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Assessing sample extraction efficiencies for the analysis of complex unresolved mixtures of organic pollutants: A comprehensive non-target approach

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Abstract

The comprehensive extraction recovery assessment of organic analytes from complex samples such as oil field produced water (PW) is a challenging task. A targeted approach is usually used for recovery and determination of compounds in these types of analysis. Here we suggest a more comprehensive and less biased approach for the extraction recovery assessment of complex samples. This method combines conventional

7 targeted analysis with a non-targeted approach to evaluate the extraction recovery
8 of complex mixtures. Three generic extraction methods: liquid-liquid extraction (Lq),
9 and solid phase extraction using HLB cartridges (HLB), and the combination of ENV+
10 and C8 (ENV) cartridges, were selected for evaluation. PW was divided into three
11 parts: non-spiked, spiked level 1, and spiked level 2 for analysis. The spiked samples
12 were used for targeted evaluation of extraction recoveries of 65 added target analytes
13 comprising alkanes, phenols, and polycyclic aromatic hydrocarbons, producing abso-
14 lute recoveries. The non-spiked sample was used for the non-targeted approach, which
15 used a combination of the F-ratio method and apex detection algorithm. Targeted
16 analysis showed that the use of ENV cartridges and the Lq method performed better
17 than use of HLB cartridges, producing absolute recoveries of 53.1 ± 15.2 for ENV and
18 46.8 ± 13.2 for Lq versus 19.7 ± 6.7 for HLB. These two methods appeared to produce
19 statistically similar results for recoveries of analytes, whereas they were both differ-
20 ent from the produced recoveries via the HLB method. The non-targeted approach
21 captured unique features that were specific to each extraction method. This approach
22 generated 26 unique features (mass spectral ions), which were significantly different
23 between samples and were relevant in differentiating each extract from each method.
24 Using a combination of these targeted and non-targeted methods we evaluated the
25 extraction recovery of the three extraction methods for analysis of PW.

26 Introduction

27 Comprehensive extraction recovery assessments of complex mixtures of organic analytes are
28 extremely difficult. This is caused mainly by the complexity of the sample and lack of
29 knowledge regarding the chemical constituents of the sample. Consequently, a generic/wide
30 range extraction method is typically employed for the analysis of complex mixtures such as
31 produced water (PW; reviewed by Oetjen¹). Often, different extraction methods are tested
32 on a small number of potential target analytes (compared to the number of chemicals in

33 a complex mixture) in order to define an optimized extraction method.^{1,2} This approach
34 assumes that the fate and behavior of each chemical constituent in the complex mixture can
35 be linearly extrapolated by the behavior of the target analytes and that there are no inter-
36 actions between different chemicals. Such an approach is perhaps questionable, for example,
37 when an examination of PW for naphthenic acids is made, since these compounds also be-
38 have as surfactants. Another method used for the extraction recovery assessment of complex
39 mixtures is the gravimetric approach.^{1,3} This method focuses on the total non-volatile ex-
40 tractable material. In this case if the amount of a certain chemical in the sample is smaller
41 than the experimental error (e.g. $\pm 10\%$) then it is impossible to capture any mass loss for
42 that chemical caused by different extraction methods. Therefore, both mentioned methods
43 are not applicable to comprehensively evaluate the recovery of different extraction methods
44 when dealing with complex mixtures such as PW.

45

46 PW is one of the largest streams of treated industrial wastewater in the world⁴ and its dis-
47 charge into the marine environment is of ecological relevance. For example from Norwegian
48 off shore activities PW volumes are $140 \text{ mil m}^3 \text{ y}^{-1}$.⁵ PW is a complex mixture contain-
49 ing a diverse range of chemical constituents.^{1,6-8} Organic compounds in PW, typically vary
50 from oil droplets to large organic acids.⁶⁻⁸ Thus, PWs exhibit a wide range of chemical and
51 physical properties, fate and behaviors. As a consequence of this chemical diversity and the
52 fact that not all of its chemical constituents are known, extraction of PW typically reveals
53 complex mixtures that are largely unresolved by typically used techniques (e.g. unit mass
54 GC-MS).⁹⁻¹¹

55

56 High resolution mass spectrometry coupled with different chromatographic technologies
57 (gas and/or liquid chromatography) has shown great potential in partially resolving the un-
58 resolved complex mixture (UCM).¹²⁻¹⁵ However, when dealing with UCMs, these analytical
59 techniques are not capable of comprehensively characterize the analyzed samples.¹⁴ Conse-

60 quently, chemometric tools such as principal component analysis (PCA), F-ratio, and N-way
61 partial least-squares in combination with HRMS are usually employed to tackle the com-
62 plexity of these UCMs.¹⁵⁻¹⁸

63

64 The combination of F-ratio method and the apex detection algorithm has been shown to
65 be a powerful tool when dealing with complex environmental samples, including petroleum
66 related matrix.^{17,20} F-ratio is a parametric supervised method, which uses the ratio of the
67 between-groups variability and within each group variability to define the significance of
68 each variable.^{19,20} Therefore, it identifies the features in the samples which are statistically
69 significant, while the apex detection algorithm reduces the redundancy in those features
70 by grouping them as unique statistically significant feature. PW was selected as the
71 test/validation matrix for the applicability of this approach in comprehensive recovery as-
72 sessment of complex mixtures due to its complexity.

