The Effect of Extraction Methodology on the Recovery and Distribution of Naphthenic Acids of oilfield Produced Water

Saer Samanipour,[*,†,§] Maryam Hooshyari,[‡,§] Jose A. Baz-Lomba,[†] Malcolm J. Reid,[†] Monica Casale,[‡] and Kevin V. Thomas[†,¶]

†*Norwegian Institute for Water Research (NIVA), 0349 Oslo, Norway*

‡*Department of Pharmacy, Genova University, Viale Cembrano 4 – 16147 Genova Italy*

¶*Queensland Alliance for Environmental Health Science (QAEHS), University of Queensland, 20 Cornwall Street, Woolloongabba, QLD, 4102 Australia*

§*These authors contributed equally*

E-mail: saer.samanipour@niva.no

Abstract

Comprehensive chemical characterization of naphthenic acids (NAs) in oilfield produced water is a challenging task due to sample complexity. The recovery of NAs from produced water, and the corresponding distribution of detectable NAs are strongly influenced by sample extraction methodologies. In this study, we evaluated the effect of the extraction method on chemical space (i.e. the total number of chemicals present in a sample), relative recovery, and the distribution of NAs in a produced water sample. Three generic and pre-established extraction methods (i.e. liquid-liquid extraction (Lq), and solid phase extraction using HLB cartridges (HLB), and the combination of ENV+ and C8 (ENV) cartridges) were employed for our evaluation. The ENV method

produced the largest number of detected NAs (134 out of 181) whereas the HLB and Lq methods produced 108 and 91 positive detections, respectively, in the tested produced water sample. For the relative recoveries, the ENV performed better than the other two methods. The uni-variate and multi-variate statistical analysis of our results indicated that the ENV and Lq methods explained most of the variance observed in our data. When looking at the distribution of NAs in our sample the ENV method appeared to provide a more complete picture of the chemical diversity of NAs in that sample. Finally, the results are further discussed.

1 Introduction

Naphthenic acids (NAs) are naturally occurring compounds in petroleum, with a highly variable composition depending on the source of the oil.[1] The concentration of NAs in petroleum can range from non-detectable to 3% by weight.[2] NAs constitute a complex mixture of chemicals, due to the multiple possible chemical structures (i.e. structural isomers) for the same chemical formula. For example for an NA with the formula of $C_{10}H_{18}O_2$, assuming 6 component rings, there are more than 37 isomers. Many of these isomers have a similar structure and thus similar chemical and physical properties. Therefore, a mixture of NAs becomes an extremely challenging matrix to resolve and characterize.[2] As a consequence, the composition of NAs in a complex matrix such as oilfield produced water (PW) is unknown.

Oil production PW is one of the largest streams of industrial treated wastewater in the world.[3] PW is an unresolved complex mixture and consists of a wide variety of chemicals from metals to organic pollutants, including NAs.[3–7] Moreover, multiple studies have reported that the NAs are one of the toxic components of the oilfield PW to a variety of organisms.[2,3,8–10] For example, NAs have been shown to be weak estrogen receptor agonists and androgen receptor antagonists.[3,10–12] Little is, however, known about the chemical composition NAs as well as their environmental fate and behavior. Consequently, an effective

assessment of the risk they pose to the environments receiving oilfield PW difficult. An understanding of the chemical composition of the NAs in the oilfield PW is therefore warranted.

The chemical characterization of NAs in PWs is typically performed on the acidic fraction of the total extract of PW.[2–4,9] Typically, liquid-liquid extraction, solid phase extraction, or a combination of both are used in order to tackle the sample complexity provided by both the NAs and PW.[2,13,14] The extraction method used to produce these extracts are compared/validated either via total extractable material measurement or through the use of a limited number of surrogates as reviewed by Kovalchik et al.[13,15–17]Both mentioned methods have shown to be unable to comprehensively assess the extraction efficiency of one method compared to another.[2,13] For example, in our previous study we demonstrated that the choice of the extraction procedure changes the explored chemical space of the sample.[18] In that study even though two out of three extraction methods showed similar performance for the surrogate chemicals, more detailed chemical characterization revealed substantial differences among tested extraction methods. However, that study was focused on the volatile and semi-volatile fraction of PW. With regards to NAs, to our knowledge there has not been a detailed extraction recovery assessment based on individual NAs.

