix effects in the UPLC-ESI$^-$-QTOF-MS analysis, resulting in an increase in sensitivity down to detection limits in the ng/L range. To provide structural information within each oxy-NA isomer class, a new method was developed by derivatizing oxy-NAs with dansyl chloride by UPLC-ESI$^+$-QTOF-MS. The molecular ion dansyl derivatives from the corresponding oxy-NAs and characteristic fragmentation ions, not detected before derivatization, were observed in the extracts of oilfield wastewater, providing evidence that O_3−NAs and O_4−NAs were mainly composed of OH−NAs and $(OH)_2$−NAs, respectively. Semiquantification of oxy-NAs and NAs in various oilfield wastewaters revealed NAs, O_3−NAs, and O_4−NAs present at concentrations of 187−397, 44−146, and 40−108 μg/L, respectively. Significantly different profiles of NA mixtures were observed in petroleum refinery wastewater and oil sands extraction water, but the profile of oxy-NAs was similar to NAs in different wastewaters suggesting the existence of biotransformation between NAs and oxy-NAs in the environment, and hydroxylation could be one of the major biotransformation pathways of NAs.



INTRODUCTION

Naphthenic acids (NAs) are natural components of petroleum (<3% by weight) and have broad applications such as in wood preservatives, paint additives, etc.[1−5] NAs have been found in wastewaters from petroleum industry,[1−4] and some NAs in oil sands process-affected waters (OSPW) were persistent in the tailing ponds with in situ degradation half-lives of 13 years.[6] Some NAs elicit acute toxicity toward various aquatic organisms including bacteria, phytoplankton, and fish[7−10] and have been identified as xeno-estrogens and antiandrogens, accounting for 65% of the estrogenic receptor agonist potency in North Sea offshore-produced water discharges.[11]

NAs are the primary toxic components in OSPW produced at the oil sands extraction plants in northeastern Alberta, Canada, which must be stored in large ponds until they are successfully remediated due to the no-discharge policy in Canada. Ozone treatment can rapidly diminish NA concentrations,[12] but their estrogenicity was not attenuated after the treatment.[13] This was postulated to be due to generation of numerous byproducts (e.g., oxy-NAs), which might have structures more similar to estrogens compared with the parent NAs.[14] These results highlighted the need for broader identification and determination of NA byproducts or analogues in the complex mixtures.[15]

Oxy-NAs, considered as a potential marker for the degradation of NAs in aquatic samples, were observed during aerobic microbial biodegradation of commercial NAs.[6] Focusing on oxy-NAs will improve the current knowledge of how these NAs interact with the environment.[16] Oxy-NAs with formulas of $C_nH_{2n+Z}O_x$ (x = 3, 4, 5), which "n" means carbon number and "Z" means cylinder number, were identified in OSPW by electrospray ionization with Fourier transform ion cyclotron resonance (FTICR) mass spectrometry due to its inherent ultrahigh resolving power and mass accuracy.[6,17,18] Matrix effects could influence the analytical accuracy of infusion

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MS techniques without chromatographic separation, and only molecular weights were obtained by the high resolution mass spectrometry without any structure information.[19] Identification of oxy-NAs was also tried by capillary HPLC-QTOF-MS, but it is not possible to confirm the chemical nature (e.g., ketone or hydroxyl) by the current analytical methods;[19] and the sample pretreatment method of NAs developed in previous studies (ENV+ and Oasis HLB cartridges)[19,20] was not specific to acids and could not exclude various interferences (e.g., ketones, esters) in the complex NA mixtures. In addition, while the occurrences of NAs in OSPW are widely studied, little is known about the characterization of oxy-NAs and NAs in petroleum refinery wastewater. The lack of information on the structures and occurrences of oxy-NAs in oilfield wastewaters limited the interpretations of the biotransformation pathways of NAs and structure-specific toxicity.

In this study, we developed a solid-phase extraction (SPE) method which could simultaneously extract oxy-NAs together with NAs from oilfield wastewater samples with low matrix effects and improved the method for analyzing the target compounds with high sensitivity using ultrahigh-pressure liquid chromatography (UPLC) coupled to a quadrupole time-of-flight mass spectrometer (QTOF-MS). A derivatization method of hydroxylated NAs with dansyl chloride was further developed to identify the chemical nature of oxy-NAs in the MS/MS mode of the QTOF-MS analysis. Based on the semiquantitative analytical methods, concentrations of oxy-NAs and NAs were compared in petroleum refinery wastewater from Hebei Province and oil sands extraction water from Xinjiang Province, China, revealing the different characterization of the compounds from various oilfield wastewaters.

MATERIALS AND METHODS

Chemicals and Reagents. Fourteen NAs and three oxy-NAs were used as model compounds (Table S1, Supporting Information (SI)): 4-propylcyclohexanecarboxylic acid (*cis*- and *trans*-) ($C_{10}H_{18}O_2$), *trans*-4-pentylcyclohexane carboxylic acid ($C_{12}H_{22}O_2$), 12-oxochenodeoxycholic acid ($C_{24}H_{38}O_5$), cyclohexanecarboxylic acid ($C_7H_{12}O_2$), 1-pyrenebutyric acid ($C_{20}H_{16}O_2$), abietic acid ($C_{20}H_{30}O_2$), 1-adamantaneacetic acid ($C_{12}H_{18}O_2$), 2-hexyldecanoic acid ($C_{16}H_{32}O_2$), 12-hydroxystearic acid ($C_{18}H_{36}O_3$), and dansyl chloride (DNS) were purchased from TCI, Tokyo; 1,2,3,4-tetrahydro-2-naphthoic acid ($C_{11}H_{12}O_2$), dicyclohexylacetic acid ($C_{14}H_{24}O_2$), 5-beta-cholanic acid ($C_{24}H_{40}O_2$), cyclohexane pentanoic acid ($C_{11}H_{20}O_2$), 12-hydroxydodecanoic acid ($C_{12}H_{24}O_3$), and 4-dimethylamiopryidine (DMAP) were purchased from Sigma Aldrich (Oakville, ON, Canada); 1-methyl-1-cyclohexane carboxylic acid ($C_8H_{14}O_2$) was purchased from Alfa Aesar (Ward Hill, MA, USA); *trans*-4-tert-butylcyclohexanecarboxylic acid ($C_{11}H_{20}O_2$) was purchased from Acros Organics (New Jersey, USA); 1-adamantane carboxylic acid ($C_{11}H_{16}O_2$) was purchased from J&K Chemical (Beijing, China). Three commercial mixtures of NAs were purchased from Sigma Aldrich (Oakville, ON, Canada), Acros Organics (New Jersey, USA), and TCI American Organic Chemicals, respectively. Methanol, dichloromethane, acetonitrile, and methyl tert-butyl ether were obtained from Fisher Chemicals (New Jersey, USA), and ethyl acetate was obtained from J.T Baker Chemicals (Phillipsburg, NJ, USA). HPLC grade ammonium acetate and formic acid were purchased from Dima Technology TNC (USA). Hydrochloric acid and ammonia were purchased from Beijing Chemicals. Distilled water was prepared by a Milli-Q Synthesis water purification system (Millipore, Bedford, MA, USA).