73

74 The aim of the present study was to use the F-ratio method to comprehensively assess
75 the extraction recovery of three generic (i.e. wide range of chemical and physical property)
76 extraction methods for PW. We employed three extraction methods: liquid-liquid extraction
77 (Lq), HLB cartridges (HLB), and the combination of ENV+ and C8 cartridges(ENV) for an
78 applicability proof of concept. These methods have been widely used for recovering complex
79 mixtures of analytes from matrices including PW.²¹⁻²⁶ We employed a combination of the
80 conventional targeted and the alternative non-targeted analysis for a comprehensive recovery
81 assessments. PW was divided into three categories: non-spiked, spiked level 1, and spiked
82 level 2. For the targeted approach we used a spike solution consisting of a mixture of 65
83 target analytes that were added into the PW at two different concentrations (i.e. spiked level
84 1 and spiked level 2). The concentration differences between the two spike levels were used to
85 calculate the absolute recoveries of each target analyte. For the non-targeted approach, we
86 used the non-spiked PW. We employed the null-distribution in order to define the threshold

87 of false positive detection. Finally, we calculated the relative recovery of unique features
88 based on the average intensity of those features. This study was a proof of concept for the
89 applicability of the suggested approach in comprehensive recovery assessment of complex
90 unresolved mixtures of organic analytes.

91 **Experimental Methods**

92 **Sample Preparation and Extraction**

93 PW (20L) was obtained from the Heidrun oil platform²⁷ in the Halten bank off the coast
94 of mid-Norway during February 2017. PW was subdivided into 27 aliquots each of 400 mL.
95 These aliquots were divided into three categories: non-spiked, spiked level 1 and spiked level
96 2, thus 9 samples in each category (Figure 1). We added a predefined volume of a stan-
97 dard mixture solution to the spiked samples (i.e. spiked level 1 and spiked level 2) in order
98 to reach a certain concentration for each added component of the mixture. The standard
99 mix solution consisted of a mixture 29 alkanes (Als) from C10-C33 at 8 $\mu\text{g mL}^{-1}$ each, 19
100 alkylated phenols (ALPs) at 10 $\mu\text{g mL}^{-1}$ each, and 16 polycyclic aromatic hydrocarbons
101 (PAHs) at 2 $\mu\text{g mL}^{-1}$ each. The spiked level 1 samples (i.e. 9 out of 27) were spiked with
102 50 μL of standard mix solution resulting in addition of 0.4 μg of Als, 0.5 μg of ALPs, and
103 0.1 μg of PAHs whereas spiked level 2 samples were spiked with 100 μL of standard mix
104 solution resulting in addition of 0.8 μg of Als, 1 μg of ALPs, and 0.2 μg of PAHs. The
105 non-spiked samples were used for non-targeted recovery assessment while the spiked sam-
106 ples were employed for the targeted workflow. Detailed information regarding the standard
107 mixtures and suppliers is provided in the Supporting Information, Section S1.1 and Table S1.

108

109 Each spiked level sample group was extracted using one of three different extraction
110 methods: liquid-liquid extraction (Lq), HLB cartridges, or the combination of ENV+ and
111 C8 cartridges (ENV), each in triplicates, Figure 1. The Lq method resulted in recovering

112 a dichloromethane extract of acidified PW (pH 2). This method is the official method rec-
113 ommended by the Norwegian Oil and Gas for extraction of PW.²⁵ On the other hand, use
114 of the HLB cartridge is a solid phase extraction (SPE) approach, where the solid phase is
115 a universal polymeric reverse phase sorbent for extraction of acidic, basic and neutral com-
116 pounds in different water-based matrices. This method has been widely used for analysis of
117 wastewater samples.²¹⁻²⁴ ENV+ is another SPE cartridge with a non-polar crosslinked hy-
118 droxylated polystyrene-divinylbenzene solid phase, reportedly adequate for extraction of po-
119 lar and semi-polar compounds from complex aqueous samples.²⁶ The combination of ENV+
120 and the reversed phase C8 cartridges enables extraction of a wide range of chemicals with
121 polarity varying from non-polar to polar. This method has been successfully used for extrac-
122 tion of PW, previously.²⁶ More detailed information regarding the extraction procedures is
123 provided in the Section S1.2 of the Supporting Information. The three tested methods all
124 are considered to be generic extraction methods, which implies that they are supposed to
125 extract a large number of chemical constituents with a wide range of chemical and physical
126 properties in the PW.

127

128 For the quality control/assurance of the analysis, we took the following steps during our
129 extractions. For application of each extraction method at a specific spiked level, a procedural
130 blank was generated, Figure 1. These procedural blanks were extracts of either the unloaded
131 cartridges or the glassware used for Lq method. All the glassware used during the extractions
132 and analyses was oven baked at 450 °C over-night. Additionally, all the final extracts were
133 spiked with 50 ng of diazepam-d5 as injection standard in order to monitor the performance
134 of the instrumentation.

