Impact of oil matrix on anionic and nonionic surfactants separation using Ultra-High- Performance-Liquid-Chromatography hyphenated to High-Resolution Mass Spectrometry

Alizée Dufour^{1,2,}, Didier Thiébaud², Matthieu Loriau¹, Leticia Ligiero¹, Jérôme Vial²*

¹Total S.A. Exploration & Production - Lacq Research Center (PERL), 64170 Lacq, France

²LSABM, CBI, UMR 8231, ESPCI Paris - PSL Université Paris - CNRS, 10 rue Vauquelin,
75005 Paris, France

KEYWORDS

Surfactants, Crude oil, Mixed-mode chromatography, High-Resolution Mass Spectrometry,
Petroleum industry

ABSTRACT

For the first time, to our knowledge, anionic and nonionic surfactants were analyzed in oil matrix by Ultra-High-Performance Liquid Chromatography hyphenated to High-Resolution Mass Spectrometry (UHPLC-HRMS). The feasibility of their analysis was studied using synthetic

mixtures of surfactants prepared in water (Quality controls), in a binary THF/Toluene 50/50 v/v (Surfactant+THF/Toluene) and in a binary THF/Toluene containing 1 and 10% of crude oil (Crude1% and Crude10%). These compositions were chosen in order to be as close as possible to petroleum related samples to be investigated in the future. Analyses were carried out by UHPLC methods using both reverse phase and anion-exchange mechanisms with a mixed mode column. Despite the complexity of the oil matrix and the presence of organic solvents used for dilution, the retention times of the surfactants were not affected whatever the concentration of crude oil present in the sample. Nevertheless, a significant matrix effect caused a loss of signal when the concentration of oil reached 10% in mass. For the analysis of samples with this crude oil concentration range, it would be advisable to dilute the sample.

1. Introduction

Surfactants, also called surface active agents, are composed of a hydrophilic head and a hydrophobic chain. In order to modulate their hydrophilic-lipophilic balance, ethylene oxide (EO) or/and propylene oxide (PO) can be added between these two parts¹. Synthesis of surfactants involves polymerization mechanisms, such as the Guerbet reaction², which are not always fully controlled, thus producing polymeric surfactants with a high polydispersity^{3,4}. Thanks to their amphiphilic nature, they are used ubiquitously in the petroleum industry for their foaming⁵, wetting⁶ and dispersing properties. For example, they help the chemically enhance oil recovery (cEOR) by reducing interfacial tension⁷ between oil and water or/and by modifying the wettability of rocks^{2,8}. Several groups listed most common surfactants used for cEOR^{9–12}. Surfactants are also used for hydraulic fracturing in order to increase natural resource extraction^{13,14}. They are mixed with oil fluids to reduce the viscosity during transportation¹⁵. When hydrocarbon-based fuel fires

are declared, mixtures containing hydrocarbon surfactants are used to extinguish the fire¹⁶. In the context of the high volatility of the crude oil market, it is essential to optimize surfactants use by monitoring their injection strategy, quantity and potential losses during the processes. For this purpose, performant analytical tools are necessary to insure an accurate analysis (detection, identification, and quantification) of the different families of surfactants (anionic, cationic, nonionic, etc.).

Currently, in the literature, only a few articles report the analysis of surfactants in oil samples. Eide *et al.* characterized and compared four crude oil samples by HRMS¹⁷. For the first time, in 2006, they reported an analytical method by direct injection of 2 μ L samples without chromatographic separation, to identify and quantify surfactants in oil¹⁸. In view of the nature of the surfactants and the complexity of the matrix, liquid chromatography appeared a relevant step before the HRMS detection. Currently, many analytical methods using liquid chromatography hyphenated with mass spectrometry are described in the literature to analyze these types of molecules, but in aqueous samples only^{19–22}. Motteran *et al.* reported a simultaneous determination of anionic and nonionic surfactants in commercial laundry wastewater²³. Luo *et al.* developed a method to detect nonionic surfactants in textiles samples²⁴. In the petrochemical industry, hydraulic fracturing fluids flowbacks and produced water were analyzed to detect surfactants by HPLC-HRMS. Thurman *et al.* characterized ethoxylated surfactants thanks to their “fingerprinting”^{14,25}. Heyob *et al.* focused their efforts on the detection and quantification of nonionic surfactants and their degradation products by HPLC-HRMS¹³. García *et al.* reported an analytical method to follow surfactants in groundwater due to their use to extinguish fuel fires¹⁶. In our previous paper²⁶, we evaluated the retention behavior of different polydisperse surfactants (cationic, anionic, nonionic) under three separations modes: reverse phase (RP), hydrophilic interaction (HILIC) and mixed-mode. With

RP mode, surfactants were separated according to their class (sulfonate, carboxylate, alcohol, etc). With HILIC mode, surfactants were separated according to their EO or/and PO units number. The mixed mode column involved simultaneously two mechanisms: hydrophobic interaction and ion-exchange. The combination of both mechanisms improved the resolution between classes of surfactants and the resolution between homologues. For these reasons, this column was chosen for the present study.

Samples from the petroleum industry are generally complex^{27,28}: more than a thousand of compounds, high viscosity, organic solvent etc. THF and toluene are commonly used as organic solvent, to dilute the oil matrix²⁹. These solvents have a higher elution strength than organic solvents currently used in RPLC, *i.e.* ACN, MeOH etc. Injecting samples diluted into these solvents in mixed mode could generate distorted peak, and retention variations³⁰, because analytes can have more affinity with the THF/Toluene mixture than with the mobile phase ; thus, the interactions with the stationary phase would be modified. Moreover, THF and its degradation impurities are known for their aggressive effect on the stationary phase³¹. This could ruin the quality of separations. For the same reasons, the presence of crude oil in the samples can disturb the LC system and modify the separation, thus causing losses of efficiency or even dysfunction. Moreover, matrix effect can be observed.

The present study focused on the ruggedness of the analysis when commercial surfactants were injected on a mixed mode column in water (Quality Controls), in a binary THF/Toluene and in crude oil diluted in THF/Toluene at two concentrations, 1 and 10% in mass (denoted Crude1% and Crude10%).

2. Materials and methods

2.1. Chemicals

LC-MS grade acetonitrile (ACN), water (W), tetrahydrofuran (THF), and formic acid (FA - 99%) were purchased from Carlo Erba (Val-de-Reuil, France). Ammonium formate (98%) was supplied by Fluka Chemie AG, CH-9470 Buchs. Toluene for HPLC was purchased from VWR Chemicals (France). Ultra-pure water was obtained from a Milli-Q Purification system from Millipore (Molsheim, France) and used for the preparation of all standard solutions. Crude oil was supplied by TOTAL S.A.. Brij®C10 (Brij, Mn 683) and glycolic acid ethoxylate lauryl ether (Carboxylate, Mn 360) were purchased from Sigma Aldrich (Saint-Quentin-Fallavier, France). These surfactants have already been used for retention behavior investigations²⁶ and their composition was verified by HRMS. Their mass spectra are provided as supplementary data (Figure S1).

Brij is an ethoxylated alcohol, with the molecular formula $C_xH_{2x+1}(OC_2H_4)_nOH$, x being the number of carbons in the alkyl chain, and n the number of ethylene oxide units (EO). It was detected in positive mode with a majority of ammonium adduct. After data processing, the carbon number of the alkyl chain appeared to be sixteen ($C_{16}H_{33}$) and the EO units ranged from 3 to 25. Two distributions composed the mass spectrum. The first one (Figure S1-red) corresponding to homologues with EO units number between 3 and 19 were attributed to monocharged ions $[M+NH_4]^+$. The second one (Figure S1-blue) was attributed to doubly charged ions, $[M+2NH_4]^{2+}$, with EO units number ranging from 14 to 25.

The glycolic acid ethoxylated lauryl ether was ionized in both ionization mode, *i.e.* (+) HESI and (-) HESI. Two distributions were observed in negative mode: (i) $[C_{11}H_{23}(OC_2H_4)_nCOOH-H]^-$ with n ranging from 1 to 13, noted C_{11} , and (ii) $[C_{13}H_{27}(OC_2H_4)_nCOOH-H]^-$, with n ranging from 1 to 10, noted C_{13} . In positive mode, the EO units' number of the C_{11} family ranged from 3 to 15 and

from 3 to 14 for the C₁₃ family. The smaller homologues were not detected in this mode probably due to their lower stability with ammonium adduct²⁴.