To answer that question, we employed three generic and well established extraction methods a liquid-liquid extraction method and two solid phase extraction (SPE) approaches to assess the relative recoveries each NA. We evaluated the effect of each extraction method on both the distribution and the relative recoveries of NAs in PW. The extracts were analyzed as such (i.e. no fractionation) via liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS), which was essential to accurate identification of NAs in the PW samples.[19]

2 Methods

2.1 Sample Preparation and the Experimental Setup

A sample of PW (total volume of 5 L) was obtained from an oil platform in the Halten bank off coast of mid-Norway in February 2017.[20] The sample was divided into 9 aliquots, each of 400 mL. These samples were extracted using three generic extraction methods: liquid-liquid extraction (Lq); Hydrophilic-Lipophilic-Balanced cartridges, here referred to as HLB; and the combination of C8 and ENV+ cartridges, which we refer to as ENV. The HLB cartridges were a combination of two monomers, the hydrophilic N-vinylpyrrolidone and the lipophilic divinylbenzene whereas the ENV cartridges consisted of hydroxylated polystyrene-divinyl benzene copolymer. Both of these methods are considered wide range extraction methods for a combination of polar and non-polar chemicals. The details of the extraction procedure for all three methods are provided elsewhere.[18] In short, the Lq method was the dichloromethane (DCM) extract of the acidified PW, repeated three times, with a final volume of 2 mL. A solution of 1N hydrochloric acid was used for acidification of the PW samples. For the solid phase extraction methods (SPE), both cartridges were conditioned with a combination of methanol and water as recommended by the vendors. The preconditioned cartridges then were loaded with 400 mL of PW using a vacuum pump. These, then, were eluted with two times the volume of the cartridges employing a mixture of hexane, DCM, and 2-propanol. This mixture was selected based on the fact that it appeared inert towards the extracted NAs. The final extracts of 2 mL were stored in the freezer until the analysis. This combination of eluents was previously shown to be effective for extraction of analytes with a wide range of chemical and physical properties in complex samples.[18]

Three procedural blanks were generated for each extraction method. For Lq method, these blanks were the extract of the glassware using a mixture of DCM and a 1N solution of HCl. Regarding the SPE methods, the blanks were the extracts of the preconditioned

cartridges with the same solvent mixture used for extraction of the samples.

The final extracts, including the blanks, were spiked with 100 ng of diazepam-D5 as the injection standard for monitoring the instrument performance during the analysis. The detailed list of chemicals and suppliers are provided in the Supporting Information, section S1.

2.2 Instrumental Conditions and Analysis

Seven μL of each extract was injected into a Waters Acquity UPLC system (Waters Milford, MA, USA) equipped with UPLC HSS C18 column (2.1×150 mm, particle size 1.8 mm) (Waters, Milford, MA, USA). More details regarding the chromatographic method is provided in the Supporting Information, section S2.

The UPLC system was coupled to an Xevo G2-S Q-TOF-MS (Waters Milford, MA, US) time of flight high resolution mass spectrometer. The Mass spectrometer was operated with a nominal mass resolution of 35,000 and a sampling frequency of 2.3 Hz. This system was equipped with electron spray ionization source (ESI) operated in negative mode. During each cycle the mass spectrometer acquired a full-scan spectrum between 60 Da and 600 Da employing a collision energy of 6 eV.

All the samples including the blanks and quality control/assurance were analyzed using the above instrumental conditions.

2.3 Quality Control/Assurance (QC)

For the purpose of QC, all the glassware used in this study were baked at 450°C overnight. The samples were divided into sets of three extracts, which were followed by a solvent injection to avoid the carryover from previous injections. Additionally, the signal of the injection

standard (i.e. diazepam-D5) was monitored in order to assess the stability of the instrument during the analyses. We observed less than 20% variability in the signal of the injection standard. This suggested that all the samples showed similar levels of ion suppression for the injection standard. Therefore, we interpreted that the chromatograms were adequate for our data processing workflow without any correction for the ion suppression.

2.4 Data Processing Workflow

All the chromatograms, including the samples and blanks, went through the following data processing steps sequentially. The acquired chromatograms were converted to an open MS format (i.e. netCDF) employing DataBridge provided via MassLynx (Waters, Milford, the US). The converted data were imported into the Matlab[21] environment (Matlab R2015b) for further processing. The imported data were mass calibrated prior to evaluation for the NAs. The details of the mass calibration are reported elsewhere.[22–25] In short, for the mass calibration, the measured mass of the calibrant injected into the source in 20 S intervals were compared to the exact mass of the same compound. The observed mass errors were used to calculate the needed mass shift over the whole chromatogram using a third order polynomial. The estimated mass shift then was applied to the data in order to produce the calibrated chromatograms. The mass calibrated data were used for the identification and signal extraction of NAs.

2.5 Identification and Signal Extraction

Each NA in a PW sample is representative of the mixture of all the structural isomers with the same molecular formula. An increase in the size of the NAs (i.e. the number of carbons) is exponentially correlated with the number of potential structural isomers of NAs.[1,2] Consequently, in the literature, NAs are typically considered as a group of isomers rather than individual compounds.[2] Similarly to the previous reports, we employed the mixture of isomers approach rather than individual compound ones.