135 **Instrumental Conditions and Analysis**

136 The final extracts of non-spiked samples and all the blanks were analyzed via Thermo
137 ScientificTM QExactiveTM GC Hybrid Quadrupole-OrbitrapTM Mass Spectrometer (Ther-

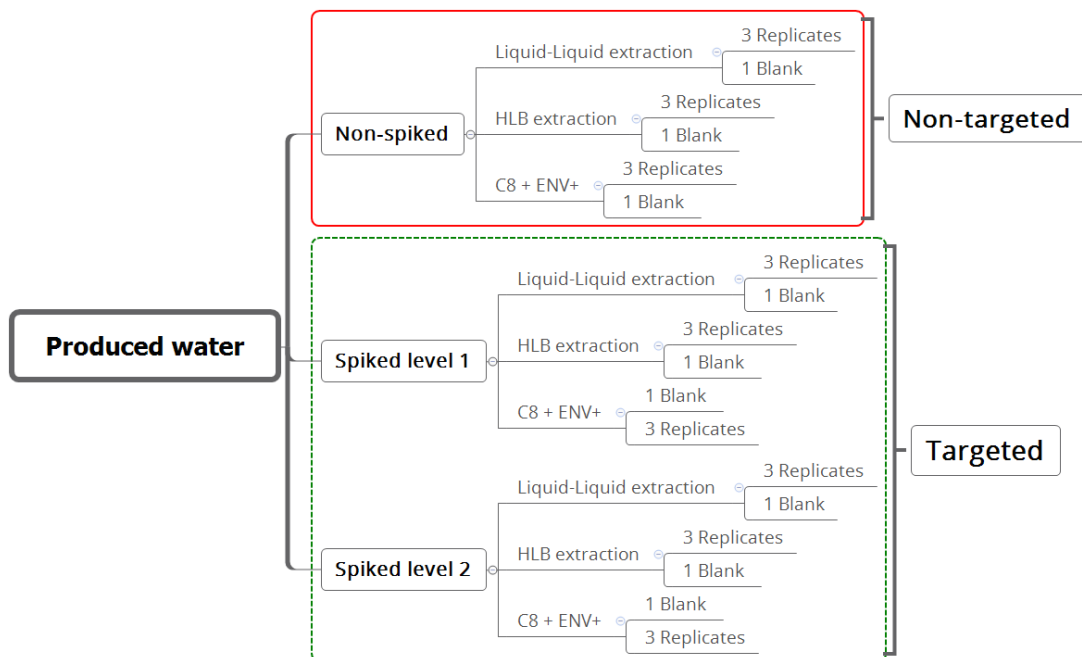


Figure 1: Schematic of the design of the experiment employed in this study depicting the extraction methods, number of replicates, number of spiking levels and data processing approach.

138 moFisher Scientific, USA) with an electron impact ionization source (EI), hereafter referred
 139 to as GC-Orbi. One μL of each extract was injected in splitless mode at $320\text{ }^\circ\text{C}$ of inlet tem-
 140 perature. The samples were separated on a $30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$ TraceGOLD (TG-
 141 5MS) by ThermoFisher Scientific, USA. We employed Thermo ScientificTM TraceFinderTM
 142 software (ThermoFisher Scientific, USA) for the data acquisition of the non-spiked samples.

143

144 The extracts of spiked levels 1 and 2 samples as well as all the blanks were analyzed
 145 employing GC coupled to a high resolution time of flight mass spectrometry (GC-HR-
 146 TOFMS; GCT Premier, Waters, USA) equipped with EI source. The samples were examined
 147 using a DB-5 column ($30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$, Agilent) with an injection volume of
 148 $1\text{ }\mu\text{L}$. The TOFMS was operated with a sampling frequency of 2 Hz between 50 and 650
 149 Da with a resolution of 9000 at half width full range. The chromatograms of these samples
 150 were acquired via MassLynxTM (Waters, USA). These settings were optimized previously for

151 analysis of PW extracts.²⁸ The details regarding the temperature program used for these
152 separations are provided in the Section S1.3 of the Supporting Information.

153 **Target Analysis and Absolute Recovery Assessment**

154 Target screening was employed for the analysis of the spiked level 1 and 2 samples. De-
155 tails of the detection and quantification procedure are provided elsewhere.²⁸ In brief, we
156 used the retention time, accurate mass of the parent ion and the accurate masses of two
157 fragments for confident identification of the target analytes while using a five point external
158 standard calibration curve with three replicates at each level for the quantification of the
159 target analytes. The differences in the average concentration of the analytes between spiked
160 level 2 and spiked level 1 were used for the absolute recovery calculations. Throughout this
161 document we refer to the recoveries calculated via target analysis as absolute recoveries. It
162 should be noted that the analytes which produced a negative or zero absolute recoveries were
163 considered to have a recovery of zero.

164 **Data Processing for Non-targeted Recovery Assessment**

165 The raw chromatograms of the non-spiked samples were converted to mzXML format em-
166 ploying the MSConvert package implemented via ProteoWizard.²⁹ The converted data files
167 were imported into Matlab (R2015b)³⁰ for further processing. During the non-targeted data
168 processing the imported data went through five consecutive steps: 1) data binning, 2) re-
169 tention alignment, 3) F-ratio calculation, 4) null distribution, and finally 5) Apex detection
170 (Figure S1). The F-ratio method, being a parametric test, assumes normal distribution of
171 the tested dataset. Typically, the data produced via LC-MS and/or GC-MS are more than
172 65% normally distributed, which implies the adequacy of a parametric method for the anal-
173 ysis.³¹ This is particularly the case for the raw LC-MS and GC-MS data due to inherent
174 nature of the raw data, which consist of a combination of gaussian peaks for analytical signal
175 and noise. Therefore, the F-ratio method can be applied to these datasets. We selected a

176 very large F-ratio threshold with a very small probability of false positive detection of 0.01%.
177 The reason behind this choice of F-ratio value was the fact that this study is only a proof of
178 concept, and therefore, we preferred to focus on a limited number (i.e. sub-sample) of the
179 unique statistically relevant features rather than all of them. This workflow has been shown
180 to be able to capture the statistically meaningful differences between different sample sets.¹⁷
181 The details of all the steps in the non-targeted workflow is available in the Section S2 of the
182 Supporting Information.