2.2. Samples preparation

For this study, different samples had to be prepared and analyzed. Each sample composition and their respective objectives are recapped in Table 1. The blank was only composed of water. Quality Control contained surfactants diluted at 0.5 mg.L⁻¹ in water. To study the ruggedness of the method when submitted to organic and oil samples, surfactants at 0.5 mg.L⁻¹ were diluted in a binary THF/Toluene mixture (50/50 v/v). To mimic the analysis of real petroleum samples, crude was added to surfactants in THF/Toluene at two levels, 1 and 10% in mass. The order and the number of injections are recapped in the list row of Table 1. Blank was noted B, Quality control was noted QC, THF/Toluene was noted S, Surfactant in THF/Toluene was noted TAS, Surfactant in THF/Toluene with Crude at 1% was noted C1 and at 10% was noted C2.

Table 1: List of samples analyzed with their goals in the study and injection sequences.

Name		Composition	Goals	Number of injections
Blank	B	Water	Memory effect	19
Quality Control (QC)	QC	Brij and Carboxylate at 0.5 mg.L ⁻¹ in water	Column performance monitoring	11
THF/Toluene	S	Binary mixture of THF/Toluene 50/50 v/v	Column integrity	5
Surfactant + THF/Toluene	TAS	Brij and Carboxylate at 0.5 mg.L ⁻¹ in THF/Toluene	Impact of THF/Toluene on chromatographic separation	5
Crude1%	C1	Brij and Carboxylate at 0.5 mg.L ⁻¹ , Crude oil at 1% in THF/Toluene	Chromatographic separation in oil sample	6
Crude10%	C2	Brij and Carboxylate at 0.5 mg.L ⁻¹ , Crude oil at 10% in THF/Toluene	Chromatographic separation in oil sample	6

Injection order

Impact of solvent:

B – QC – B – S – B – QC – B – 4×S – B – QC – B – TAS – B – QC – B – 4×TAS – B – QC – B

Impact of crude oil:

B – C1 – B – QC – B – 5×C1 – B – 2×QC – B – C2 – B – QC – B – 5×C2 – B – 2×QC – B

2.3. LC-HRMS analysis

The analytical method used in this study was developed previously by Dufour et al.²⁶. The UHPLC system was a Vanquish UHPLC (ThermoFisher Scientific) equipped with a binary pump, an autosampler and a Mixed-Mode column: Surfactant Plus (250 mm × 2.1 mm × 3 μm – Thermo Scientific). The mobile phases were: A, ammonium formate 10 mM pH 3.5, and B, ACN. The flow rate was 0.4 mL.min⁻¹ and the injection volume 3 μL. After an equilibration step (10 min) at 30% of B, a linear gradient from 30 to 100% B was used during 20 min. A 10 min isocratic plateau at 100% of B was systematically applied at the end of the gradient to elute the most retained compounds. Column and pre-heater temperatures were set at 40°C. The detection was performed with a hybrid quadrupole ion trap-Orbitrap mass spectrometer (Exactive Plus Orbitrap HRMS, ThermoFisher Scientific) equipped with a heated electrospray ionization source (HESI-II). The electrospray voltage was set at 3 kV, and the capillary and probe temperatures at 350°C. Nitrogen (purity>99%) was used for the sheath, auxiliary, and spare gases, and were set at, respectively, 40 psi, 15 arbitrary unit and 0 arbitrary unit. The S-Lens was set at 50. The orbitrap mass analyzer performed full MS scans in both negative and positive ionization modes (dual mode) over the m/z range 125-1100 with a mass resolution of 70,000 (@m/z 200). The automatic gain control target (AGC) value was 10⁶ with a maximum injection time set at 200 ms. Xcalibur 4.2 software (Thermo Scientific) was used for instrument control and data acquisition. FreeStyle (Thermo Scientific) was used for data processing. All samples (QC, Surfactant in THF/Toluene, Surfactants in THF/Toluene with 1 and 10% crude) were processed as follows: Extracted ion chromatogram (EIC) were collected in positive mode for Brij, and in positive and negative modes for Carboxylate,

with ammonium adduct $[M+NH_4]^+$ in positive mode. The width of ion-extraction window was set at 10 ppm. All surfactants homologues were monitored, *i.e.* 26 homologues for Brij and 20 for Carboxylates. The retention time, peak area, and their relative standard deviation (RSD) were computed from EIC.

3. Results and discussion

3.1. Study checkpoints

Before discussing the impact of solvent or crude oil on surfactants analysis, different checkpoints were done. As 52 injections were done with different compositions, the variation of the column backpressure was followed in order to detect any obstructions in the column. The overlay of the 52 pressure curves (blanks, QC and samples) illustrated in Figure 1 showed a perfect superposition, no variations were observed. From this point of view, the column was not damaged.

Figure 1: Overlay of the pressure monitored at the head of the mixed mode column for 52 injections

Moreover, blanks were inserted regularly to monitor the possibility of a memory effect. Indeed, due to their amphiphilic characters, surfactants could be adsorbed on the stationary phase but also in the different parts of the instruments³². Figure 2 shows the evolution of the global (summation

over all the homologues) peak areas for Brij (green) and Carboxylate (blue) homologues. No trend could be noticed. Moreover, the Figure S2 (Supplementary Data) demonstrates the stability of the column by showing the extracted ion chromatogram of Brij homolog with $m/z=744.5836$ obtained in twelve blanks (due to residual signal) and in one QC samples. In the blank, the residual signal of Brij and Carboxylates corresponded to less than 1% of the signal obtained in samples (QC, Surfactants in THF/toluene, Surfactants in THF/Toluene with crude). It was considered as acceptable for the present work.

Figure 2: Evolution of Brij (green) and Carboxylate (blue) peak area in blank injections with the average (m), limits are defined as average value $\pm 2 \times$ standard deviation according to the injection number (Analytical conditions: see section 2.3.)

3.2. Analysis of anionic and nonionic surfactants by UHPLC-HRMS in aqueous sample

In our previous work, anionic and nonionic surfactants were already analyzed²⁶. These two surfactants diluted in water, were separated in mixed mode using a Surfactant Plus column. In the present study, this separation served as Quality Control. This column provided both reverse phase and anion-exchange mechanisms. As expected, the retention order was the same as the one obtained in our previous work²⁶: Brij eluted between 9 and 12 min, Carboxylate families with an

alkyl chain of 11 carbons (noted C₁₁) eluted between 10 and 15 min, and with an alkyl chain of 13 carbons (noted C₁₃) between 12 and 17 min. The retention of homologues for Brij, C₁₁ Carboxylate and C₁₃ Carboxylate followed the decreasing number of EOs units. Ethylene oxide increased the hydrophilic character³³. Contrary to carboxylate homologues, the resolution between Brij homologues was poor. Figure 3 shows the obtained chromatograms for neutral and anionic surfactants (ammonium adducts, positive mode).

Figure 3: Overlay of mixed mode chromatograms of Carboxylate (blue-C₁₃ and red-C₁₁) and Brij (green) in UHPLC-HRMS (Analytical conditions: see section 2.3.)

3.3. Analysis of anionic and nonionic surfactants in THF/Toluene samples by UHPLC-HRMS

As explained in the introduction, THF and toluene are commonly used in the petroleum industry as diluents. Diluted in THF/Toluene, the separation of surfactants could be ruined owing to the difference of solvent strength between the injected sample and the mobile phase³⁰. Thus, the robustness and the stability of the separation had to be studied by injecting first THF/Toluene alone and then surfactants diluted in THF/Toluene.

Five injections with only THF/Toluene were carried out: one isolated and then quadruplicate. QC were injected between these two series in order to observe the impact on the QC separation. The retention times and peak areas were the same between QCs. No impact of the injection of a THF/Toluene mixture on the separation was observed. This might be explained by the injection of a small volume (3 μ L) with no remnant effect on the stationary phase.

Afterwards, injections of surfactants in the binary THF/Toluene were carried out in order to evaluate THF/Toluene impact on the separation. Five injections were done: one isolated and four in a row. Raw values for retention time, peak area and their relative standard deviation are recapped in Table S1 for Brij and in Table S2 for Carboxylate. RSDs of retention time and peak area above 20% are highlighted by bold italic style. In order to establish if differences between QC and Samples were significant, Analysis of Variance tests were carried out³⁴. Difference was considered significant when the p-value was lower than 0.05. In case the difference (Δ) was significant, it was estimated both in absolute units and relative value. Results are presented in Table 2 for Brij and in Table 3 for Carboxylate (both in positive ionization mode).