Sample Collection. Oilfield wastewater was collected in methanol-rinsed glass bottles from oil production platforms in Hebei Province and oil sands extraction plants in Xinjiang Province, China. The petroleum refinery wastewaters were collected after the anoxic−oxic (AO) processes of the production platforms and represented the last sample point before discharge to the environment. The oil sand wastewater was collected from the sewage outfall of the oilfield plants. Samples were stored at 4 °C and extracted within 4 h in the local laboratory, and surrogate standards were added before extraction.

Sample Preparation. After filtration with a 1.2 μm pore size Whatman GF/C glass fiber pad (Maidstone, UK), 500 mL of distilled water and 200 mL of oilfield wastewater (including petroleum refinery wastewater and oil sands extraction water) were spiked with 0.1−0.2 μg of surrogate standards (12-oxochenodeoxycholic acid and 1-pyrenebutyric acid, 1−2 ng/μL) before extraction with MAX cartridges (Oasis MAX, 6 mL, 150 mg, Waters, USA). The MAX cartridge was conditioned with 6 mL of methanol followed by 6 mL of distilled water. The water samples were passed through the conditioned MAX cartridge at a flow rate of 5−10 mL/min. The cartridge was then further washed with 6 mL of 5% ammonia and dried under a flow of nitrogen gas. The MAX cartridge was first prewashed with 6 mL of methanol which was discarded, and 12 mL of ethyl acetate saturated with hydrochloric acid (2 M HCl:ethyl acetate = 1:10, v/v) was finally used to elute the analytes. The extract was washed with pure water three times, dried under a gentle nitrogen stream, and reconstituted with methanol (0.2 mL for distilled water, 0.1 mL for oilfield wastewater) for UPLC-ESI$^-$-QTOF-MS analysis.

Derivatization. After the analysis of NAs and oxy-NAs, the extracts were further derivatized with DNS to identify the chemical nature of oxy-NAs. The extract was dried under a stream of nitrogen, then dissolved in 2 mL of methyl tert-butyl ether, and washed with pure water three times. The water content of the extracts was removed by passing through a Pasteur pipet filled with anhydrous sodium sulfate. Then the extracts were blown to dryness and dissolved with 1 mL of acetonitrile, followed by the addition of 0.2 mL of pyridine and a mixture (0.2 mL) of 30 mg/mL of dansyl chloride and 30 mg/mL of catalyst (4-dimethylamiopryidine) dissolved in dichloromethane. The mixture was shaken with a vortex device for 1 min and incubated at 65 °C for 60 min. The residuals were blown to dryness and then dissolved with 0.1 mL of acetonitrile for UPLC-ESI$^+$-QTOF-MS analysis.

UPLC-QTOF-MS Analysis. An ACQUITY UPLC system (Waters, Milford, MA) coupled to a Xevo QTOF-MS (G2, Waters) equipped with an electrospray ionization (ESI) source was used in the analysis of naphthenic acids. Instrument control was performed using MassLynx Software (Waters, software version V4.1). All the model compounds were separated on a Waters ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 × 50 mm). The column was maintained at 40 °C, and the injection volume was 3 μL. The flow rate was 0.2 mL min^{-1}. The mobile phases consisting of ultrapure water containing 10 mM ammonium acetate (A) and methanol (B) were used with gradient elution. The initial conditions were 10% B for 2 min, ramped to 60% by 3 min, ramped to 70% by 7 min, then ramped to 100% by 13 min, and finally held for 1 min before returning to initial conditions, which were equilibrated for 5