183

184 For the non-targeted recovery assessment, hereafter referred to as relative recoveries, the
185 average signal of the method with highest intensity for a certain feature is assumed to be
186 the total extractable material for that feature. Therefore, the ratio of the average signal
187 of a certain feature for all the extraction methods and the total extractable material could
188 be considered the relative recovery of that feature via that extraction method. In Eq. 1,
189 Rec_{Rel} represents the relative recovery, $\hat{S}_{i,j}$ represents the average signal of i^{th} feature and
190 j^{th} extraction method, and $\hat{S}_{i,total}$ represents the total extractable material for i^{th} feature.
191 Using this approach we were able to capture the relative amount of signal lost for a feature
192 due to a specific extraction method.

$$Rec_{Rel} = 100 \times \frac{\hat{S}_{i,j}}{\hat{S}_{i,total}} \quad (1)$$

193 Computations

194 All the mentioned data processing steps were performed via Matlab, employing a Windows
195 7 Professional version (Microsoft Inc, USA) workstation computer with 12 CPUs and 128
196 GB of memory.

197 **Results and discussion**

198 We comprehensively evaluated the extraction recovery of a complex unresolved mixture,
199 such as PW, via the combination of targeted and non-targeted analysis. Through the target
200 screening we examined the absolute recovery of 65 analytes with three different extraction
201 methods. This was carried out by spiking the PW with a standard mixture at two concentra-
202 tion levels. The concentration differences between the two spike levels were used to calculate
203 the absolute recovery of each target analyte. Additionally, as a quality assurance step we
204 evaluated the concentration of the 65 target analytes in the blanks. For all 65 target analytes
205 the sample concentrations were at least 10 times higher than their blank concentrations. The
206 non-targeted approach, on the other hand, was used to capture the statistically meaningful
207 features in the samples which differentiated each extraction method from the others. We
208 used the F-ratio method in order to select the relevant features in each sample.^{17,32,33} The
209 F-ratio method was combined with the null distribution approach to calculate the probabilit-
210 ity of false positive detection for each F-ratio.^{17,20} During the F-ratio analysis, the blanks for
211 each extraction method (i.e. the non-spiked and the two spike levels) were grouped together
212 as triplicates. These blank triplicates were included in the dataset used for F-ratio analysis
213 as separate groups. This procedure enabled us to assure that the finally selected features
214 are unique to the samples. This study is a proof of concept for the applicability of this
215 approach to comprehensively assess the extraction recovery of unresolved complex mixtures,
216 particularly for non-targeted structural elucidation and/or retrospective analysis.

217 **Targeted Recovery Assessment**

218 The ENV method resulted in the largest number of analytes (i.e. 48 out of 65; 74%) with an
219 absolute recovery larger than zero whereas the HLB method produced the smallest number
220 of positive recovery analytes, 34 out of 65 (52%), Table 1. A similar trend was observed for
221 the average absolute recovery of each extraction method across all three chemical families

222 (Table 1 and Figure 2). The ENV method was able to extract Als from dodecane to octa-
223 cosane while the Lq method was more successful in extraction of smaller Als such as decane,
224 Figure S3. In case of ENV method the C8 sorbant had a similar level of affinity towards the
225 Als with different molecular size. Therefore, the higher volatility of these smaller Als com-
226 pared to the larger ones caused lower recoveries for those analytes. For the Lq method the
227 observed trend was attributed to the higher solubility of smaller Als in the DCM compared
228 to the larger analytes. For these analytes (i.e. Als) the HLB method was less successful
229 than both ENV and Lq methods in extracting the small Als and n-pentadecane was the
230 smallest extracted Al. consequently, for the larger Als, this method fared better than Lq
231 method while performing in a similar way to the ENV method. For ALPs, similarly to
232 the Als, the ENV method extracted the largest number of target analytes (i.e. 13) when
233 compared to the other two methods, Table 1. We were not able to find a consistent trend
234 between the molecular size or hydrophobicity of target analytes and their absolute recoveries.
235 However, all three methods appeared to be more successful in extraction of smaller ALPs
236 (Figure S4). For PAHs, the ENV and Lq methods were able to produce positive recoveries
237 for all 16 target analytes whereas the HLB method was only able to extract 12 analytes out
238 of 16 (Table 1 and Figures 2 and S5). Overall, the ENV and Lq methods performed bet-
239 ter than the HLB method based on the observed number of analytes with positive recoveries.

240

241 Regarding the absolute recoveries, the ENV and Lq methods with average absolute re-
242 coveries of 53.1 ± 15.2 for ENV and 46.8 ± 13.2 for Lq performed better than the HLB
243 method with an average absolute recovery of 19.7 ± 6.7 (Table 1 and Figure 2). The ENV
244 method with an observed within replicates' variability of 59% appeared to be the most sta-
245 ble extraction method compared to HLB method with 85% observed variability and Lq with
246 198% observed variability (Figures S3, S4 and S5). The Lq method includes more manual
247 steps than the SPE methods. Both ENV and HLB methods showed more uniform recover-
248 ies (i.e. closer to the average recovery) across all the target analytes compared to the Lq

249 method, whereas the Lq method resulted in larger levels of variability in the recoveries as
 250 a function of analyte molecular size and DCM solubility (e.g. Als, Figure S3). In terms
 251 of absolute recoveries, the ENV and Lq methods performed in a similar way for all three
 252 chemical families while the HLB method fared the worst.