Table 1: ANOVA results for the homologues of Brij in THF/Toluene vs in water (QC) in positive ionization mode (* significant value $p < 0.05$)

Homologues			Retention time			Peak Area		
m/z	EO units	Tr (QC)	p-value	Δ (Samples-QC) Absolute Percentage (min)		p-value	Δ (Samples-QC) Absolute $\times 10^5$ Percentage	
392.374	3	11.70	0.0033*	-0.060	-0.51%	0.0005*	8.66	91%
436.400	4	11.40	0.0005*	-0.066	-0.58%	0.0005*	14.46	98%
480.426	5	11.15	0.0002*	-0.068	-0.61%	0.0003*	18.64	106%
524.453	6	10.93	0.0002*	-0.074	-0.68%	0.0003*	22.39	104%
568.479	7	10.74	0.0006*	-0.058	-0.54%	0.0002*	25.10	101%
612.505	8	10.57	0.0001*	-0.066	-0.62%	0.0001*	26.67	102%

656.531	9	10.41	0.0015*	-0.052	-0.50%	0.0001*	25.40	97%
700.557	10	10.27	0.0001*	-0.060	-0.58%	0.0001*	22.82	91%
744.584	11	10.14	0.0026*	-0.048	-0.47%	0.0001*	19.06	83%
788.610	12	10.02	0.0071*	-0.044	-0.44%	0.0001*	15.41	77%
832.636	13	9.91	0.0042*	-0.050	-0.50%	0.0001*	11.86	71%
876.662	14	9.80	0.0009*	-0.040	-0.41%	0.0001*	7.99	66%
920.688	15	9.71	0.0002*	-0.050	-0.51%	0.0001*	27.79	228%
964.715	16	9.62	0.0027*	-0.044	-0.46%	0.0001*	26.32	222%
1008.741	17	9.54	0.0010*	-0.044	-0.46%	0.0001*	21.27	215%
1052.767	18	9.46	0.0010*	-0.040	-0.42%	0.0001*	17.61	229%
1096.793	19	9.38	0.0004*	-0.038	-0.41%	0.0001*	11.79	207%
579.427	20	9.30	0.0094*	-0.024	-0.26%	0.0001*	4.04	52%
601.440	21	9.23	0.0820	/	/	0.0001*	2.52	48%
623.453	22	9.17	0.1894	/	/	0.0001*	1.48	45%
645.466	23	9.10	0.1280	/	/	0.0001*	0.87	44%
667.479	24	9.05	0.0101*	-0.028	-0.31%	0.0001*	0.50	42%
689.492	25	8.97	0.8465	/	/	0.0002*	0.20	30%
711.506	26	8.93	0.7707	/	/	0.0002*	0.09	24%
733.519	27	8.87	0.5274	/	/	0.0007*	0.07	45%
755.532	28	8.81	0.8961	/	/	0.0313*	0.02	54%

Table 2: ANOVA results for the homologues of Carboxylate in THF/Toluene vs in water (QC) in positive ionization mode (* significant value $p < 0.05$)

Homologues			Retention time			Peak Area		
m/z	EO unit s	Tr (Qc)	p-value	Δ (Samples-QC) Absolute Percentage (min)		p-value	Δ (Samples-QC) Absolute $\times 10^5$ Percentage	
350.291	3	15.00	0.0004*	-0.082	-0.54%	0.0027*	0.86	6.9%
394.317	4	14.20	0.0002*	-0.077	-0.54%	0.0010*	4.50	8.6%
438.343	5	13.58	0.0002*	-0.077	-0.57%	0.0178*	3.50	5.9%
482.369	6	13.04	0.0008*	-0.064	-0.49%	0.0685	/	/
526.395	7	12.58	0.0018*	-0.064	-0.51%	0.9786	/	/
570.422	8	12.19	0.0012*	-0.044	-0.36%	0.0171*	1.66	7.3%
614.448	9	11.84	0.0032*	-0.052	-0.44%	0.0001*	2.44	16%
658.474	10	11.53	0.0084*	-0.056	-0.49%	0.0001*	2.49	27%
702.500	11	11.25	0.0056*	-0.047	-0.42%	0.0001*	1.92	38%
746.527	12	11.00	0.0066*	-0.051	-0.46%	0.0001*	1.43	51%

Homologues			Retention time			Peak Area		
m/z	EO unit s	Tr (Qc)	p-value	$\Delta(\text{Samples-QC})$ Absolute Percentage (min)		p-value	$\Delta(\text{Samples-QC})$ Absolute $\times 10^5$ Percentage	
378.322	3	17.15	0.0001*	-0.091	-0.53%	nd	nd	nd
422.348	4	16.29	0.0001*	-0.113	-0.69%	0.0005*	0.46	11%
466.374	5	15.60	0.0004*	-0.085	-0.55%	0.0011*	1.54	9.4%
510.401	6	15.02	0.0004*	-0.057	-0.38%	0.0021*	1.70	9.0%
554.427	7	14.54	0.0009*	-0.078	-0.54%	0.2097	/	/
598.453	8	14.10	0.0005*	-0.065	-0.46%	0.3464	/	/
642.479	9	13.71	0.0050*	-0.065	-0.47%	0.0037*	0.12	1.7%
686.505	10	13.39	0.0068*	-0.060	-0.45%	0.0001*	0.44	9.4%
730.532	11	13.08	0.0013*	-0.069	-0.52%	0.0001*	0.57	19%
774.558	12	12.79	0.0053*	-0.056	-0.44%	0.0001*	0.48	29%

3.3.1. THF/Toluene impact on retention time

Repeatability

Within a group of samples, *i.e.* over the 5 repetitions of surfactants in THF/Toluene, repeatability of retention time was good (Table S1 and Table S2). RSDs ranged between 0.05% and 0.38% for all homologues and both surfactants. These RSD were similar to those of QC (ranging from 0.13 to 0.34%). Then, retention time between QC and Surfactants in THF/Toluene samples were compared.

Brij homologues (Table 2)

A significant but slight diminution of retention times was observed for the whole distribution. A 2 ways ANOVA showed that these differences were dependent on the homologue considered. Therefore, for example, homologues with 10 and 26 EO units did not exhibit the same behavior. The difference was significant (p-value: $1.0 \cdot 10^{-4} < 0.05$) for $\text{C}_{16}\text{H}_{23}\text{EO}_{10}\text{OH}$ (m/z=700.557) and was not significant (p-value: $0.77 > 0.05$) for $\text{C}_{16}\text{H}_{33}\text{EO}_{26}\text{OH}$ (m/z=711.506). Globally the differences

ranged from 0 (in case it was not significant) to -0.68% ($C_{16}H_{33}EO_6OH$ with $m/z=524.453$ at 10.93 min).

Carboxylates homologues (Table 3)

A significant but slight diminution of retention time was also observed for the whole distribution. Contrary to Brij homologues, this diminution was independent of the homologue considered and could be, in average, estimated at -0.07 min, which is in fact very few.

Globally, this retention time diminution was quite moderate, and remained within the usual variability of retention times in chromatography.

3.3.2. THF/Toluene impact on peak area

Repeatability

Over the 5 repetitions of the surfactants diluted in THF/Toluene, repeatability of peak area was satisfactory: RSDs ranged from 0.8% to 10.0% for both surfactants with a pooled RSD $\approx$ 5.5%. Most of the RSDs did not exceed 6%, but homologues at the end of the distribution presented a high RSD (up to 20%). For example, 17.8%, for $C_{16}H_{33}EO_{28}OH$ ($m/z=755.532$) and 11.5% for $C_{13}H_{27}EO_9COO^-$ ($m/z=623.437$). This behavior was not surprising because the highest RSDs concerned low intensity signals. So, a slight absolute variation could mechanically induce high RSDs values. Repeatability of Brij peak areas in QC samples was not as good as expected: RSD were between 20 and 36% for homologues with EO unit number ranging from 3 to 12, which is higher than the RSDs observed with THF/Toluene Samples. Indeed, a signal drop was observed for these homologues throughout the sequence. Blanks, added before each sample injection, showed a lower signal (10^4 for Brij and 10^5 for Carboxylate). One possible explanation could be the MS source fouling. Indeed, after this study, a cleaning step had to be added to recover the

original signal. To avoid this problem, a control chart based on QC samples could be used to determine when a cleaning procedure must be implemented.