In order to identify the NAs in our samples, a list of NAs using their general formula (i.e. $C_nH_{2n-z}O_2$) was generated. In this list the number of carbons (i.e. n) ranged between 8 to 35 while the number of rings ranged from zero to 6 (i.e. z= 0 : -2 : -20). This range was selected based on the previously reported analyzable range of NAs via LC-HRMS.[2] In addition to these conventional NAs, we added several sulfur containing NAs based on the literature reports[26], which enabled us to produce a comprehensive list of detectable NAs in PW. This resulted in a total of 181 NAs to be screened for in the samples (Table S1). For the identification of NAs, we generated the extracted ion chromatogram (XIC) of each NA in the list, employing a mass accuracy of ± 3 mDa. This mass window was selected based on the observed mass resolution measured using the signal of the calibrant. The generated XICs were integrated over the whole chromatogram to produce the signal specific to each NA in the list. This procedure was carried out for all the calibrated chromatograms including the blanks. The signal of each NA after the blank subtraction was used for the comparison of the performance of the three extraction methods employed in this study. During the identification, we performed a noise removal step which consisted of elimination of the NAs that produced a signal smaller than 500 counts and the NAs that were detected only in one out of three replicates. These eliminated NAs were considered non-detects for that method. This approach enabled us to accurately detect the tested NAs and compare the three extraction methods investigated in this study.

2.6 Relative Recovery Calculations

We calculated the relative recovery of each NA using the approach proposed by Samanipour et al.[18] This approach was selected due to the large number of NAs analyzed and the lack of analytical standards for individual NAs in the sample.[1,2,13,16] As an example, for an NA with formula of $C_{10}H_{18}O$ there is need for more than 37 individual analytical standards in order to define the absolute recovery of that NA. Therefore, we used the cumulative signal

approach where the signal of all possible isomers of one NA are summed up to define the produced signal for that NA via an extraction method. Each NA, in this study, resulted in 9 cumulative signal values (i.e. the integrated XIC for each extract 3 methods $\times$ 3 replicates) generated via three different extraction methods. The largest method averaged cumulative signal was considered the total extractable material for that NA. Therefore, the recovery of each NA was calculated based on its signal from each extract divided by the total extractable material for that NA. Using this approach we were able to evaluate the performance of different extraction methods for each NA.

2.7 Statistical Analysis

In order to further evaluate the performance of the three extraction methods, we performed both uni-variate and multi-variate statistical analysis. For the uni-variate test, we employed the non-parametric test Kruskal-Wallis.[27] A $\rho < 0.05$ was selected as the threshold for the rejection of null-hypothesis with 95% confidence interval. With regards to multi-variate test, principal component analysis (PCA) was used in this investigation.[28] Prior to our PCA analysis our data was scaled utilizing Pareto scaling.[29] This approach has shown to be effective in keeping the data structure intact while reducing the importance of large signals. For the PCA, the singular value decomposition (SVD) was employed in order to isolate the statistically relevant components.[30] This algorithm (i.e. SVD) is effective in dealing with datasets where the number of variables is larger than the number of observations. This procedure was previously shown to be effective in separating different extraction methods from each other while isolating the variables that were causing the separation.[25]

3 Results and Discussions

3.1 Detection of NAs

The ENV method with 134 positive detections out of 181 total tested NAs, performed the best, when looking at the number of positively detected NAs in the samples via different extraction methods. The HLB and Lq methods resulted in positive detection of 108 and 81 NAs, respectively (Fig. 1). We further examined the effect of the number of rings and the number of carbons on the detection frequency of NAs produced via each extraction method.

The ENV method systematically produced larger detection frequencies for all 7 z values when compared to the other two methods, Fig. 1. The largest detection frequency for both ENV and HLB was observed for NAs with a z value of -4 (i.e. 2 rings) with positive detection of 23 and 19 NAs, respectively. On the other hand, the Lq method showed to be unaffected by the number of rings in terms of the detection frequency resulting in an average of 11 NAs detected for all seven cases. The non-parametric Kruskal-Wallis test [27] results (i.e. $\rho < 0.05$) indicated that the differences observed in the detection frequencies versus the ring number were statistically significant. Further examination of these results suggested that the two SPE methods performed in a similar way whereas the Lq method appeared to be different from those two. Overall, all three methods covered a range of NAs from aliphatic chains (i.e. z=0) up to 6 rings (i.e. z=-12) while all three methods were unable to detect NAs with larger number of rings, thus z values between -14 and -20. Furthermore, none of the methods detected the sulfur containing NAs, which may suggest their absence and/or lower than instrumental limit of detection concentrations in the analyzed sample.