253

254 The methods ENV and Lq were not statistically distinguishable when looking at all 65 tar-
 255 get analytes while they both appeared to be different from the HLB method (Kruskal-Wallis
 256 test³⁴ p value < 0.01). We used the non-parametric Kruskal-Wallis test³⁴ to differentiate the
 257 investigated extraction methods from each other. The observed result of the statistical test
 258 was in agreement with the observed trends of recoveries for different chemical families and
 259 extraction methods.

Table 1: Lists the number of analytes with positive absolute recoveries as well as the average absolute recoveries for each extraction method and chemical family.

Number of chemicals with positive recoveries ^a			
Chemical family	Extraction methods		
	ENV	HLB	Lq
Al ^b	19	15	19
ALP ^c	13	7	9
PAH ^d	16	12	16
Total	48	34	44

Average absolute recoveries ^a			
Chemical family	Extraction methods		
	ENV	HLB	Lq
Al	52.4±10.2	17.1±7.0	50.0±16.2
ALP	41.1±17.3	14.8±6.4	37.9±6.9
PAH	63.5±17.4	26.1±5.7	48.1±12.0
Total	53.1±15.2	19.7±6.7	46.8±13.2

^a This parameter was calculated using only the analytes with positive recoveries; ^b The total number of alkanes (Als) in this study was 29; ^c The total number of investigated alkylated phenols (ALPs) was 19; and ^d The total number of PAHs in this study was 16 compounds.

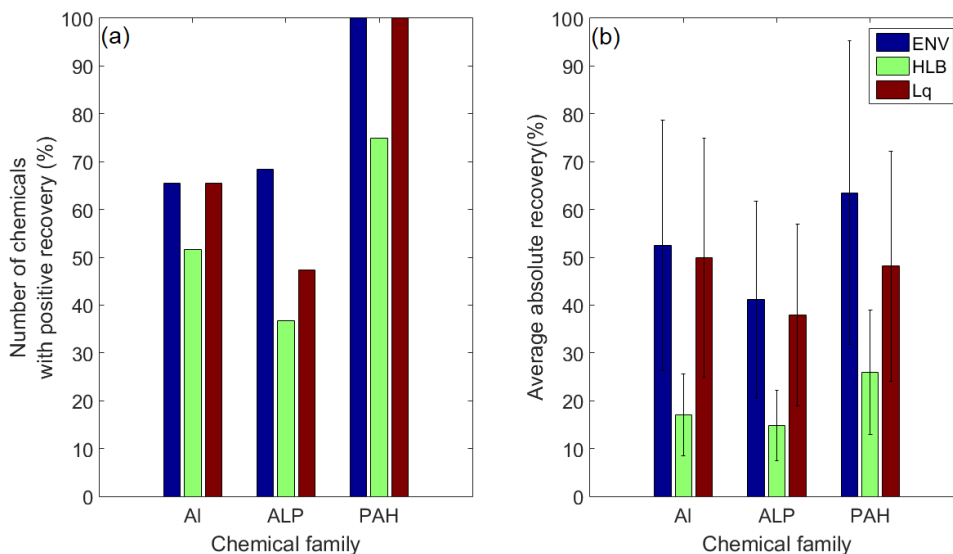


Figure 2: (a) Percentage of the target analytes with positive recoveries and (b) average absolute recoveries of target analytes with positive absolute recoveries. In panel "b" the error bars represent $\pm 2 \times$ standard deviation of the recoveries for a chemical family via an extraction method.

260 Non-targeted Recovery Assessment

261 The F-ratio approach was employed for capturing the statistically meaningful features in the
 262 chromatograms. The features/fragments and/or molecular ions in the mass spectra that were
 263 causing the differentiation among investigated extraction methods were singled out through
 264 the combination of F-ratio analysis and apex detection. For the purpose of this proof of
 265 concept and to minimize false positives detection, we utilized a false positive detection prob-
 266 ability value of 0.01% for the F-ratio, which corresponded to an F-ratio value of 3180, (Figure
 267 S6). Further optimization of the F-ratio value will be subject of future studies. This F-ratio
 268 value reduced the number of variables in the dataset by a factor of 95% and enabled us to
 269 focus only on the statistically significant features (Figure S7). After F-ratio correction, each
 270 chromatogram contained ~ 2000 features. These features were a combination of redundant
 271 analytical signal (i.e. multiple features representing one unique feature, Figure S8), unre-
 272 solved signal (i.e. signal which goes across a large section of chromatogram and does not

273 have a peak shape, Figure S7), and finally the noise, Figure S8. Those statistically signifi-
274 cant features then were grouped, noise removed and unique features obtained by employing
275 the apex detection algorithm. The apex detection resulted in 26 features which appeared
276 to be highly relevant in differentiating the three extraction methods from each other. From
277 those 2000 initial features, 67.4% were removed during the grouping process (i.e. redundant
278 analytical signal), 28.9% of those features were unresolved signal and finally 3.7% of those
279 features were classified as noise. The number of features belonging to redundant signals was
280 in agreement with our expectations considering the sampling rate provided by the GC-Orbi
281 (i.e. ~ 10 Hz based on the number of scans in an average peak). For example for each unique
282 feature, on average, around 55 redundant analytical signals were observed that after group-
283 ing were represented by one unique feature (Figure S8). The unresolved features/signals
284 and noise were excluded from the final unique feature list for further evaluation due to the
285 difficulties in associating a chemical formula to them. Thus we used the relative recoveries
286 (Eq. 1) of the final 26 unique features generated via the combination of F-ratio method and
287 the apex detection algorithm for recovery assessment of different extraction methods.