Brij homologues (Table 2)

When comparing THF/Toluene and QC samples, a significant increase of the peak area was observed for the whole distribution from 24% ($C_{16}H_{33}EO_{26}OH$ with $m/z=711.506$) to 229% ($C_{16}H_{33}EO_{18}OH$ with $m/z=1052.767$); in other words, the signal increase by factor of 1.2 to 3.3. For example, the peak area of $C_{16}H_{33}EO_{19}OH$ ($m/z=1096.763$) in QC was $5.69 \cdot 10^6$ whereas it was $1.75 \cdot 10^7$ in THF/Toluene. This increase could be induced by a solvent effect due to THF/Toluene presence. At this moment, we do not have any explanation concerning this effect. Consequently, for quantitative analysis, calibration must be done within the same matrix in order to take into account this effect.

Carboxylates homologues (Table 3).

When comparing THF/Toluene and QC samples, a significant increase (but less pronounced than for Brij) of the peak area was observed globally and could reach up to 51% ($C_{11}H_{23}EO_{12}COOH$ with $m/z=746.527$). A 2 ways ANOVA showed that these differences were dependent of the homologue considered. For example, homologues with 5 and 6 EO units did not exhibit the same behavior. The difference was significant (p-value: $0.0178 < 0.05$) for $C_{11}H_{23}EO_5COOH$ ($m/z=438.343$) and was not significant (p-value: $0.0685 > 0.05$) for $C_{11}H_{23}EO_6COOH$ ($m/z=482.369$).

In conclusion, retention times of surfactants diluted in THF/Toluene were marginally affected but, the signal of nonionic surfactants showed a considerable “matrix” effect causing an exacerbation of the signal.

3.4. Analysis of anionic and nonionic surfactants in oil matrix by UHPLC-HRMS at 1% of crude oil

In view of the results obtained for injection of surfactants in the THF/Toluene, samples containing crude were studied because real petroleum fluids, for example produced water during hydraulics fracturing process, may sometimes contain traces to percent of crude oil. In order to be as close as possible to samples containing crude oil, 1% in mass of crude oil was added to THF/Toluene. The surfactants concentrations were unchanged, 0.5 mg.L^{-1} . As in the previous part, the impact of crude oil on the separation was studied by comparing retention times and signals between aqueous (QC) and oil containing samples (Crude1%). The overlays of the extracted ion chromatograms obtained in positive mode for Brij (Figure 4-A) and the carboxylate family in C_{11} (Figure 4-B) in the three samples are given in Figure 4.

Figure 4: Overlay of mixed mode chromatograms (A) Brij and (B) Carboxylate C_{11} in (black) QC, (red) Crude 1% and (green) Crude 10%. (Analytical conditions are recapped in section 2.3.)

Samples in THF/Toluene with 1% of crude oil were injected 6 times: one single, then five in a row. Raw values for retention time, peak area and their relative standard deviation are recapped in Table S3 for Brij and in Table S4 for Carboxylate. RSDs of retention time and peak area above 20% are highlighted by bold italic style. Differences between QC and Samples were again evaluated by Analysis of Variance tests. Differences were considered significant when the p-value was lower than 0.05. In case the difference (Δ) was significant, it was estimated both in absolute units and relative value. Results are presented in Table 4 for Brij and in Table 5 for Carboxylate (both in positive ionization mode).

Table 3-3: ANOVA results for the homologues of Brij in THF/Toluene with 1% of Crude oil vs in water (QC) in positive ionization mode (* significant value $p < 0.05$)

Homologues			Retention time			Peak Area		
m/z	EO units	Tr (QC)	p-value	Δ (Samples-QC) Absolute Percentage (min)		p-value	Δ (Samples-QC) Absolute $\times 10^5$ Percentage	
392.374	3	11.70	0.0008*	-0.069	-0.59%	0.0001*	17.07	689%
436.400	4	11.40	0.0010*	-0.062	-0.54%	0.0001*	24.15	632%
480.426	5	11.15	0.0038*	-0.057	-0.51%	0.0001*	33.72	666%
524.453	6	10.94	0.0029*	-0.060	-0.55%	0.0001*	40.87	598%
568.479	7	10.74	0.0001*	-0.056	-0.52%	0.0001*	42.89	481%
612.505	8	10.57	0.0002*	-0.061	-0.57%	0.0001*	43.03	399%
656.531	9	10.42	0.0001*	-0.059	-0.56%	0.0001*	39.38	322%
700.557	10	10.27	0.0005*	-0.051	-0.49%	0.0001*	34.49	267%
744.584	11	10.14	0.0040*	-0.043	-0.43%	0.0001*	28.26	215%
788.610	12	10.03	0.0003*	-0.056	-0.56%	0.0001*	22.34	180%
832.636	13	9.92	0.0002*	-0.060	-0.60%	0.0001*	16.96	155%
876.662	14	9.82	0.0002*	-0.053	-0.54%	0.0001*	11.07	132%
920.688	15	9.72	0.0001*	-0.057	-0.59%	0.0001*	34.01	381%
964.715	16	9.63	0.0009*	-0.039	-0.41%	0.0001*	30.84	346%
1008.741	17	9.55	0.0001*	-0.045	-0.48%	0.0001*	24.89	320%
1052.767	18	9.47	0.0002*	-0.047	-0.50%	0.0001*	18.87	311%
1096.793	19	9.38	0.0012*	-0.038	-0.40%	0.0001*	13.49	293%
579.427	20	9.32	0.0007*	-0.048	-0.52%	0.0001*	5.69	89%
601.440	21	9.24	0.0002*	-0.039	-0.43%	0.0001*	3.17	72%

623.453	22	9.18	0.0126*	-0.037	-0.41%	0.0001*	2.08	74%
645.466	23	9.12	0.0953	/	/	0.0001*	1.19	70%
667.479	24	9.06	0.0520	/	/	0.0002*	0.59	58%
689.492	25	9.01	0.0172*	-0.044	-0.49%	0.0001*	0.41	75%
711.506	26	8.94	0.4918	/	/	0.0106*	0.21	79%
733.519	27	8.90	0.0249*	-0.037	-0.41%	0.0064*	0.06	34%
755.532	28	8.85	0.1496	/	/	0.3728	/	/

Table 4: ANOVA results for the homologues of Carboxylate in THF/Toluene with 1% of Crude oil vs in water (QC) in positive ionization mode (* significant value $p < 0.05$)

Homologues			Retention time			Peak Area		
m/z	EO units	Tr (Qc)	p-value	$\Delta(\text{Samples-QC})$ Absolute Percentage (min)		p-value	$\Delta(\text{Samples-QC})$ Absolute $\times 10^5$ Percentage	
350.291	3	15.01	nd	nd	nd	nd	nd	nd
394.317	4	14.18	0.0011*	-0.058	-0.41%	0.0001*	-7.08	-13%
438.343	*5	13.55	0.0005*	-0.050	-0.37%	0.0001*	-14.98	-24%
482.369	6	13.03	0.0002*	-0.056	-0.43%	0.0001*	-20.32	-43%
526.395	7	12.59	0.0134*	-0.064	-0.51%	0.0001*	-12.97	-38%
570.422	8	12.19	0.0018*	-0.056	-0.46%	0.0001*	-11.62	-49%
614.448	9	11.82	0.0166*	-0.030	-0.26%	0.0001*	-7.73	-50%
658.474	10	11.51	0.1404	/	/	0.0001*	-5.31	-56%
702.500	11	11.23	0.1219	/	/	0.0001*	-3.12	-57%
746.527	12	10.99	0.0332*	-0.035	-0.32%	0.0001*	-2.06	-70%
378.322	3	17.18	nd	nd	nd	nd	nd	nd
422.348	4	16.27	0.0007*	-0.078	-0.48%	0.9103	/	/
466.374	5	15.61	0.0001*	-0.093	-0.60%	0.0001*	-3.25	-17%
510.401	6	15.01	0.0031*	-0.063	-0.42%	0.0001*	-9.10	-61%
554.427	7	14.53	0.0022*	-0.080	-0.55%	0.0001*	-4.04	-37%
598.453	8	14.11	0.0102*	-0.079	-0.56%	0.0001*	-4.00	-51%
642.479	9	13.72	0.0336*	-0.049	-0.36%	0.0001*	-2.44	-49%
686.505	10	13.39	0.0047*	-0.065	-0.49%	0.0001*	-1.48	-47%
730.532	11	13.05	0.3260	/	/	0.0001*	-0.98	-53%
774.558	12	12.82	0.0322*	-0.075	-0.58%	0.0001*	-0.74	-76%

3.4.1. Impact of crude at 1% in mass on retention time

Repeatability

Within a group of samples, *i.e.* over the 6 repetitions of surfactants in THF/Toluene with 1% crude oil, repeatability of retention times was good (Table S3 and Table S4): RSD of retention time was between 0.04% and 0.49% (with a pooled value at 0.20%) for both surfactants. These RSD were similar to those of QC (0.02 to 0.41%). Then, retention times between QC and Surfactants in crude at 1% in mass samples were compared.