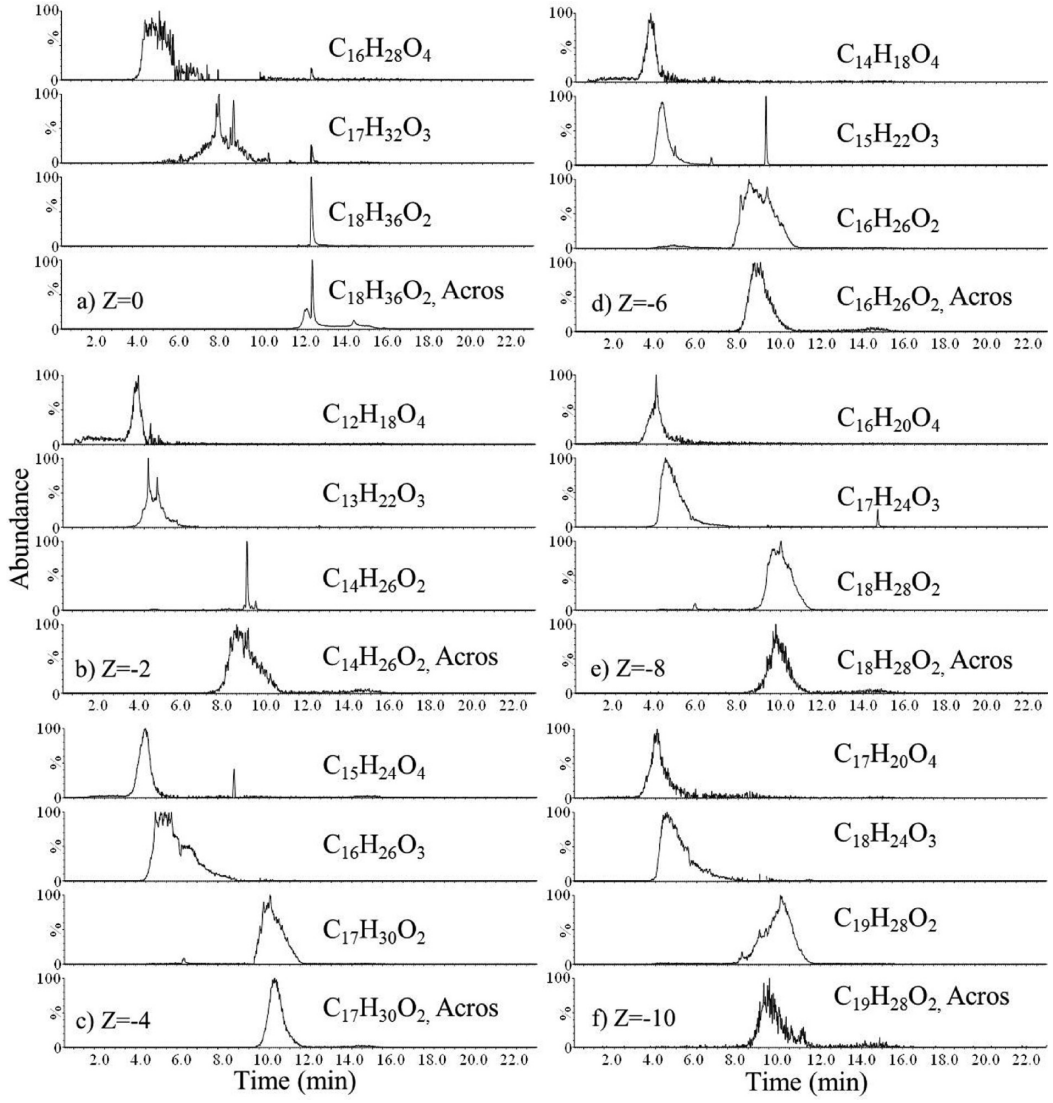

Figure 1. Extracted ion chromatograms for NAs, O_3−NAs, and O_4−NAs in oilfield wastewater and Acros commercial mixtures. Oxy-NAs were not detected in Acros commercial mixtures.

min before injection of the next sample. The UPLC conditions for analyzing the derivatized oxy-NAs were similar with that of underivatized NA mixtures except that ultrapure water containing 0.1% formic acid was used as the mobile phase (A).

The mass spectra of NAs and oxy-NAs were acquired in the negative ion mode. The analysis was performed in full scan mode in the mass range of 80−700 Da with a 1 s scan time. According to our preliminary experiments, the optimized parameters were as follows: source capillary voltage of 2.0 kV, sampling cone voltage of 45 V, extraction cone voltage of 4.0 V, source temperature 100 °C, desolvation temperature 250 °C, cone gas flow rate 50 L/h, and desolvation gas flow rate 600 L/h. The $[M-H]^-$ ion of leucine-enkephalin (200 pg/μL infused at 5 μL/min) was used as a reference lock mass (m/z 554.2615). The QTOF detector was calibrated with a sodium formate solution to achieve mass accuracy lower than 3 ppm by using leucine-enkephalin as the lock mass in negative mode. The accuracy of mass measurement in combination with the retention times in UPLC were used to calculate empirical formulas of NAs and oxy-NAs, and the mass errors of all model compounds were −2.4−2.1 ppm in the present study.

The mass spectra of derivatized oxy-NAs were acquired in the positive ion mode. The analysis was performed in full scan mode in the mass range of 80−1200 Da with a 1 s scan time. The optimized parameters were as follows: source capillary voltage of 3.5 kV, sampling cone voltage of 45 V, extraction cone voltage of 4.0 V, source temperature 110 °C, desolvation temperature 350 °C, cone gas flow rate 50 L/h, and desolvation gas flow rate 600 L/h. The $[M+H]^-$ ion of leucine-enkephalin was used as a reference lock mass (m/z 556.2771).

For MS/MS analysis, the experimental conditions were the same as above except for the collision energy, which was optimized for different compounds (Tables S3 and S4).

Quality Control. Purified water (200 mL) was used for field blanks treated simultaneously with all the wastewater samples. Total concentrations of NAs and oxy-NAs in field blank samples were less than 6 μg/L. Recoveries of the surrogate standards in field blank samples ranged from 65 to 110%.

RESULTS AND DISCUSSION

Method Development. Three model oxy-NAs and fourteen NAs (SI Table S1), representing a range of n and Z,

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Table 1. Recoveries and Method Detection Limits (MDLs, ng/L) for Model Compounds by UPLC-QTOF-MS in Various Aqueous Matrices

	model compounds		recovery (%) ± RSD (%)		MDLs (ng/L)	
			distilled water	oilfield wastewater[b]	distilled water	oilfield wastewater[b]
NAs	$C_{16}H_{32}O_2$	$Z = 0$	84.6 ± 3.9	97.7 ± 9.2	5.7	1.9
	$C_7H_{12}O_2$	$Z = -2$	67.9 ± 2.6	90.6 ± 5.1	14.9	23.5
	$C_8H_{14}O_2$	$Z = -2$	69.0 ± 8.4	67.8 ± 8.6	5.5	8.6
	$C_{10}H_{18}O_2$[a]	$Z = -2$	96.5 ± 7.3	81.6 ± 3.5	4.9	9.9
	$C_{11}H_{20}O_2$	$Z = -2$	98.4 ± 3.9	74.9 ± 3.7	8.2	6.2
	$C_{11}H_{20}O_2$-butyl	$Z = -2$	91.2 ± 1.6	75.4 ± 5.0	4.6	10.8
	$C_{12}H_{22}O_2$	$Z = -2$	102.1 ± 4.3	88.0 ± 8.1	4.6	8.6
	$C_{11}H_{12}O_2$	$Z = -10$	93.3 ± 2.7	60.4 ± 4.7	6.6	14.0
	$C_{14}H_{24}O_2$	$Z = -4$	104.3 ± 1.9	87.1 ± 4.4	3.9	3.0
	$C_{20}H_{16}O_2$	$Z = -14$	77.3 ± 1.6	53.4 ± 2.8	6.1	12.2
	$C_{20}H_{30}O_2$	$Z = -10$	86.2 ± 1.4	113.7 ± 3.6	4.6	5.9
	$C_{24}H_{40}O_2$	$Z = -8$	63.8 ± 4.0	52.2 ± 4.3	6.7	7.3
	$C_{11}H_{16}O_2$	$Z = -6$	97.3 ± 4.3	97.7 ± 4.5	3.1	6.9
	$C_{12}H_{18}O_2$	$Z = -6$	94.4 ± 2.0	80.3 ± 1.5	2.1	5.1
oxy-NAs	$C_{24}H_{38}O_5$	$Z = -10$	90.8 ± 1.9	63.9 ± 2.8	0.7	4.1
	$C_{18}H_{36}O_3$	$Z = 0$	90.2 ± 0.5	84.3 ± 0.5	3.2	2.9
	$C_{12}H_{24}O_3$	$Z = 0$	89.1 ± 3.9	61.4 ± 2.9	2.8	1.3

[a] $C_{10}H_{18}O_2$ (4-propylcyclohexanecarboxylic acid) was a mixture of *cis*- and *trans*-compounds. [b] Petroleum refinery wastewater was used in the matrix spike experiment since most of the model compounds were detected with high concentration in oil sands extraction water. Spiked level was 0.5 μg/L, $n = 3$. Sample volume was 500 mL for distilled water and 200 mL for oilfield wastewater.