288

289 The ENV method produced a relative recovery of 100% for all 26 unique features (i.e.
290 the maximum averaged signal for all 26 unique features) whereas the Lq and HLB methods
291 produced relative recoveries larger than zero for only 3 out of 26 unique features (Figure 3).
292 The signal of 23 out of 26 unique feature was zero in the extraction methods Lq and HLB
293 whereas a meaningful signal was produced in the chromatogram obtained from the ENV
294 method (Figure S9). The low variability ($\leq 20\%$) observed for all the extraction methods
295 and all the unique features further indicated the meaningfulness of these features. We also
296 predicted the chemical formula of each of these unique features using the ChemCal online
297 tool.³⁵ Additionally, another online tool (i.e. Isotope Distribution Calculator and Mass Spec
298 Plotter³⁶) was used to calculate the isotopic distribution of the predicted formula in order
299 to provide further confirmation (Table S2). Based on the predicted chemical formulas of

300 the unique features (molecular fragment ions), most of those features contained one or more
301 heteroatom (i.e. O, N, and S), which could be considered as an indication that these ana-
302 lytes were among the more "polar" compounds. Furthermore, the three features where the
303 methods Lq and HLB produced larger than zero relative recoveries all appeared to be simple
304 hydrocarbons without any heteroatoms. Therefore, the ENV method appeared to be more
305 successful in extracting more "polar" components of PW. Further investigation is necessary
306 in order to identify confidently the compounds which produced these unique features. None
307 the less, the suggested approach was shown to be effective in capturing the relevant features
308 that were causing the differentiation among the studied extraction methods. Also our results
309 indicate the overall better performance of the ENV method in extracting PW compared to
310 the other two methods. Finally, it should be noted that these 26 unique features are only a
311 sub-sample of the unique statistically significant features in this dataset. In order to make
312 sure that all the statistically significant features in differentiating these samples are captured
313 an optimization of the F-ratio threshold is necessary. The optimization of this parameter
314 will be subject of future studies.

315

316 The non-targeted approach was able to comprehensively evaluate the extraction recovery
317 of PW via the three different methods. This method was effective where the traditional
318 approaches (e.g. targeted method) failed to distinguish the best extraction method (e.g. the
319 ENV and Lq methods were statistically similar).

320 **Implications and Limitations**

321 The combination of the F-ratio method and the apex detection algorithm was shown to be
322 effective in isolating those features which allowed the differentiation of complex samples. In
323 this study, we used this approach to evaluate the recovery of three widely used extraction
324 methods for analysis of produced water. Our results suggested that one of the methods

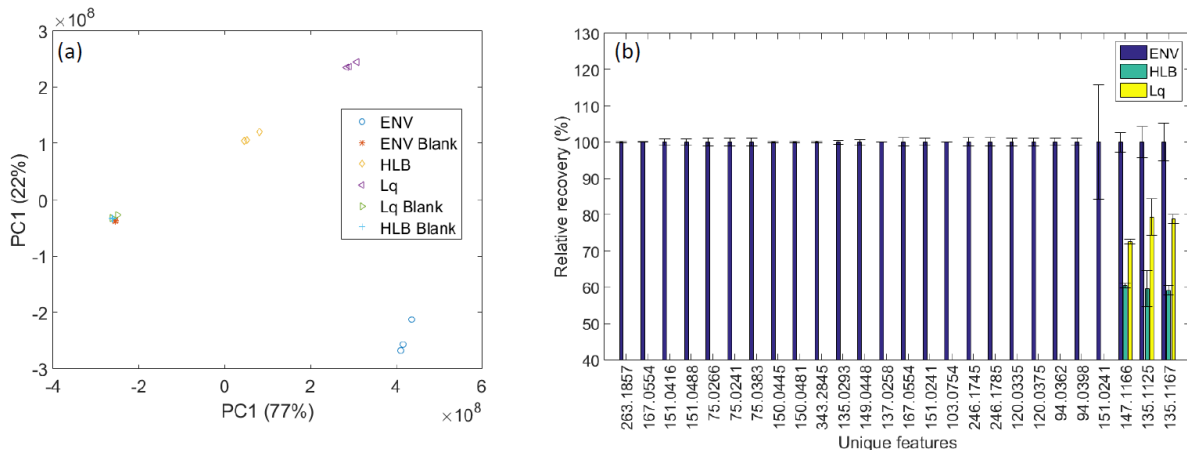


Figure 3: Depicting (a) the score plot of the first two principal components with percentage variability described and (b) relative recoveries of all 26 unique features using Eq. 1. The error bars in this figure represent \pm standard deviation of the recoveries for a unique feature via an extraction method.