Brij homologues (Table 4)

A significant but slight diminution of the retention time was observed for nearly the whole distribution from -0.40% ($C_{16}H_{33}EO_{19}OH$ with $m/z=1096.793$ at 9.30 min) to -0.60% ($C_{16}H_{33}EO_{15}OH$ with $m/z=920.688$ at 9.72 min). A 2 ways ANOVA showed that these differences were dependent on the homologue considered. Therefore, for example, homologues with 10 and 26 EO units did not exhibit the same behavior. The difference was significant (p-value: $1.0 \cdot 10^{-4} < 0.05$) for $C_{16}H_{33}EO_{10}OH$ ($m/z=700.557$) and was not significant (p-value: $0.49 > 0.05$) for $C_{16}H_{33}EO_{26}OH$ ($m/z=711.506$).

Carboxylates homologues (Table 5)

A significant but slight diminution of retention time was also observed for nearly the whole distribution from -0.36% ($C_{11}H_{23}EO_8COOH$ with $m/z=570.422$) to -0.69% ($C_{13}H_{27}EO_4COOH$ with $m/z=422.348$). A 2 ways ANOVA showed that these differences were dependent of the homologue considered. Therefore, for example, C_{11} carboxylate homologues with 9 and 10 EO units did not exhibit the same behavior. The difference was significant (p-value: $0.02 < 0.05$) for $C_{11}H_{23}EO_9COOH$ ($m/z=614.448$) and was not significant (p-value: $0.14 > 0.05$) for $C_{11}H_{23}EO_{10}COOH$ ($m/z=658.474$).

As in the THF/toluene section, despite these differences were significant, they were quite moderate from an analytical point of view because they were within the usual variability of retention times in chromatography.

3.4.2. Impact of crude at 1% in mass on peak area

Repeatability

Over the 6 repetitions of the surfactants diluted in THF/Toluene with 1% of crude oil, the variation of the repeatability of peak areas was rather low. Indeed, for nonionic surfactant, RSDs did not exceed 5% for homologues with EO units number ranging from 3 to 25. Above 25 EO units, RSD raised from 8% to 25% probably because the absolute signal was low. This evolution was also observed for carboxylate distribution. Most of the RSDs did not exceed 6%, but homologues with only three EO units were not detected, and the homologues with EO units' number above 12 had RSD above 6%.

Brij homologues (Table 4)

A significant increase of the peak area was observed for the whole distribution from 34% ($C_{16}H_{33}EO_{27}OH$ with $m/z=733.519$) to 689% ($C_{16}H_{33}EO_3OH$ with $m/z=382.374$); in other words, the signal increased by a factor of 1.3 to 7.9. This difference was significant for all the homologues because all p-value were below 0.05 except the last one. For example, $C_{16}H_{33}EO_9OH$ ($m/z=656.531$) peak area in QC was $1.22 \cdot 10^6$ whereas in THF/Toluene with 1% of crude, it was $5.16 \cdot 10^6$. This increase could be induced by a matrix effect or/and crude compounds which had the same m/z contributed to signal. A resolution of 70,000 at $m/z=200$ allows a distinction at 0.003 u.a., which in this case, was not sufficient to differentiate Brij homologues from oil compounds. Using a Ultra Higher Resolution Mass Spectrometer could remove these confusions³⁵⁻³⁷. Indeed,

Tsybin's group worked with a mass spectrometry resolution approaching to 700 000 @m/z 200 to analyze organic pollutants by GC-MS³⁸. Their Ultra-High-Resolution allowed distinction at 0.0003 u.a. UHMRS could rule out the compound's contribution hypothesis.

Consequently, for quantitative analysis, calibration must be done within the same matrix in order to consider this effect.

Carboxylates homologues (Table 5)

A significant but slight diminution of the peak area was observed for the whole distribution from -17% (C₁₃H₂₇EO₅COOH with m/z=466.374) to -70% (C₁₃H₂₇EO₁₂COOH with m/z=774.558). A 2 ways ANOVA showed that, in absolute value, these differences were independent of the considered homologue. The presence of crude oil affected the signal by ion suppression or/and because of a possible instrument fouling.

In conclusion, crude at 1% of mass in the matrix did not affect critically the separation. However, its impact on the signal was significant, and its magnitude could be problematic. Nonionic surfactant signal was exacerbated (by a factor up to 8) by matrix effect or/and crude compounds contribution. Anionic surfactant signal was suppressed (by a factor up to 2) by matrix effect and instrument fouling.

3.5. Analysis of anionic and nonionic surfactants in oil matrix by UHPLC-HRMS at 10% of crude oil

Then, the concentration in crude oil added to THF/Toluene was increased to 10% in mass. The surfactants concentrations were unchanged, 0.5 mg.L⁻¹. As in the previous part, the impact of 10% in mass of crude oil on the separation was studied by comparing retention times and signals between aqueous (QC) and oil containing samples (Crude10%). The overlays of the extracted ion

chromatograms obtained in positive mode for Brij (Figure 4-A) and the carboxylate family in C₁₁ (Figure 4-B) in the three samples are given in Figure 4.

As samples in THF/Toluene with 1% of crude oil, samples with 10% of crude oil were injected 6 times: one single, then five in a row. Raw values for retention time, peak area and their relative standard deviation are recapped in Table S5 for Brij and in Table S6 for Carboxylate. RSDs of retention time and peak area above 20% are highlighted by bold italic style. Here again ANOVA tests were used to determine if differences were significant. In case the difference (Δ) was significant, it was estimated both in absolute units and relative value. Results are presented in Table 6 for Brij and in Table 7 for Carboxylate (both in positive ionization mode).

Table 5: ANOVA results for the homologues of Brij in THF/Toluene with 10% of Crude oil vs in water (QC) in positive ionization mode (* significant value $p < 0.05$)

Homologues			Retention time			Peak Area		
m/z	EO unit s	Tr Qc	p-value	Δ (Samples-QC) Absolute Percentage (min)		p-value	Δ (Samples-QC) Absolute $\times 10^5$ Percentage	
392.374	3	11.73	0.0001*	-0.079	-0.67%	0.0001*	15.41	1278%
436.400	4	11.42	<i>nd</i>	<i>nd</i>	<i>nd</i>	<i>nd</i>	<i>nd</i>	<i>nd</i>
480.426	5	11.17	0.0001*	-0.076	-0.68%	0.0001*	32.80	1044%
524.453	6	10.95	0.0001*	-0.067	-0.61%	0.0001*	39.90	921%
568.479	7	10.75	0.0001*	-0.063	-0.59%	0.0001*	41.04	705%
612.505	8	10.58	0.0008*	-0.061	-0.58%	0.0001*	47.81	674%
656.531	9	10.44	0.0001*	-0.070	-0.67%	0.0001*	41.68	523%
700.557	10	10.29	0.0001*	-0.066	-0.64%	0.0001*	39.76	456%
744.584	11	10.16	0.0001*	-0.066	-0.65%	0.0001*	32.96	369%
788.610	12	10.03	0.0001*	-0.057	-0.57%	0.0001*	26.53	312%
832.636	13	9.93	0.0044*	-0.055	-0.56%	0.0001*	21.95	288%
876.662	14	9.83	0.0002*	-0.057	-0.58%	0.0001*	14.51	242%
920.688	15	9.73	0.0001*	-0.049	-0.50%	0.0001*	36.70	554%
964.715	16	9.64	0.0001*	-0.051	-0.53%	0.0001*	30.91	456%
1008.741	17	9.55	0.0023*	-0.038	-0.39%	0.0001*	23.75	399%
1052.767	18	9.47	0.0431*	-0.027	-0.29%	0.0001*	17.20	354%