For the effect of the number of carbons on the detection frequency of NAs, the ENV method covered all n values ranging from 8 to 35, Fig. 1. The HLB method produced zero positive detections for n values of 8 and 25 while the Lq method was limited in an n value

range of 9-29. The ENV method resulted in the largest detection frequency of NAs for 20 out of 27 n values across the tested range. For cases where Lq method was the best performing approach with n values of 11, 12, 15, and 17, the mentioned NAs appeared to be aliphatic NAs. Moreover, they all were removed during the noise removal (i.e. their signal was smaller than 500 counts). For the remaining three cases with n values of 28, 29, and 34, HLB method performed better than ENV extraction method. For these cases, the missing NAs were: a one ring NA for the n value of 28, a two ring NA for the n value of 29, and finally, a five ring NA for the n of 34. Also for these cases, the noise removal step caused the elimination of these NAs from the detection list of ENV. Based on the fact that all these discrepancy cases where generated during the noise removal step, we interpreted that the sample complexity/matrix effect was the main cause of these observations. Finally, we preformed the non-parametric Kruskal-Wallis test to evaluate the trend observed in the detection frequency versus the n values. The $\rho < 0.05$ of this test suggested a statistically significant difference between the methods. Further investigation in the outcome of this statistical test showed the similarity of the SPE methods when compared to the Lq method.

Overall, the ENV method appeared to perform the best by extracting the largest number of NAs across all the z values and n values. Additionally, this method showed a consistent performance when looking at the z and n values compared to the other two methods (i.e. HLB and Lq).

3.2 Extraction Recoveries

The ENV method resulted in an average relative recovery of 49.6 % across all the tested NAs whereas HLB and Lq produced average relative recoveries of 44.7% and 42.1%, respectively. We also evaluated the recoveries of the NAs for each method based on the number of carbons and the number of rings.

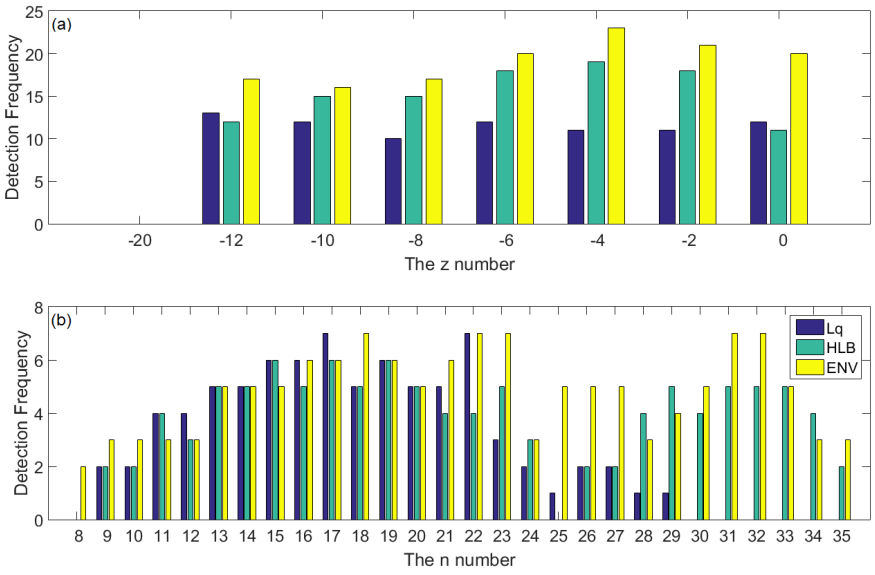


Figure 1: showing the detection frequency of NAs versus (a) the z value (i.e. the number of aliphatic rings) and (b) the n number (i.e. the number of carbons).

For the aliphatic NAs (i.e. z=0), the Lq method performed better than the other two methods resulting in 100% relative recoveries for 12 out of 27 NAs, Fig. 2. The other two methods (i.e. HLB and ENV) produced a larger level of variability in the relative extraction recoveries across the analyzed NAs, ranging from non-detect for n=12 and 17 to 100% for n larger than 29. However, the ENV method was the only method that extracted the largest number of NAs compared to the other two methods. Additionally, this method showed to be successful in capturing the smallest and the largest NAs in this group. For small NAs with n ranging from 8 to 10 both HLB and Lq resulted in zero recoveries, which was attributed to the low affinity of these NAs for HLB resin and DCM. However, further structural elucidation is necessary to confirm this hypothesis. On the other hand, for NAs having n values larger than 22, the two SPE methods were able to isolate those NAs while the Lq failed in this task. This trend was associated with the lower solubility of larger NAs in DCM. However, in this case also further structural elucidation is necessary to confirm this hypothesis. For NAs with z values between -2 and -10 (i.e. 1 to 5 rings), the ENV method systematically

produced higher relative recoveries compared to the other two methods, Fig. 2, S1, S2, S3, and S4. Among these cases, for z values of -2, -4, and -6 both ENV and Lq preformed better than HLB in extracting smaller NAs. However, for NAs with n values larger than 22 the two SPE methods perform better both in terms of number of detected NAs and the relative recovery of individual NAs. Finally, for NAs with a z value of -12, thus 6 rings, the Lq performs better than the other two methods producing 100% relative extraction recoveries for 13 out of 17 NAs, Fig. 2. This method however was unable to isolate the NAs with number of carbons larger than 31. Overall, none of the methods were able to extract all the tested NAs. However, the ENV method appeared to perform better than the other two methods when looking at the relative recoveries and the number of detected of NAs.