were applied to optimize UPLC-QTOF-MS parameters and method recoveries. Since ESI is largely dependent on the solvent conditions, the mobile phase composition and the additive were investigated. In this study, a methanol/water mixture containing NH_4Ac was used since this mobile phase composition produced a good peak shape and a 2−3-fold increase in signal intensity compared to the methanol/water mixture containing acetic acid. Initial runs showed good separation and sensitivities for all the model compounds, and the retention times of oxy-NAs were shorter than those of NAs (SI Figure S1). The instrumental detection limits (IDLs) of oxy-NAs were 0.4−1.0 μg/L, which is similar to those of most NAs (0.3−5.0 μg/L except for $C_{16}H_{32}O_2$ and $C_{10}H_{18}O_2$, SI Table S2). The optimized UPLC-QTOF-MS method was applied to the commercial mixtures of NAs purchased from Acros. Characteristic 'humps' of unresolved acids were found for each NA isomer class, but no hump peak of oxy-NAs was observed in the extraction for the molecular weights with the formula of $C_nH_{2n+Z}O_x$ ($x = 3, 4$) in the commercial mixture (Figure 1 and S2a in SI). While the peak widths for model compounds were about 0.2 min at base, the peak widths for each NA isomer class in the unresolved mixtures were about 2 min in the commercial mixture purchased from Acros.

Sample preparation was further refined to quantitatively extract oxy-NAs together with NAs in environmental samples while minimizing interferences before injections. Solid-phase extraction has been used to preconcentrate NAs for large volume water samples (>100 mL) and exhibits high extraction efficiency compared to liquid−liquid extraction.[19] In this study, a simultaneous extraction of oxy-NAs together with NAs from various water samples was developed. After water samples pass through the MAX cartridge, nonacid interferences in the SPE cartridge due to hydrophobic sorption interactions were removed by a prewash of 6 mL of methanol, in which the model compounds were confirmed to be nondetected (SI Figure S3); and the prewash could contain non-NA components that would be reported by earlier methods as part of total NAs. The acids absorbed in the MAX cartridge by electrostatic interactions were eluted by acidified solvents. For all the elution solvents tested, recoveries of the model compounds increased with the percentage of acids in the solvent and were 69−109% for elution with 12 mL of ethyl acetate saturated with 2 mol/L of hydrochloric acid (2 mol/L of hydrochloric acid:ethyl acetate = 1:10, v/v) (SI Figure S3). The results of the spiking experiments for various water matrices are shown in Table 1, and the absolute recoveries of model compounds ranged from 68 to 104% from distilled water and from 52 to 114% from oilfield wastewaters using the optimized method. ENV+ and Oasis HLB cartridges have been used for extracting NAs from water samples, and the recoveries of NAs were reported to be highly pH-dependent,[19,20] and the fluctuating pH in water samples could cause high variations of the results. In the present study, since the 6 mL of methanol prewash was used to remove the nonacid interferences, the developed extraction based on the MAX cartridge was more specific for acids with low matrix effects, as exemplified by relatively low signal suppression/enhancement for the model oxy-NAs and NAs observed in oilfield wastewater samples (2−20% (except for 36% with $C_{20}H_{16}O_2$)). We further compared MAX and HLB cartridges for extracted ion mass chromatography using the model NAs at the same-fold concentration (2000-fold). As shown in SI Figure S4, the recoveries of model compounds using the HLB cartridge were generally lower than those eluted from the MAX cartridge, especially for 2-hexyldecanoic acid (48.7% for HLB), 1-pyrenebutyric acid (55.5%), and abietic acid (33.2%).

The optimized MAX SPE coupled to the UPLC-QTOF-MS method was applied to the analysis of oilfield wastewaters. The characteristic humps for each NA isomer class were observed in the wastewater samples within the same retention time range as observed for the NAs in the commercial Acros mixtures (SI Figure S2b). Besides NAs, the mass ions of oxy-NAs ($C_nH_{2n+Z}O_x$, $x = 3, 4$) were also found with a short retention time in the UPLC-QTOF-MS chromatogram of oilfield