Homologues			Retention time			Peak Area		
m/z	EO units	Tr Qc	p-value	$\Delta(\text{Samples-QC})$ Absolute Percentage (min)		p-value	$\Delta(\text{Samples-QC})$ Absolute $\times 10^5$ Percentage	
1096.793	19	9.39	0.0123*	-0.035	-0.38%	0.0001*	12.50	344%
579.427	20	9.32	0.0389*	-0.030	-0.32%	0.0001*	6.49	132%
601.440	21	9.26	0.5255	/	/	0.0003*	2.81	83%
623.453	22	9.18	0.4523	/	/	0.0002*	2.35	107%
645.466	23	9.13	0.0055*	-0.033	-0.37%	0.0003*	2.35	161%
667.479	24	9.06	0.7524	/	/	0.1098	/	/
689.492	25	8.99	0.9461	/	/	0.0471*	0.29	62%
711.506	26	8.96	0.3477	/	/	0.6840	/	/
733.519	27	8.91	0.5092	/	/	0.0192*	-0.03	-29%
755.532	28	8.84	nd	nd	nd	nd	nd	nd

Table 6: ANOVA results for the homologues of Carboxylate in THF/Toluene with 10% of Crude oil vs in water (QC) in positive ionization mode (* significant value $p < 0.05$)

Homologues			Retention time			Peak Area		
m/z	EO units	Tr Qc	p-value	$\Delta(\text{Samples-QC})$ Absolute Percentage (min)		p-value	$\Delta(\text{Samples-QC})$ Absolute $\times 10^5$ Percentage	
350.291	3	15.02	nd	nd	nd	nd	nd	nd
394.317	4	14.20	0.0004*	-0.062	-0.43%	0.0001*	-20.67	-38%
438.343	5	13.57	0.0001*	-0.067	-0.49%	0.0001*	-10.22	-17%
482.369	6	13.03	0.0049*	-0.042	-0.32%	0.0001*	-33.58	-72%
526.395	7	12.59	0.0398*	-0.050	-0.40%	0.0001*	-4.93	-14%
570.422	8	12.19	0.0243*	-0.045	-0.37%	0.0001*	-7.43	-31%
614.448	9	11.87	0.0065*	-0.075	-0.63%	0.0002*	-2.65	-17%
658.474	10	11.54	0.0058*	-0.044	-0.38%	0.3546	/	/
702.500	11	11.25	0.1810	/	/	0.8014	/	/
746.527	12	11.00	0.7303	/	/	0.0074*	-0.94	-32%
378.322	3	17.16	nd	nd	nd	nd	nd	nd
422.348	4	16.28	0.0010*	-0.087	-0.54%	0.0001*	-12.27	-75%
466.374	5	15.61	0.0028*	-0.085	-0.55%	0.0001*	-4.92	-26%
510.401	6	15.01	nd	nd	nd	nd	nd	nd
554.427	7	14.55	0.0090*	-0.078	-0.54%	0.0001*	-3.59	-34%
598.453	8	14.13	0.0056*	-0.074	-0.52%	0.0001*	-6.03	-79%
642.479	9	13.71	0.0076*	-0.050	-0.36%	0.0001*	-1.29	-26%

686.505	10	13.37	0.2362	/	/	0.0157*	-1.09	-35%
730.532	11	13.12	0.0680	/	/	0.6836	/	/
774.558	12	12.81	0.8077	/	/	0.4961	/	/

3.5.1. Impact of crude at 10% in mass on retention time

Repeatability

Within a group of samples, *i.e.* over the 6 repetitions of surfactants in THF/Toluene with 1% crude oil, repeatability of retention time was good (Table S5 and Table S6): RSD of retention time was between 0.06% and 0.68% (with pooled value at 0.25%) for both surfactants. These RSDs were similar to those of QC (0.02 to 0.41%). Then, retention time between QC and Surfactants in crude at 10% in mass samples were compared.

Brij homologues (Table 6)

A significant but slight diminution of the retention times was observed for the whole distribution and could reach up to -0.68% ($C_{16}H_{33}EO_5OH$ with $m/z=480.426$ at 11.17 min). A 2 ways ANOVA showed that these differences were dependent of the homologue considered: For example, homologues with 10 and 26 EO units did not exhibit the same behavior; the difference was significant ($p\text{-value: } 1.0 \cdot 10^{-4} < 0.05$) for $C_{16}H_{33}EO_{10}OH$ ($m/z=700.557$), and was not significant ($p\text{-value: } 0.35 > 0.05$) for $C_{16}H_{33}EO_{26}OH$ ($m/z=711.506$).

Carboxylates homologues (Table 7)

A significant but slight diminution of retention time was observed for the whole distribution and could reach up to -0.63% ($C_{11}H_{23}EO_9COOH$ with $m/z=614.448$). A 2 ways ANOVA showed that these differences were also dependent of the homologue considered: For example, C_{11} carboxylate homologues with 10 and 11 EO units did not exhibit the same behavior. The difference was

significant (p-value: $0.005 < 0.05$) for $C_{11}H_{23}EO_{10}COOH$ ($m/z=658.474$), and was not significant (p-value: $0.18 > 0.05$) for $C_{11}H_{23}EO_{11}COOH$ ($m/z=702.500$).

As observed for samples in THF/Toluene and THF/Toluene with 1% of crude, these differences remained moderate and acceptable because they were within the usual variability of retention times in chromatography.

3.5.2. Impact of crude at 10% in mass on peak area

Repeatability

For surfactants diluted in THF/Toluene with 10% of crude oil, the repeatability RSD of peak area increased for both surfactants (compared with QC repeatability). Indeed, for nonionic surfactant (Table S5), RSDs ranged between 3.3% and 60.8% with a pooled value at 14.1%. For carboxylate (Table S6), RSD ranged between 1.6% and 43.5% with a pooled value at 14.1%. As observed with 1% of crude oil, homologues with high EO numbers ($24 < EO$ for Brij and $11 < EO$ for Carboxylate) presented a high variability ($20\% < RSD < 60\%$) due to accumulation of instrument fouling, matrix effect and homologues initially absolute signal.

Brij homologues (Table 6)

A significant change of the peak area was observed for the whole distribution from -29% ($C_{16}H_{33}EO_{27}OH$ with $m/z=733.519$) to 1275% ($C_{16}H_{33}EO_3OH$ with $m/z=382.374$), in other words, the signal could decrease or increase by factor up to 13.8. This difference was dependent of the homologue considered. Indeed, homologues with more than 24 EO units showed a quite different behavior. The homologue $C_{16}H_{33}EO_5OH$ (with $m/z=480.426$) had a p-value at $1.0 \cdot 10^{-4}$ whereas the homologue $C_{16}H_{33}EO_{27}OH$ (with $m/z=733.519$) had a p-value at 0.02. With the same example as in the previous section (with 1% of crude oil), the peak area of the $C_{16}H_{33}EO_9OH$ homologue

($m/z=656.531$) increased by a factor of 4 in samples with 1% whereas with 10% of crude, it increased by a factor of 6 ($7.98 \cdot 10^6$ vs $4.97 \cdot 10^7$). The explanation of this enhanced raise was the same as the previous part: matrix effect or/and contribution of crude compounds in signal.

Carboxylates homologues (Table 7)

A significant decrease of peak areas was observed for the whole distribution, and could reach up to 79% ($C_{13}H_{27}EO_8COOH$ with $m/z=598.453$). A 2 ways ANOVA showed that these differences were dependent of the homologue considered. The difference was not significant for homologues with EO units' number above 10. This behavior could confirm the matrix effect observed at 1% and ion suppression or/and instrument fouling. However, this behavior is ionization mode dependent as, in negative mode, peak areas increased by a factor up to 12 (Results not shown). This phenomenon was not observed with 1% of crude. It could be the result of the matrix effect which affected anionic surfactant at higher level of crude oil.

In conclusion, surprisingly, 10% of crude in the matrix still did not strongly affect retention times of TA homologues. However, the impact of 10% crude oil on the signal intensity was more important than that of 1% crude oil for both surfactants and it was found to be strongly related to the ionization mode. In positive mode, nonionic surfactant signal was exacerbated (by a factor up to 14) by matrix effect or/and crude compounds contribution. Anionic surfactant signal was suppressed (by a factor up to 5) in positive mode and exacerbated (by a factor up to 12) in negative mode. This variation of the signal intensity, caused by an important matrix effect, was compound and ionization mode dependent.

4. Conclusion

Faced to these results, several conclusions can be drawn. A good and unexpected point is that the presence of organic solvent (THF/Toluene), but also of crude oil, whatever the level (1 or 10% in mass), did not critically affect the separation because retention times were very similar to those obtained in aqueous samples (QC). However, the presence of an organic phase had an important (and difficult to predict) impact on the MS signal, which was even more evident with crude oil. Depending on the mode and the surfactant (nonionic or anionic), the signal could be exacerbated or suppressed. Despite these effects, semi-quantitative analysis could be considered if a rather large uncertainty on the mass balance could be accepted.