325 (i.e. using ENV method) performed far better than the other two methods, even though
 326 the traditional targeted approach failed to reveal the differences between these methods (i.e.
 327 ENV and Lq methods). This method captured the features that were statistically meaning-
 328 ful and also were extracted only using the ENV extraction method. Better understanding
 329 of the chemical space explored via each extraction method is highly relevant for the toxicity
 330 risk assessment, chemical processes/process engineering, and retrospective suspect and non-
 331 target screening. This method should enable analysts to evaluate qualitatively the extraction
 332 recovery of different methods and at the same time to explore the chemical space sampled
 333 via each extraction method. This would result in an optimized method, which would cover
 334 a wide area of chemical space. Additionally, the method proposed here has the potential to
 335 be applied to all cases where a change in the process may cause the generation of different
 336 outputs. For example, this method could be applied to the output of treated wastewater
 337 with different advanced oxidation processes, given the differences in the reaction pathways.

338

339 The main limitations of the present approach are the sensitivity towards high levels of
 340 variability, the computational cost, and the necessary MS resolution. For example, we cal-

341 culated the F-ratio values for the 65 target analytes in this study and those values ranged
342 between 18 to 543, which were too small for them to be captured by the non-targeted ap-
343 proach. This was mainly caused by the high level of variability observed in the Lq extraction
344 method (i.e. 198%). Therefore, this data processing method should be combined with the
345 conventional targeted method in order to be able to evaluate its effectiveness, specially when
346 expecting a larger level of variability in the dataset. In terms of the computational cost,
347 the cloud computation (i.e. the use of a cluster of computers) should be considered in order
348 to make these types of analysis possible in a timely fashion. The F-ratio method can be
349 applied to data produced via both unit resolution MS^{32,33} as well as high resolution data.¹⁷
350 The necessary MS resolution for F-ratio analysis depends on the level of complexity of
351 the evaluated sample. In other words for highly complex samples such as produced water the
352 F-ratio applied to low resolution GC-MS or LC-MS (i.e. unit mass) data may fail. Therefore,
353 the analyst must choose the adequate MS resolution for the F-ratio analysis, based on the
354 prior knowledge of the sample complexity. However, all considered, this approach (i.e. the
355 combination of F-ratio method and the apex detection algorithm) appears to be a powerful
356 tool for dealing with complex samples and chemical space problems.

357

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361 providing us with the produced water samples.

362 **Supporting Information Available**

363 The Supporting Information including details regarding the sample preparation, analysis,
364 steps taken during the data processing, and figures is available free of charge on the ACS

365 Publications website. Table S1 (an external file) containing the list of target analytes is also
366 available free of charge on the ACS Publications website.

367 **Associated Content**

368 **Author Information**

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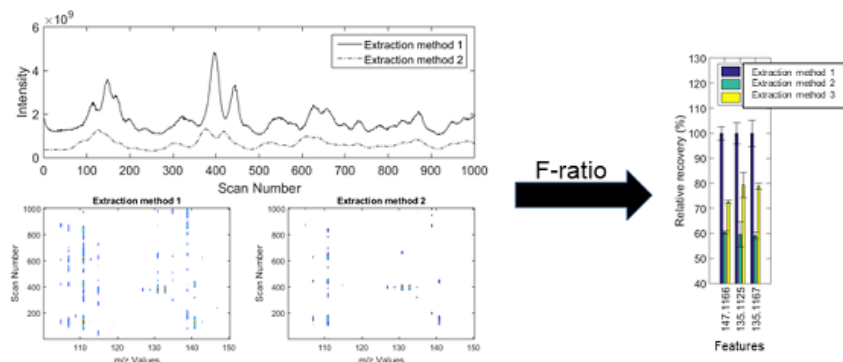
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References

- (1) Oetjen, K.; Giddings, C. G.; McLaughlin, M.; Nell, M.; Blotevogel, J.; Helbling, D. E.; Mueller, D.; Higgins, C. P. *Trends Environ. Anal. Chem.* **2017**, *15*, 12–23.
- (2) Robson, W. J.; Sutton, P. A.; McCormack, P.; Chilcott, N. P.; Rowland, S. J. *Anal. Chem.* **2017**, *89*, 2919–2927.
- (3) Jones, D.; Scarlett, A.; West, C.; Frank, R.; Gieleciak, R.; Hager, D.; Pureveen, J.; Tegelaar, E.; Rowland, S. *Chemosphere* **2013**, *93*, 1655–1664.
- (4) Thomas, K. V.; Balaam, J.; Hurst, M. R.; Thain, J. E. *J. Environ. Monit.* **2004**, *6*, 593–598.
- (5) Oil, N.; Gas, Environmental Report 2016. <https://www.norskoljeoggass.no/no/Publikasjoner/Miljorap2016/>, 2016.
- (6) Thomas, K.; Langford, K.; Petersen, K.; Smith, A.; Tollefsen, K. *Environ. Sci. Technol.* **2009**, *43*, 8066–8071.
- (7) Thomas, K. V.; Balaam, J.; Hurst, M. R.; Thain, J. E. *Environ Toxicol. Chem.* **2004**, *23*, 1156–1163.
- (8) Balaam, J. L.; Chan-Man, Y.; Roberts, P. H.; Thomas, K. V. *Environ. Toxicol. Chem.* **2009**, *28*, 1159–1167.
- (9) Booth, A. M.; Scarlett, A. G.; Lewis, C. A.; Belt, S. T.; Rowland, S. J. *Environ. Sci. Technol* **2008**, *42*, 8122–8126.
- (10) Booth, A. M.; Sutton, P. A.; Lewis, C. A.; Lewis, A. C.; Scarlett, A.; Chau, W.; Widdows, J.; Rowland, S. J. *Environ. Sci. Technol.* **2007**, *41*, 457–464.