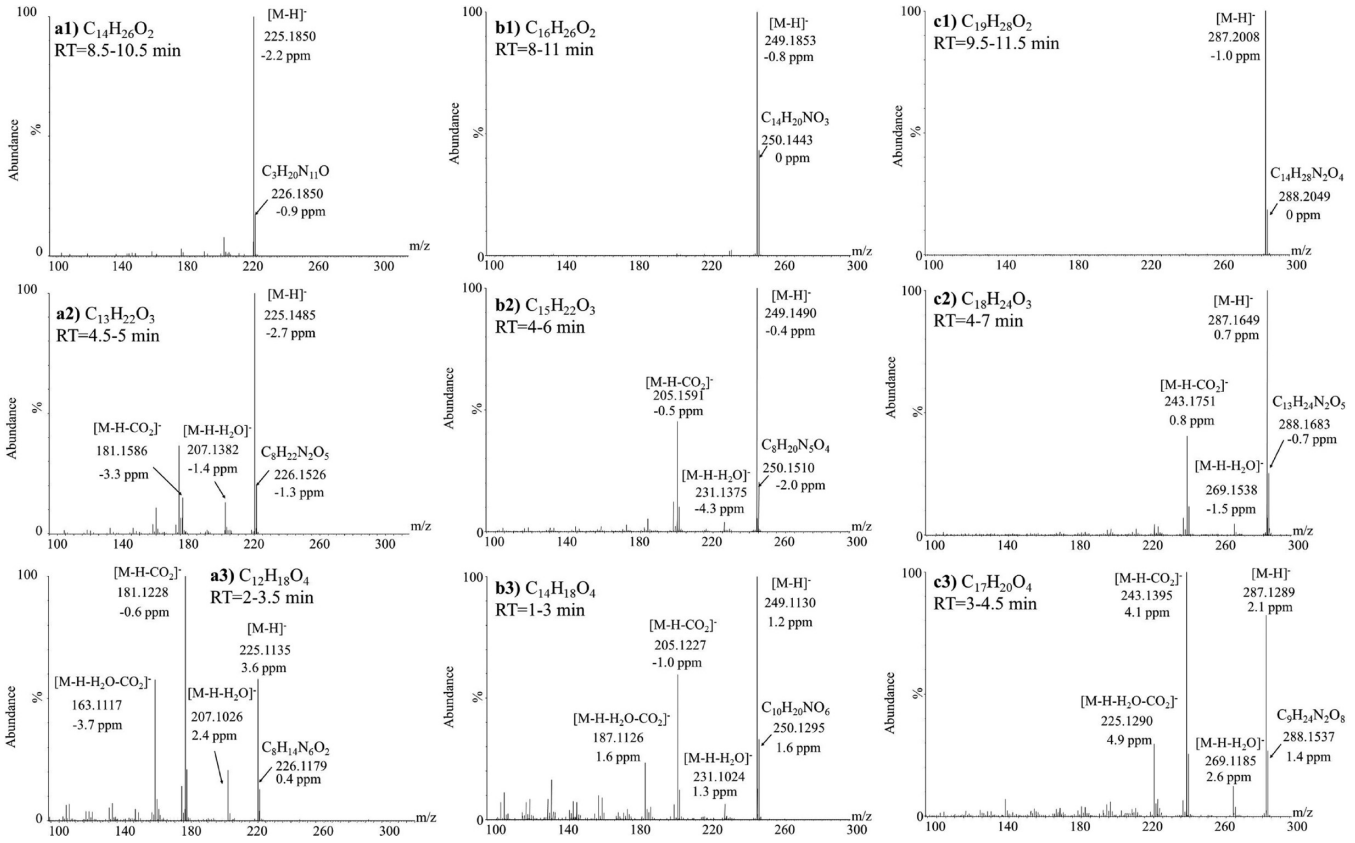

Figure 2. MS/MS spectra of NAs, O_3−NAs, and O_4−NAs with precursor ions of 225 (a1-a3), 249 (b1-b3), and 287 (c1-c3) in extracts of oilfield wastewater.

wastewater (Figure 1). We also found similar humps by extracting the molecular weights of NAs ($C_nH_{2n+Z}O_2$) in the methanol-prewashed fraction, while no model acids were recovered in the fraction, suggesting that nonacid compounds (e.g., esters, ketones) with the same molecular weight were present the samples. It is possible that the prewash contains components that would be reported by earlier methods in the literature as part of total NAs, and this fraction might be critical for assessment of the overall toxicity of the wastewater.

Identifying Classes of Oxy-NAs. The structures of $C_nH_{2n+Z}O_x$ (x = 3, 4) were characterized in the MS/MS mode of the QTOF-MS analysis. MS/MS spectra as shown in SI Figure S5 were obtained for three model compounds of oxy-NAs to investigate their ESI mass spectral fragmentation pathways. For mono-oxidized compounds (12-oxochenodeoxycholic acid and 12-hydroxystearic acid), two diagnostic fragments were observed involving the loss of the CO_2 and H_2O moiety. The neutral loss of 2 Da corresponding to molecular hydrogen has also been observed for fatty acids in previous studies.[21,22] For 12-oxochenodeoxycholic acid, four diagnostic fragments were generated by the loss of more H_2O or both CO_2 and H_2O moieties. Thus neutral loss of CO_2 and H_2O moieties were the characteristic mass fragmentations for oxy-NAs, which is similar with the fragmentation ions of commercial NAs by ESI^--MS/MS analysis reported previously.[23] The MS/MS mode of QTOF-MS was applied to characterize the structures of $C_nH_{2n+Z}O_x$ (x = 3, 4) in the extracts of oilfield wastewater by MAX cartridge as described above. Since the quadrupole yields a lower mass spectral resolution than TOF-MS, identification of NAs and oxy-NAs with the same precursor ion can be accomplished for the compounds with different Z values in the MS/MS mode (e.g., precursor 283: $C_{18}H_{36}O_2$ (Z = 0), $O{-}C_{17}H_{32}O_2$ (Z = −2), $O_2{-}C_{16}H_{28}O_2$ (Z = −4)). The ions giving intense mass signals for NAs with Z values ranging from −10 to 0 detected in oilfield wastewater were selected as precursor ions, and the ramp of collision energy was adjusted to generate abundant mass fragment ions. As shown in Figure 2 and Table S3, only molecular ions ($[M{-}H]^-$) were observed for NAs ($C_nH_{2n+Z}O_2$), but $C_nH_{2n+Z}O_3$ species generated fragment ions of $[M{-}H{-}H_2O]^-$ and $[M{-}H{-}CO_2]^-$, and $C_nH_{2n+Z}O_4$ species generated one more fragment ion ($[M{-}H{-}H_2O{-}CO_2]^-$) compared to $C_nH_{2n+Z}O_3$. With the same precursor, molecular ions of NAs and oxy-NAs were found together with some interference ions, which are compounds containing different elements (e.g., nitrogen) from NAs and oxy-NAs (Figure 2). Since the parent ion interference contains heteroatoms (i.e., nitrogen), it is unlikely that the major product ions result from the interferences. The loss of H_2O and CO_2 moieties in $C_nH_{2n+Z}O_3$ and H_2O, CO_2, and H_2CO_3 moieties in $C_nH_{2n+Z}O_4$ indicated that these compounds were hydroxylated NAs or ketonic NAs not compounds with ester or diacid groups. The retention times of O_4−NAs were found to be the shortest, followed by O_3−NAs and NAs (Figure 1), which is consistent with the elution sequence of the three model compounds of oxy-NAs (SI Figure S1).

To identify the chemical nature of oxy-NAs (hydroxyl or ketone) in the extracts, a derivatization method with DNS was further developed. In the optimized derivatization conditions, DNS is only reactive with hydroxyl groups, which is proved by the fact that DNS did not react with the model NAs and only reacted with one hydroxyl groups of the model oxy-NAs (SI

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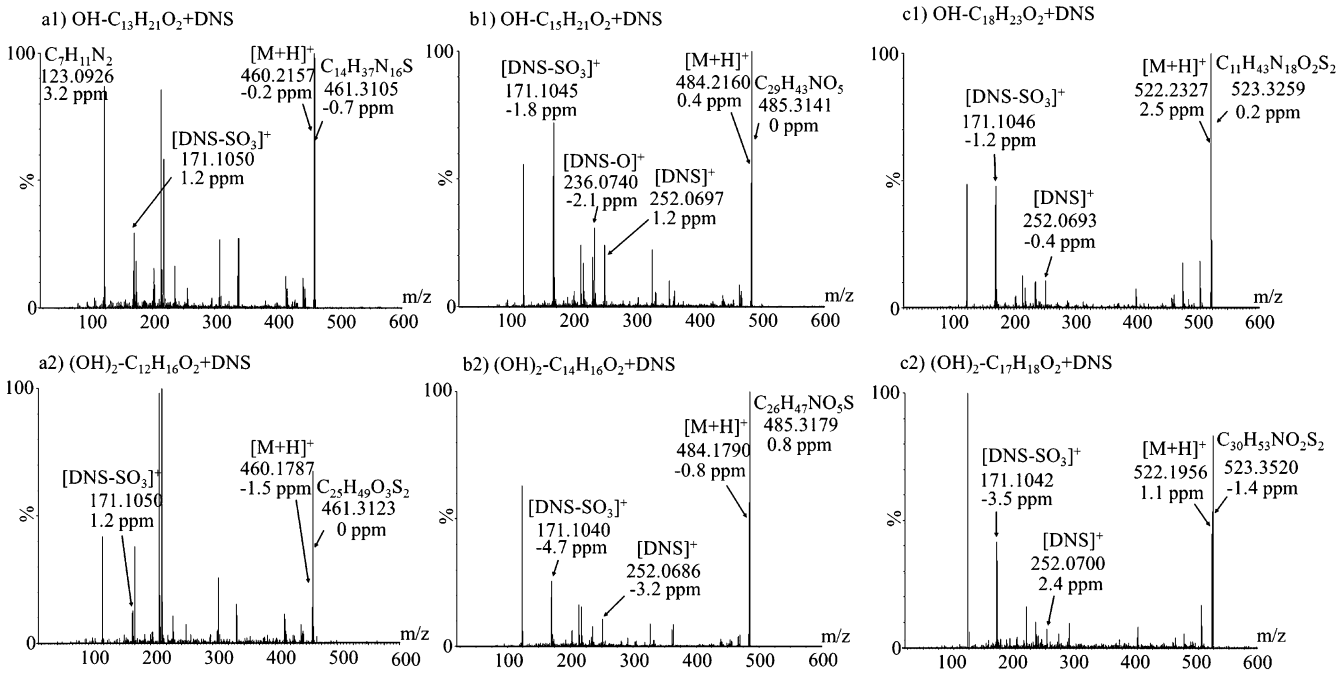