The PCA of the scaled and mean centered relative recoveries was able to clearly distinguish the three extraction methods from each other, Fig. S5. The first two PCs successfully described ∼62% of variability in our dataset. When looking at the loading plot, also in this case three different clusters of variables were observed. These clusters indicated the variables that were causing the separation of the methods from each other. When looking at the loadings plot, we focused on the variables that had a weight value of larger than 30%, which reduced the number of relevant variables to 79 rather than 172. From those 79, 41 were associated with the NAs where the ENV method performed better than the other two whereas 34 belonged to the method HLB. For the Lq method, there were only four statistically relevant variables (i.e. NAs with masses of 326.3218, 338.3376, 348.3534, and 426.4482), which indicated the worse performance of this method compared to the other two extraction approaches. The results of PCA suggested that the ENV method performed the best when compared to the other two methods. This was in agreement with our assessment of the recoveries based on individual NAs explained in details above.

The ENV method also produced the largest total signal of NAs compared to the other two

methods, Fig. 3. We also evaluated the blank subtracted and injection standard normalized total signal of all detected NAs using each extraction method in order to evaluate the overall recovery of each method. Based on the absolute signal, the Lq and HLB methods extracted $\sim$ 80% of total extractable material, assuming the ENV method extracting 100%. The outcome of the total signal was comparable to the previous reports for Lq and SPE methods.[13]

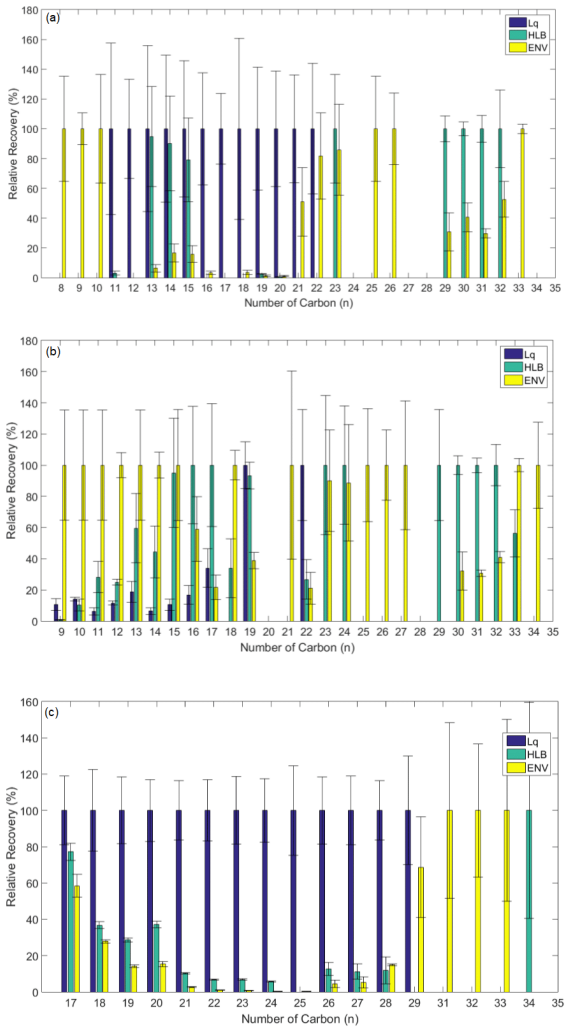


Figure 2: showing the relative recoveries of NAs versus the n value for (a) the z=0 (i.e. no ring), (b) the z=-4 (i.e. two rings), and (c) the z=-12 (i.e. six rings).

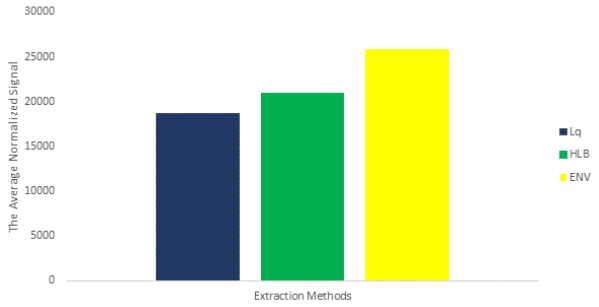


Figure 3: showing the blank subtracted and injection standard normalized total signal of all detected NAs using each extraction method.

3.3 NA Distribution in Produced Water

We further evaluated the effect of the extraction method on the overall distribution of tested NAs in the analyzed produced water. The noise removed extracted signal of the NAs for each extraction method was utilized for these evaluations.

When looking at the distribution of NAs in the analyzed produced water via SPE methods, the NAs with z values ranging from -4 to -12 appeared to be the most abundant ones. On the other hand, via Lq method the NAs with z value of -12 were the most abundant group while for other z values, this method produced relatively similar abundances, Fig. 4. All three extraction methods produced the smallest relative abundances for the aliphatic NAs. All the methods, for z values between -2 and -10, resulted in higher relative abundances for n values between 13 and 18, which was in agreement with previous reports regarding the distribution of NAs in produced water or similar matrices.[9,31,32] For a z value of -12, the most abundant NAs were those with n values between 16 and 20 for all three tested extraction methods.