Several strategies to limit these phenomena can be proposed. When the crude level is above 1%, dilutions should be advised. In order to limit the risk of instrument fouling (both column and mass spectrometer), a rinsing step could be added. Column could be rinsed using a back flush with a strong organic solvent (like THF). QC injections must be made before and after, in order to check the effectiveness of rinsing but also the resistance of the stationary phase. At the same time, infusion in mass spectrometer with for example MeOH or Isopropanol could be done in order to clean the probe and the transfer line. Moreover, it could be very interesting to study other ionization modes (such as APPI or APCI) as they should be less prone to matrix effects ³⁹. Regardless, the main conclusion was that qualitative analysis could be done in oil matrix.

For an accurate quantitative analysis, even with a HRMS, several precautions must be taken. So, a calibration in the matrix is unavoidable if real petroleum samples are considered. Approach by standard addition method could be investigated.

The present study was a first insight into the behavior of the HRMS hyphenated to LC for the analysis of surfactants in petroleum matrices. It should be of interest to complete this study by an investigation of the long-term impact of crude oil in a routine use.

AUTHOR INFORMATION

Corresponding Author

* Email address: alizee.dufour@espci.psl.eu (Alizee Dufour)

ORCID

DUFOUR Alizée: 0000-0003-0014-2351

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ACKNOWLEDGMENT

This work was a collaboration between TOTAL S.A. at Lacq, France and the École Supérieure de Physique et de Chimie Industrielles de la ville de Paris (ESPCI-Paris), France. The authors acknowledge TOTAL S.A. for the permission to publish these results as well as for the financial support.

REFERENCES

- (1) Lin, I.J.; Marszall, L. CMC, HLB, and effective chain length of surface-active anionic and cationic substances containing oxyethylene groups. *Journal of Colloid and Interface Science* **1976**, *57* (1), 85–93. DOI: 10.1016/0021-9797(76)90178-8.
- (2) Yan, L.-m.; Li, Y.-l.; Cui, Z.-g.; Song, B.-l.; Pei, X.-m.; Jiang, J.-z. Performances of Guerbet Alcohol Ethoxylates for Surfactant–Polymer Flooding Free of Alkali. *Energy Fuels* **2017**, *31* (9), 9319–9327. DOI: 10.1021/acs.energyfuels.7b01843.
- (3) Ma, Q.; Zhang, Y.; Zhai, J.; Chen, X.; Du, Z.; Li, W.; Bai, H. Characterization and analysis of non-ionic surfactants by supercritical fluid chromatography combined with ion mobility spectrometry-mass spectrometry. *Analytical and bioanalytical chemistry* **2019**, *411* (13), 2759–2765. DOI: 10.1007/s00216-019-01777-3.
- (4) Belgacem, N.; Gandini, A., Eds. *Monomers, polymers and composites from renewable resources*; Elsevier, 2008.
- (5) Beneventi, D.; Carre, B.; Gandini, A. Role of surfactant structure on surface and foaming properties. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2012**, *410* (1-10).
- (6) Jarrahan, K.; Seiedi, O.; Sheykhan, M.; Sefti, M. V.; Ayatollahi, S. Wettability alteration of carbonate rocks by surfactants: A mechanistic study. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2012**, *410*, 1–10. DOI: 10.1016/j.colsurfa.2012.06.007.
- (7) Bai, Y.; Xiong, C.; Shang, X.; Xin, Y. Experimental Study on Ethanolamine/Surfactant Flooding for Enhanced Oil Recovery. *Energy Fuels* **2014**, *28* (3), 1829–1837. DOI: 10.1021/ef402313n.

- (8) Saxena, N.; Kumar, A.; Mandal, A. Adsorption analysis of natural anionic surfactant for enhanced oil recovery: The role of mineralogy, salinity, alkalinity and nanoparticles. *Journal of Petroleum Science and Engineering* **2019**, *173*, 1264–1283. DOI: 10.1016/j.petrol.2018.11.002.
- (9) Negin, C.; Ali, S.; Xie, Q. Most common surfactants employed in chemical enhanced oil recovery. *Petroleum* **2017**, *3* (2), 197–211. DOI: 10.1016/j.petlm.2016.11.007.
- (10) Mahdavi, S. Z.; Aalaie, J.; Miri, T.; Razavi, S.M.R.; Rahmani, M. R. Study of polyacrylamide-surfactant system on the water–oil interface properties and rheological properties for EOR. *Arabian Journal of Chemistry* **2017**, *10* (8), 1136–1146. DOI: 10.1016/j.arabjc.2016.05.006.
- (11) Olajire, A. A. Review of ASP EOR (alkaline surfactant polymer enhanced oil recovery) technology in the petroleum industry: Prospects and challenges. *Energy* **2014**, *77*, 963–982. DOI: 10.1016/j.energy.2014.09.005.
- (12) Raffa, P.; Broekhuis, A. A.; Picchioni, F. Polymeric surfactants for enhanced oil recovery: A review. *Journal of Petroleum Science and Engineering* **2016**, *145*, 723–733. DOI: 10.1016/j.petrol.2016.07.007.
- (13) Heyob, K. M.; Blotevogel, J.; Brooker, M.; Evans, M. V.; Lenhart, J. J.; Wright, J.; Lamendella, R.; Borch, T.; Mouser, P. J. Natural Attenuation of Nonionic Surfactants Used in Hydraulic Fracturing Fluids: Degradation Rates, Pathways, and Mechanisms. *Environmental science & technology* **2017**, *51* (23), 13985–13994. DOI: 10.1021/acs.est.7b01539.

- (14) Thurman, E. M.; Ferrer, I.; Blotevogel, J.; Borch, T. Analysis of hydraulic fracturing flowback and produced waters using accurate mass: identification of ethoxylated surfactants. *Analytical chemistry* **2014**, *86* (19), 9653–9661. DOI: 10.1021/ac502163k.
- (15) Al-Roomi, Y.; George, R.; Elgibaly, A.; Elkamel, A. Use of a novel surfactant for improving the transportability/transportation of heavy/viscous crude oils. *Journal of Petroleum Science and Engineering* **2004**, *42* (2-4), 235–243. DOI: 10.1016/j.petrol.2003.12.014.
- (16) García, R. A.; Chiaia-Hernández, A. C.; Lara-Martin, P. A.; Loos, M.; Hollender, J.; Oetjen, K.; Higgins, C. P.; Field, J. A. Suspect Screening of Hydrocarbon Surfactants in AFFFs and AFFF-Contaminated Groundwater by High-Resolution Mass Spectrometry. *Environmental science & technology* **2019**, *53* (14), 8068–8077. DOI: 10.1021/acs.est.9b01895.
- (17) Eide, I.; Zahlsen, K. A Novel Method for Chemical Fingerprinting of Oil and Petroleum Products Based on Electrospray Mass Spectrometry and Chemometrics. *Energy Fuels* **2005**, *19* (3), 964–967. DOI: 10.1021/ef049743m.
- (18) Eide, I.; Zahlsen, K.; Kummernes, H.; Neverdal, G. Identification and Quantification of Surfactants in Oil Using the Novel Method for Chemical Fingerprinting Based on Electrospray Mass Spectrometry and Chemometrics. *Energy Fuels* **2006**, *20* (3), 1161–1164. DOI: 10.1021/ef050423s.
- (19) Zembrzuska, J.; Budnik, I.; Lukaszewski, Z. Monitoring of selected non-ionic surfactants in river water by liquid chromatography-tandem mass spectrometry. *Journal of environmental management* **2016**, *169*, 247–252. DOI: 10.1016/j.jenvman.2015.12.034.