Figure 3. MS/MS spectra of OH−NAs and $(OH)_2$−NAs derivatized with DNS with precursor ions of 460 (a1-a2), 484 (b1-b2), and 523 (c1-c2) in extracts of oilfield wastewater.

Figure S6); and derivatized oxy-NAs were analyzed in positive ion mode, which excluded the interferences of acids in the sample extracts, providing a new method to identify classes of oxy-NAs prior to UPLC-QTOF-MS/MS analysis. The sample extracts were dried by anhydrous sodium sulfate and added with pyridine for improving the derivatization efficiencies of wastewater samples, since the reaction occurred under anhydrous alkaline conditions. As expected, ionization and fragmentation of the isolated dansyl derivatives from oilfield wastewater resulted in protonated molecular ions $[M+H]^+$ and produced the same major product ions at m/z 252.0694 and m/z 171.1048 (Figure 3). The ion m/z 252.0694 is the protonated molecular ions of DNS, and the ion m/z 171.1048 originates from a cleavage of a C−S bond in the dansyl portion of the molecule. Figure 3 and Table S4 shows the MS/MS fragment ions of dansyl derivatives from the corresponding oxy-NAs (Figure 2 and Table S3) in the extracts of oilfield wastewater, and the major dansyl derivative parent and product ions were not observed before derivatization (SI Figure S7). While some interference ions (e.g., $C_{14}H_{37}N_{16}S$) and fragmentation ions existed, the molecular ion dansyl derivatives and characteristic fragmentation ions were observed with relatively high abundance, and these ions were not originated from the interference ions based on the molecular composition obtained by the high resolution mass spectrometry. The estimated concentrations of derivatized hydroxlyated NAs in Table S4 (3.7−7.6 μg/L) were similar with that of oxy-NAs (0.1−7.7 μg/L) before derivatization, suggesting that the O_3−NAs and O_4−NAs detected in the oilfield wastewater were mainly composed of hydroxylated NAs (OH−NAs) and dihydroxylated NAs ($(OH)_2$−NAs), respectively. The hydroxylated structures of oxy-NAs are also consistent with that of the aromatic steroidal hydroxyl acids, which might resemble the sex steroids compounds and exhibited endocrine disrupting activities in NA mixtures.[13,14]

In addition, oxy-NAs were found to easily generate abundant fragment ions possibly due to that oxidized groups with high electron affinities would reduce stability of the naphthenic structures (Figure 2), while an extremely low abundance of fragment ions could be found for NAs, including NA model compounds and commercial mixtures under suitable collision energy; and dansyl derivatives of oxy-NAs generated more fragment ions. For example, fragmentation ions of $[M-C_6H_9COOH+H]^+$, $[M-C_3H_8+H]^+$, and C_6H_9 were observed for compound OH−$C_{16}H_{25}O_2$+DNS from petroleum refinery wastewater, suggesting that the mixtures compound contained one cyclohexane connected with −OH, $-C_3H_7$, and $-C_6H_8COOH$ (Figure 4a); and compound OH−$C_{13}H_{21}O_2$+DNS has the fragmentation ion of C_6H_9 and C_6H_{11}, showing that the compounds contained two cyclohexanes with different substitutions (Figure 4b). Thus, this provided a new method of identifying classes of oxy-NAs.

Characterization of Oxy-NAs. The commercial NAs and oilfield NAs clearly contain many isomers with the same molecular weight and similar retention times, of which structures and response factors will not be the same as those of the model compounds. Currently, concentrations of NA mixtures were generally semiquantified based on integration of the hump peak of each NA isomers assuming that the responses for individual compounds in the hump peaks were similar,[18,19,24−27] since the separation method and standards for all the individual NAs were not available. In the present study, concentrations of NA mixtures in environmental samples were semiquantified by using commercial NA mixtures as the standards, and suitable model NA compounds served as an automatic correction or losses of analytes during extraction or sample preparation as well as for variations in instrument response from injection to injection. Calibration curves based on UPLC peaks of model compounds in the concentration ranges 1−1000 μg/L had r^2 ranging from 0.98 to 0.999 (SI Table S2). The intraday RSDs (n = 5) were below 8.6% and the