The ENV method appeared to cover the largest NA chemical space compared to the other two methods, where the chemical space is defined as the total number of tested NAs, Fig.

4. The performance of the other SPE method, thus HLB, appeared to be more similar to the ENV rather than the Lq method. For Lq method the distribution of the NAs appeared to be affected mainly by their solubility in DCM. As a consequence, the boundaries of the explored chemical space via Lq method were dominated by the molecular size. In other words, the non-extracted NAs via the Lq were either too small or too large, therefore non soluble in DCM. For the two SPE methods, the explored chemical space appeared to be less concise when compared to the Lq method. We interpret that this observed trend was mainly caused by the interactions of individual compounds with the resin, sample complexity, and the matrix effects. We observed that the HLB method, in particular, showed less affinity for the smaller NAs (i.e. n value of 8) compared to the ENV method. To further test this, we explored our chromatograms for NAs with z value of 0 and n values of 7 and 6, which were not included in our initial list of NAs. None of the three tested extraction methods detected the NA with z=0 and n=7. However, for NA with z=0 and n=6, the ENV method was the only one producing a positive detection for that particular NA, Fig. S6. This further indicated the difficulties that the Lq and HLB methods have in extracting smaller NAs.

The ENV method was able to explore the largest chemical space of NAs compared to HLB and Lq methods. Additionally, this method was the only method that produced a positive signal for hexanoic acid, which is considered the marker for the presence of NAs in produced water according to Norwegian Oil and Gas.[33] Even though this method (i.e. ENV) did not produce the highest recoveries for all the tested NAs, it resulted in 100% relative recoveries for the largest number of NAs explored in this study. Our results in overall suggested that among the tested extraction procedures the ENV method is the most effective one for analysis of NAs in produced water. However, testing the other extraction procedures is necessary and will be subject of our future study.

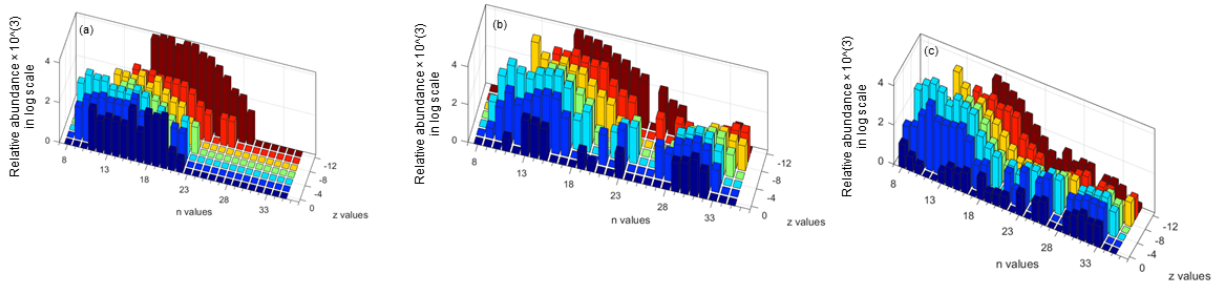


Figure 4: depicting the relative abundance of the analyzed NAs using (a) Lq, (b) HLB, and (c) ENV extraction methods. The relative abundances (i.e. "Z" axis) are multiplied to 1000 and are shown in log scale for ease of visual comparison among the three extraction methods.

4 Environmental Implications

Our results suggested that the choice of sample preparation approach may have a substantial effect on the explored chemical space of NAs. In other words, using different extraction methods may produce different toxicity profiles for the same sample. This is highly relevant for a complex mixture such as produced water and NAs with a wide variety of toxicity profiles. Consequently the risk assessment of such mixtures without a comprehensive understanding of the explored chemical space becomes impossible. Our results indicated that, when dealing with such complex mixture, the conventional methods may fall short and thus the use of more comprehensive methods are warranted. Additionally, our results indicated that when assessing the extraction recoveries, this should be done at higher detailed levels rather than the total NAs or using only a few surrogates. For example for an NA with n=24 and z=-2, this NA was detected using only one extraction method ENV, which implied that using the other two methods would not have produced an accurate toxicity profile. This is extremely important when performing the risk assessment of such complex mixtures such as NAs and PW.

5 Acknowledgments

We are grateful to the Research Council of Norway for the financial support of this study through project RESOLVE (grant number 243720). The authors are also thankful to equinor (former StatOil) for providing us the produced water samples.

6 Supporting Information

The Supporting Information including details regarding the chemicals, the list of tested NAs, and figures related to the relative recoveries and statistical analysis is available free of charge on the Publication website.

7 Author Information

Corresponding Author:

Saer Samanipour

Email: saer.samanipour@niva.no

Phone: +47 98 222 087 Address: Norwegian Institute for Water Research (NIVA)

0349 Oslo, Norway

References

(1) Tomczyk, N.; Winans, R.; Shinn, J.; Robinson, R. On the nature and origin of acidic species in petroleum. 1. Detailed acid type distribution in a California crude oil. *Energy & Fuels* **2001**, *15*, 1498–1504.