- 397 (11) Melbye, A. G.; Brakstad, O. G.; Hokstad, J. N.; Gregersen, I. K.; Hansen, B. H.;
398 Booth, A. M.; Rowland, S. J.; Tollefsen, K. E. *Environ. Toxicol. Chem.* **2009**, *28*,
399 1815–1824.
- 400 (12) Spanik, I.; Machynakova, A. *J. Sep. Sci.* **2018**, *41*, 163–179.
- 401 (13) Luek, J. L.; Gonsior, M. *Water research* **2017**, *123*, 536–548.
- 402 (14) Staš, M.; Chudoba, J. *Chemické listy* **2017**, *111*, 628–636.
- 403 (15) Headley, J. V.; Peru, K. M.; Barrow, M. P. *Mass spectrometry reviews* **2016**, *35*, 311–
404 328.
- 405 (16) Radovic, J. R.; Thomas, K. V.; Parastar, H.; Díez, S.; Tauler, R.; Bayona, J. M.
406 *Environmen. Sci. Technol.* **2014**, *48*, 3074–3083.
- 407 (17) Samanipour, S.; Reid, M. J.; Thomas, K. V. *Anal. Chem.* **2017**, *89* (10), 5585–5591.
- 408 (18) Schollee, J. E.; Schymanski, E. L.; Avak, S. E.; Loos, M.; Hollender, J. *Anal. Chem.*
409 **2015**, *87*, 12121–12129.
- 410 (19) Brereton, R. G. *Applied chemometrics for scientists*; John Wiley & Sons, 2007.
- 411 (20) Parsons, B. A.; Marney, L. C.; Siegler, W. C.; Hoggard, J. C.; Wright, B. W.; Syn-
412 ovec, R. E. *Anal. Chem.* **2015**, *87*, 3812–3819.
- 413 (21) Baz-Lomba, J. A.; Reid, M. J.; Thomas, K. V. *Anal. Chem. acta* **2016**, *914*, 81–90.
- 414 (22) Samanipour, S.; Baz-Lomba, J. A.; Alygizakis, N. A.; Reid, M. J.; Thomaidis, N. S.;
415 Thomas, K. V. *J. Chromatogr. A* **2017**, *1501* (2017), 68–78.
- 416 (23) Baker, D. R.; Kasprzyk-Hordern, B. *Journal of Chromatography A* **2011**, *1218*, 8036–
417 8059.

- 418 (24) Fatta, D.; Achilleos, A.; Nikolaou, A.; Meric, S. *TrAC Trends in Analytical Chemistry*
419 **2007**, *26*, 515–533.
- 420 (25) Noro, Norwegian Oil and Gas recommended guidelines for sampling and analysis of
421 produced water, translated version. 2003.
- 422 (26) Thomas, K. V.; Langford, K.; Petersen, K.; Smith, A. J.; Tollefsen, K. E. *Environ. Sci.*
423 *Technol.* **2009**, *43*, 8066–8071.
- 424 (27) Statoil, N. Heidrun oil platform. [https://www.statoil.com/en/what-we-do/norwegian-](https://www.statoil.com/en/what-we-do/norwegian-continental-shelf-platforms/heidrun.html)
425 [continental-shelf-platforms/heidrun.html](https://www.statoil.com/en/what-we-do/norwegian-continental-shelf-platforms/heidrun.html), 2017.
- 426 (28) Samanipour, S.; Langford, K.; Reid, M. J.; Thomas, K. V. *J. Chromatogr. A* **2016**,
427 *1463*, 153–161.
- 428 (29) Kessner, D.; Chambers, M.; Burke, R.; Agus, D.; Mallick, P. *Bioinformatics* **2008**, *24*,
429 2534–2536.
- 430 (30) MATLAB version 9.1 Natick, Massachusetts: The MathWorks Inc.,
- 431 (31) Vinaixa, M.; Samino, S.; Saez, I.; Duran, J.; Guinovart, J. J.; Yanes, O. *Metabolites*
432 **2012**, *2*, 775–795.
- 433 (32) Pierce, K. M.; Hoggard, J. C.; Hope, J. L.; Rainey, P. M.; Hoofnagle, A. N.; Jack, R. M.;
434 Wright, B. W.; Synovec, R. E. *Anal. Chem.* **2006**, *78*, 5068–5075.
- 435 (33) Pierce, K. M.; Hope, J. L.; Johnson, K. J.; Wright, B. W.; Synovec, R. E. *J. Chromatogr.*
436 *A* **2005**, *1096*, 101–110.
- 437 (34) Breslow, N. *Biometrika* **1970**, *57*, 579–594.
- 438 (35) Patiny, L.; Borel, A. ChemCalc: a building block for tomorrow’s chemical infrastruc-
439 ture. 2013.

440 (36) (SIS), S. I. S. Isotope Distribution Calculator and Mass Spec Plotter.
441 <http://www.sisweb.com/mstools/isotope.htm>, 2015; Online tool.