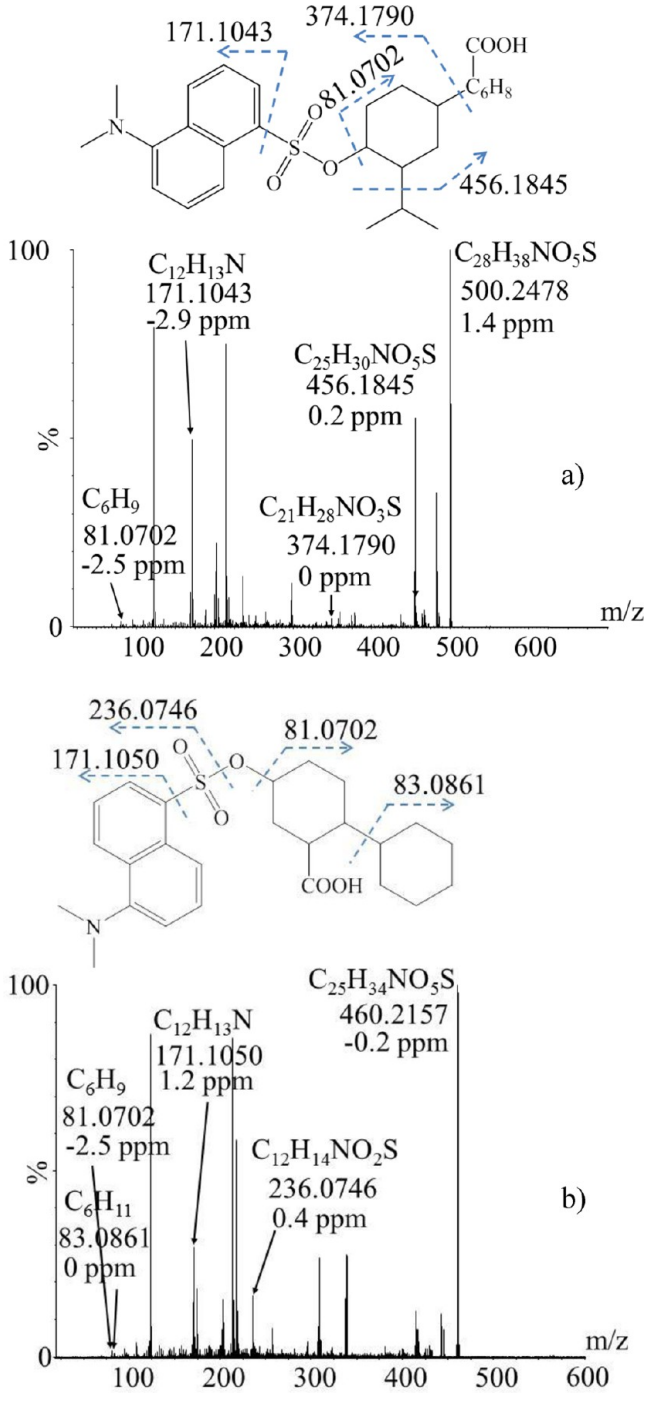

Figure 4. MS/MS spectra and possible structures of OH−NAs derivatized with DNS with precursor ions of 500 (a) and 460 (b) in extracts of oilfield wastewater.

interday RSDs calculated for 20-day periods were lower than 9.8% (SI Table S2), indicating that the UPLC-QTOF-MS method could be used semiquantitatively to analyze NA mixtures.[28]

The commercial mixtures of NAs, available from Acros, Sigma, and TCI, were good standards for obtaining the response factors relating the surrogate to each NA isomer class. Assuming the surrogate (S) was used to quantify each NA isomer class (NA_1, NA_2, ..., NA_n), the response factor (RF_i) for NA_i can be calculated by eq 1

$$RF_i = \frac{C_s/A_s}{C_{NAi}/A_{NAi}} \quad (1)$$

where C_s is the concentration of surrogate, A_s is the peak area of surrogate, C_{NAi} is the concentration of NA_i, and A_{NAi} is the area of the hump peak of NA_i. Concentrations of stock solutions of the commercial mixtures were the total concentrations of all the NA isomer classes (C_{total}), and concentrations of each NA_i isomer class (C_{NAi}) can be calculated by eq 2.

$$C_{NAi} = A_{NAi}/(A_{NA1} + A_{NA2} + ... + A_{NAn}) \times C_{total} \quad (2)$$

Thus eq 1 can be modified to eq 3.

$$RF_i = \frac{C_s/A_s}{C_{total} \times A_{NAi}/(A_{NA1} + A_{NA2} + ... + A_{NAn})/A_{NAi}} = \frac{C_s/A_s}{C_{total}/(A_{NA1} + A_{NA2} + ... + A_{NAn})} \quad (3)$$

Therefore, the response factor for each NA_i relative to the surrogate was related to the total areas of predominant NA isomer classes by using the commercial mixtures as the calibration standards. Although different profiles of the NAs were found for the three commercial mixtures, Acros, TCI, and Aldrich, similar good linearity (r^2 = 0.997−0.999, slope: 1.7−1.8 × 10^4) was obtained for the three mixtures based on the total areas of NAs (n = 5−40, Z = −14−0) and concentrations of stock solutions (1 to 100 mg/L) (SI Figure S8a). Good linearity was also reported between the peak areas of individual NA isomers and total concentrations of NAs obtained by previous studies using micro-HPLC-QTOF-MS[19] and HPLC-MS/MS analysis.[29] However, the linearity for certain NA isomers will be greatly different for commercial mixtures of NAs purchased from Acros, Fluka, and TCI due to the different profiles of the NAs (SI Figure S8b). Oxy-NAs were semiquantified by the response factors of NAs by eq 3, since oxy-NAs were not detected in the commercial mixtures, and the responses of model oxy-NAs were similar to those of model NAs (SI Table S2). 12-Oxochenodeoxycholic acid ($C_{24}H_{38}O_5$) was selected as the surrogate standard for oxy-NAs, and 1-pyrenebutyric acid ($C_{20}H_{16}O_2$) was selected for NAs, similar to a previous study,[30] since these compounds, not present in the oilfield wastewater, were separated from the hump peak of NA mixtures and have recoveries similar to other compounds. The RF values for semiquantification of oxy-NAs and NAs relative to 12-oxochenodeoxycholic acid and 1-pyrenebutyric acid were calculated to be 2.5 ± 0.10 and 3.5 ± 0.14, respectively, using the three commercial mixtures according to eq 3.

The semiquantification method was applied to triplicate oilfield wastewater samples. The total concentrations of NAs, O_3−NAs, and O_4−NAs were estimated to be 187 ± 39, 146 ± 13, and 108 ± 9 μg/L in petroleum refinery wastewater and 397 ± 94, 44 ± 3, and 40 ± 5 μg/L in oil sands extraction waters (n = 3, Figure 5). This suggested relative high concentrations of NAs in wastewater discharged by oil sand extraction processes. While the present sample treatment is different from those reported previously, the total NA concentrations in oil sand extraction water from Xinjiang Province, China (0.4 mg/L) is much lower than those reported in the tailing ponds from the oil sands region of northern Alberta, Canada (20−120 mg/L) but higher than those detected in water samples from the Athabasca river (<0.002−0.08 mg/L).[31] The most intense ions in the petroleum refinery wastewater were for the Z = −6 series (19%), followed by Z =