(2) Clemente, J. S.; Fedorak, P. M. A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. *Chemosphere* **2005**, *60*, 585–600.

(3) Thomas, K. V.; Langford, K.; Petersen, K.; Smith, A. J.; Tollefsen, K. E. Effect-directed identification of naphthenic acids as important in vitro xeno-estrogens and anti-androgens in North Sea offshore produced water discharges. *Environ. Sci. Technol.* **2009**, *43*, 8066–8071.

(4) Rowland, S. J.; Scarlett, A. G.; Jones, D.; West, C. E.; Frank, R. A. Diamonds in the rough: identification of individual naphthenic acids in oil sands process water. *Environmen. Sci. Technol.* **2011**, *45*, 3154–3159.

(5) McCormack, P.; Jones, P.; Hetheridge, M.; Rowland, S. Analysis of oilfield produced waters and production chemicals by electrospray ionisation multi-stage mass spectrometry (ESI-MSn). *Wat. Res.* **2001**, *35*, 3567–3578.

(6) Allard, A.-S.; Hynning, P.-Å.; Remberger, M.; Neilson, A. H. Environmental hazard assessment: A laboratory approach to reality? *Toxicol. Environ. Chem.* **2000**, *78*, 127–197.

(7) Jones, D.; Scarlett, A.; West, C.; Frank, R.; Gieleciak, R.; Hager, D.; Pureveen, J.; Tegelaar, E.; Rowland, S. Elemental and spectroscopic characterization of fractions of an acidic extract of oil sands process water. *Chemosphere* **2013**, *93*, 1655–1664.

(8) Gosselin, P.; Hrudey, S. E.; Naeth, M. A.; Plourde, A.; Therrien, R.; Van Der Kraak, G.; Xu, Z. Environmental and health impacts of Canada's oil sands industry. *Royal Society of Canada Ottawa, Ontario, Canada* **2010**,

(9) Swigert, J. P.; Lee, C.; Wong, D. C.; White, R.; Scarlett, A. G.; West, C. E.; Rowland, S. J. Aquatic hazard assessment of a commercial sample of naphthenic acids. *Chemosphere* **2015**, *124*, 1–9.

(10) Scarlett, A.; Reinardy, H.; Henry, T.; West, C.; Frank, R.; Hewitt, L.; Rowland, S. Acute toxicity of aromatic and non-aromatic fractions of naphthenic acids extracted

from oil sands process-affected water to larval zebrafish. *Chemosphere* **2013**, *93*, 415–420.

(11) Scarlett, A. G.; West, C. E.; Jones, D.; Galloway, T. S.; Rowland, S. J. Predicted toxicity of naphthenic acids present in oil sands process-affected waters to a range of environmental and human endpoints. *Sci. Tot. Environ.* **2012**, *425*, 119–127.

(12) Jones, D.; Scarlett, A. G.; West, C. E.; Rowland, S. J. Toxicity of individual naphthenic acids to Vibrio fischeri. *Environ. Sci. Technol.* **2011**, *45*, 9776–9782.

(13) Kovalchik, K. A.; MacLennan, M. S.; Peru, K. M.; Headley, J. V.; Chen, D. D. Standard method design considerations for semi-quantification of total naphthenic acids in oil sands process affected water by mass spectrometry: A review. *Front. Chem. Sci. Eng.* **2017**, *11*, 497–507.

(14) Barros, E. V.; Dias, H. P.; Pinto, F. E.; Gomes, A. O.; Moura, R. R.; Neto, A. C.; Freitas, J. C.; Aquije, G. M.; Vaz, B. G.; Romaao, W. Characterization of Naphthenic Acids in Thermally Degraded Petroleum by ESI (-)-FT-ICR MS and 1H NMR after Solid-Phase Extraction and Liquid/Liquid Extraction. *Energy & Fuels* **2018**, *32*, 2878–2888.

(15) Huang, R.; McPhedran, K. N.; Sun, N.; Chelme-Ayala, P.; El-Din, M. G. Investigation of the impact of organic solvent type and solution pH on the extraction efficiency of naphthenic acids from oil sands process-affected water. *Chemosphere* **2016**, *146*, 472–477.

(16) Huang, R.; Chen, Y.; Meshref, M. N.; Chelme-Ayala, P.; Dong, S.; Ibrahim, M. D.; Wang, C.; Klamerth, N.; Hughes, S. A.; Headley, J. V. Monitoring of classical, oxidized, and heteroatomic naphthenic acids species in oil sands process water and groundwater from the active oil sands operation area. *Sci. Tot. Environ.* **2018**, *645*, 277–285.

(17) Mohammed, M. A.; Sorbie, K. S. Naphthenic acid extraction and characterization from naphthenate field deposits and crude oils using ESMS and APCI-MS. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2009**, *349*, 1–18.

(18) Samanipour, S.; Baz-Lomba, J. A.; Reid, M. J.; Ciceri, E.; Rowland, S.; Nilsson, P.; Thomas, K. V. Assessing sample extraction efficiencies for the analysis of complex unresolved mixtures of organic pollutants: A comprehensive non-target approach. *Anal. Chimica Acta* **2018**, *1025*, 92–98.

(19) Martin, J. W.; Han, X.; Peru, K. M.; Headley, J. V. Comparison of high-and low-resolution electrospray ionization mass spectrometry for the analysis of naphthenic acid mixtures in oil sands process water. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 1919–1924.

(20) Statoil, N. Heidrun oil platform. https://www.statoil.com/en/what-we-do/norwegian-continental-shelf-platforms/heidrun.html, 2017.

(21) MATLAB version 9.1 Natick, Massachusetts: The MathWorks Inc., **2016**.

(22) Samanipour, S.; Reid, M. J.; Bæk, K.; Thomas, K. V. Combining a Deconvolution and a Universal Library Search Algorithm for the Nontarget Analysis of Data-Independent Acquisition Mode Liquid Chromatography- High-Resolution Mass Spectrometry Results. *Environ. Sci Technol.* **2018**, *52*, 4694–4701.

(23) Samanipour, S.; Baz-Lomba, J. A.; Alygizakis, N. A.; Reid, M. J.; Thomaidis, N. S.; Thomas, K. V. Two stage algorithm vs commonly used approaches for the suspect screening of complex environmental samples analyzed via liquid chromatography high resolution time of flight mass spectroscopy: A test study. *J. Chromatogr. A* **2017**, *1501 (2017)*, 68–78.

(24) Samanipour, S.; Langford, K.; Reid, M. J.; Thomas, K. V. A two stage algorithm for target and suspect analysis of produced water via gas chromatography coupled with

high resolution time of flight mass spectrometry. *J. Chromatogr. A* **2016**, *1463*, 153–161.

(25) Samanipour, S.; Reid, M. J.; Thomas, K. V. Statistical variable selection: An alternative prioritization strategy during the non-target analysis of LC-HR-MS data. *Anal. Chem.* **2017**, *89 (10)*, 5585–5591.

(26) Qian, K.; Robbins, W. K.; Hughey, C. A.; Cooper, H. J.; Rodgers, R. P.; Marshall, A. G. Resolution and identification of elemental compositions for more than 3000 crude acids in heavy petroleum by negative-ion microelectrospray high-field Fourier transform ion cyclotron resonance mass spectrometry. *Energy & Fuels* **2001**, *15*, 1505–1511.

(27) Breslow, N. A generalized Kruskal-Wallis test for comparing K samples subject to unequal patterns of censorship. *Biometrika* **1970**, *57*, 579–594.

(28) Brereton, R. G. *Applied chemometrics for scientists*; John Wiley & Sons, 2007.

(29) van den Berg, R. A.; Hoefsloot, H. C.; Westerhuis, J. A.; Smilde, A. K.; van der Werf, M. J. Centering, scaling, and transformations: improving the biological information content of metabolomics data. *BMC genomics* **2006**, *7*, 142.

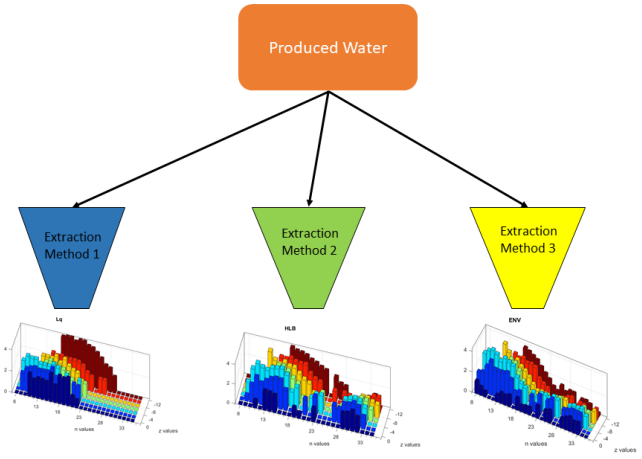
(30) Golub, G. H.; Reinsch, C. Singular value decomposition and least squares solutions. *Numerische mathematik* **1970**, *14*, 403–420.

(31) Holowenko, F. M.; MacKinnon, M. D.; Fedorak, P. M. Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. *Water Res.* **2002**, *36*, 2843–2855.

(32) Rogers, V. V.; Liber, K.; MacKinnon, M. D. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. *Chemosphere* **2002**, *48*, 519–527.

(33) Norog, Norwegian Oil and Gas recommended guidelines for sampling and analysis of produced water, translated version. 2003.

8 TOC


Produced Water
Extraction Method 1
Extraction Method 2
Extraction Method 3
Lq
HLB
ENV

TOC Art