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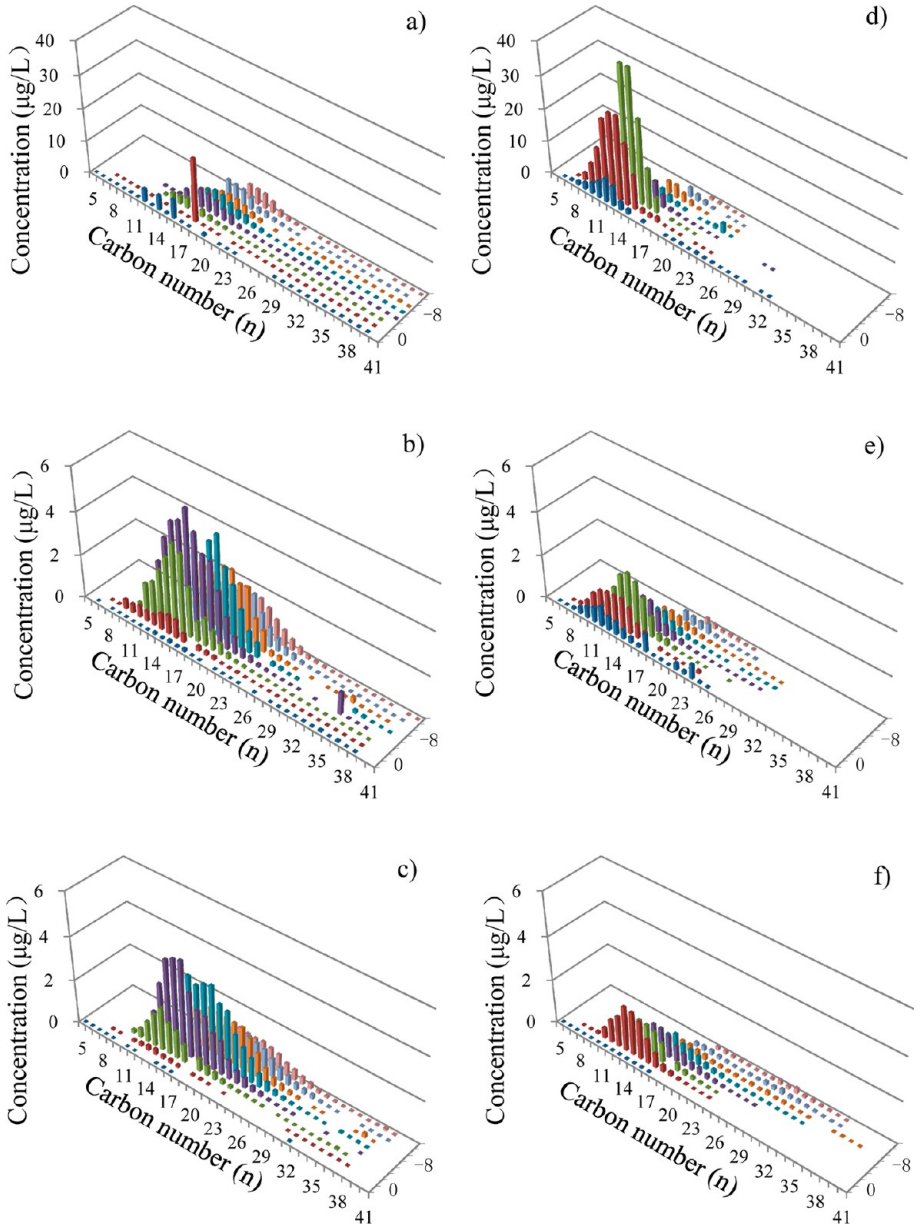

Figure 5. Three-dimensional plots of concentrations (μg/L) versus carbon number (n) and Z-series for NAs (a, d), O_3−NAs (b, e), and O_4−NAs (c, f) in extracts of petroleum refinery wastewater (a−c) and oil sands extraction waters (d−-f) analyzed by UPLC-QTOF-MS. The NAs' n is from 5 to 41, and Z is from 0 to −14.

−8 (16%), Z = −12 (13%), Z = −14 (11%), Z = −10 (11%), Z = −4 (9.0%), and Z = 0 (8.8%), and these were all centered around n = 14−18 for Z = −6 to −4 and n = 16−23 for Z = −14 to −8 (Figure 5(a)). This profile with a high abundance of three-six ring NAs with 16−21 carbons in petroleum refinery wastewater was different from those of oil sand extraction water from Xinjiang Province, China (Figure 5(d)), refined Merichem and Syncrude tailings water samples, in which the most intense compounds were one-three ring NAs with 11−16 carbons.[19]

The detected O_3−NAs and O_4−NAs had the same most intense ion with Z = −6 (O_3−NAs: 27%, O_4−NAs: 28%) and Z = −8 (O_3−NAs: 21%, O_4−NAs: 27%) in petroleum refinery wastewater from Hebei Province and the predominant ions with Z = −4 (O_3−NAs: 28%, O_4−NAs: 24%) and Z = −2 (O_3−NAs: 21%, O_4−NAs: 28%) in oil sands extraction waters from Xinjiang Province. The profile of O_3−NAs and O_4−NAs were very similar with those of NAs in both the petroleum refinery wastewater and oil sands extraction waters (Figure 5). Although oxy-NAs might be present in the original wastewater, the similar profile between oxy-NAs and NAs in different oilfield wastewater possibly suggested the existence of biotransformation between NAs and oxy-NAs in the environment, and oxy-NAs could be used as a potential marker for biodegradation of NAs. While concentrations of NAs in oil sand extraction water were higher than those of petroleum refinery wastewater, concentrations of oxy-NAs in oil sand extraction water were relatively low. This is possibly due to the fact that petroleum refinery wastewaters were generally treated with oxidation processes before discharging to the environment. Thus, the biotransformation relationship between NAs and oxy-NAs together with the identified chemical nature of oxy-NAs indicated that hydroxylation would be one of the major biotransformation pathways of NAs in the environment.

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Overall, a sensitive method was established for simultaneous determination of oxy-NAs together with NAs in environmental waters. Based on the new method developed by derivatizing oxy-NAs with dansyl chloride by UPLC-ESI$^+$-QTOF-MS, O_3−NAs and O_4−NAs were found mainly composed of OH−NAs and $(OH)_2$−NAs, respectively. Occurrences of oxy-NAs and NAs were investigated in various oilfield wastewaters, and characterization of oxy-NAs and NAs in petroleum refinery wastewater and oil sands extraction water suggested the existence of biotransformation between NAs and oxy-NAs, and hydroxylation would be one of the major biotransformation pathways of NAs in the environment.

■ ASSOCIATED CONTENT

Supporting Information

Figures and tables addressing (1) name, structure, and retention time of model compounds, (2) IDLs of model compounds, (3) MS/MS fragment ions and retention of NAs and oxy-NAs before or after derivatization with dansyl chloride in oilfield wastewater, (4) chromatography of model compounds in standard mixtures, (5) chromatography of NAs in commercial mixtures and oilfield wastewater, (6) recovery of model compounds with different eluent solvents, (7) comparisons of recoveries of model compounds in different SPE cartridges, (8) MS/MS spectra of model oxy-NAs before or after derivatization with dansyl chloride, (8) MS/MS spectra of extracts of oilfield wastewater before/after derivatization with dansyl chloride, and (10) calibration curves from three commercial mixtures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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