- (20) Park, J.-M.; Kim, H.-J.; Lee, J.-H.; Kim, J.-M. Determination of Anionic Surfactants in Dishwashing Detergents by High-performance Liquid Chromatography. *Food Eng. Prog.* **2016**, *20* (3), 211–217. DOI: 10.13050/foodengprog.2015.20.3.211.
- (21) Traverso-Soto, J. M.; Lara-Martín, P. A.; González-Mazo, E.; León, V. M. Distribution of anionic and nonionic surfactants in a sewage-impacted Mediterranean coastal lagoon: inputs and seasonal variations. *The Science of the total environment* **2015**, *503-504*, 87–96. DOI: 10.1016/j.scitotenv.2014.06.107.
- (22) Wu, H.-Y.; Shih, C.-L.; Lee, T.; Chen, T.-Y.; Lin, L.-C.; Lin, K.-Y.; Chang, H.-C.; Chuang, I.-C.; Liou, S.-Y.; Liao, P.-C. Development and validation of an analytical procedure for quantitation of surfactants in dishwashing detergents using ultra-performance liquid chromatography-mass spectrometry. *Talanta* **2019**, *194*, 778–785. DOI: 10.1016/j.talanta.2018.10.084.
- (23) Motteran, F.; Lima Gomes, P. C. F.; Silva, E. L.; Varesche, M. B. A. Simultaneous determination of anionic and nonionic surfactants in commercial laundry wastewater and anaerobic fluidized bed reactor effluent by online column-switching liquid chromatography/tandem mass spectrometry. *The Science of the total environment* **2017**, *580*, 1120–1128. DOI: 10.1016/j.scitotenv.2016.12.068.
- (24) Luo, X.; Zhang, L.; Niu, Z.; Ye, X.; Tang, Z.; Xia, S. Liquid chromatography coupled to quadrupole-Orbitrap high resolution mass spectrometry based method for target analysis and suspect screening of non-ionic surfactants in textiles. *Journal of chromatography. A* **2017**, *1530*, 80–89. DOI: 10.1016/j.chroma.2017.11.001.

- (25) Thurman, E. M.; Ferrer, I.; Rosenblum, J.; Linden, K.; Ryan, J. N. Identification of polypropylene glycols and polyethylene glycol carboxylates in flowback and produced water from hydraulic fracturing. *Journal of hazardous materials* **2017**, 323 (Pt A), 11–17. DOI: 10.1016/j.jhazmat.2016.02.041.
- (26) Dufour, A.; Thiébaud, D.; Ligiero, L.; Loriau, M.; Vial, J. Chromatographic behavior and characterization of polydisperse surfactants using Ultra-High-Performance Liquid Chromatography hyphenated to High-Resolution Mass Spectrometry. *Journal of chromatography. A* **2020**, 1614, 460731. DOI: 10.1016/j.chroma.2019.460731.
- (27) Cho, Y.; Witt, M.; Kim, Y. H.; Kim, S. Characterization of crude oils at the molecular level by use of laser desorption ionization Fourier-transform ion cyclotron resonance mass spectrometry. *Anal. Chem.* **2012**, 84 (20), 8587–8594. DOI: 10.1021/ac301615m.
- (28) Zhurov, K. O.; Kozhinov, A. N.; Tsybin, Y. O. Hexagonal class representation for fingerprinting and facile comparison of petroleomic samples. *Anal. Chem.* **2013**, 85 (11), 5311–5315. DOI: 10.1021/ac400418j.
- (29) Gateau, P.; Hénaut, I.; Barré, L.; Argillier, J. F. Heavy Oil Dilution. *Oil & Gas Science and Technology - Rev. IFP* **2004**, 59 (5), 503–509. DOI: 10.2516/ogst:2004035.
- (30) Chauve, B.; Guillarme, D.; Cléon, P.; Veuthey, J.-L. Evaluation of various HILIC materials for the fast separation of polar compounds. *Journal of separation science* **2010**, 33 (6-7), 752–764. DOI: 10.1002/jssc.200900749.
- (31) Snyder, L. R.; Kirkland, J. J.; Dolan, J. W. *Introduction to modern liquid chromatography*, 3rd edition; Wiley, 2009.

- (32) Mittal, K. L. *Surfactants in Solution*; Springer US, 1989. DOI: 10.1007/978-1-4613-0839-3.
- (33) Farsang, E.; Gaál, V.; Horváth, O.; Bárdos, E.; Horváth, K. Analysis of Non-Ionic Surfactant Triton X-100 Using Hydrophilic Interaction Liquid Chromatography and Mass Spectrometry. *Molecules (Basel, Switzerland)* **2019**, *24* (7). DOI: 10.3390/molecules24071223.
- (34) Vial, J.; Ménier, I.; Jardy, A.; Amger, P.; Brun, A.; Burbaud, L. How to better define the characteristics of dispersion of results in liquid chromatographic analyses through an interlaboratory study. *Journal of Chromatography B: Biomedical Sciences and Applications* **1998**, *708* (1-2), 131–143. DOI: 10.1016/s0378-4347(97)00668-3.
- (35) Schmidt, E. M.; Pudenzi, M. A.; Santos, J. M.; Angolini, C. F. F.; Pereira, R. C. L.; Rocha, Y. S.; Denisov, E.; Damoc, E.; Makarov, A.; Eberlin, M. N. Petroleomics via Orbitrap mass spectrometry with resolving power above 1 000 000 at m/z 200. *RSC Adv.* **2018**, *8* (11), 6183–6191. DOI: 10.1039/c7ra12509g.
- (36) Kim, D.; Kim, S.; Son, S.; Jung, M.-J.; Kim, S. Application of Online Liquid Chromatography 7 T FT-ICR Mass Spectrometer Equipped with Quadrupolar Detection for Analysis of Natural Organic Matter. *Anal. Chem.* **2019**, *91* (12), 7690–7697. DOI: 10.1021/acs.analchem.9b00689.
- (37) Zhurov, K. O.; Kozhinov, A. N.; Tsybin, Y. O. Evaluation of High-Field Orbitrap Fourier Transform Mass Spectrometer for Petroleomics. *Energy Fuels* **2013**, *27* (6), 2974–2983. DOI: 10.1021/ef400203g.

(38) Nagornov, K. O.; Zennegg, M.; Kozhinov, A. N.; Tsybin, Y. O.; Bleiner, D. Trace-Level Persistent Organic Pollutant Analysis with Gas-Chromatography Orbitrap Mass Spectrometry-Enhanced Performance by Complementary Acquisition and Processing of Time-Domain Data. *Journal of the American Society for Mass Spectrometry* **2020**, *31* (2), 257–266. DOI: 10.1021/jasms.9b00032.

(39) Jewett, B. N.; Ramaley, L.; Kwak, J. C. T. Atmospheric pressure ionization mass spectrometry techniques for the analysis of alkyl ethoxysulfate mixtures. *J Am Soc Mass Spectrom* **1999**, *10* (6), 529–536. DOI: 10.1016/S1044-0305(99)00017-3.

Figures and Tables captions:

Figure 1: Overlay of the pressure monitored at the head of the mixed mode column for 52 injections

Figure 2: Evolution of Brij (green) and Carboxylate (blue) peak area in blank injections with the average (m), limits (dashed lines) are defined as average value $\pm 2 \times$ standard deviation according to the injection number (Analytical conditions: see section 2.3.)

Figure 3: Overlay of mixed mode chromatograms of Carboxylate (blue-C13 and red-C11) and Brij (green) in UHPLC-HRMS (Analytical conditions: see section 2.3.)

Figure 4: Overlay of mixed mode chromatograms (A) Brij and (B) Carboxylate C11 in (black) QC, (red) Crude 1% and (green) Crude 10%. (Analytical conditions are given in section 2.3.)

Table 1: List of the samples analyzed with their goals in the study and injection sequences.

Table 2: ANOVA results for the homologues of Brij in THF/Toluene vs in water (QC) in positive ionization mode (* significant value $p < 0.05$)

Table 3: ANOVA results for the homologues of Carboxylate in THF/Toluene vs in water (QC) in positive ionization mode (* significant value $p < 0.05$)

Table 4: ANOVA results for the homologues of Brij in THF/Toluene with 1% of Crude oil vs in water (QC) in positive ionization mode (* significant value $p < 0.05$)

Table 5: ANOVA results for the homologues of Carboxylate in THF/Toluene with 1% of Crude oil vs in water (QC) in positive ionization mode (* significant value $p < 0.05$)

Table 6: ANOVA results for the homologues of Brij in THF/Toluene with 10% of Crude oil vs in water (QC) in positive ionization mode (* significant value $p < 0.05$)

Table 7: ANOVA results for the homologues of Carboxylate in THF/Toluene with 10% of Crude oil vs in water (QC) in positive ionization mode (* significant value $p < 0.05$)

Supplementary Data

Impact of oil matrix on anionic and nonionic surfactants separation using Ultra-High-Performance-Liquid-Chromatography hyphenated to High-Resolution Mass Spectrometry

Alizée Dufour^{1,2,}, Didier Thiébaud², Matthieu Loriau¹, Leticia Ligiero¹, Jérôme Vial²*

¹Total S.A. Exploration & Production - Lacq Research Center (PERL), 64170 Lacq, France

²LSABM, CBI, UMR 8231, ESPCI Paris - PSL Université Paris - CNRS, 10 rue Vauquelin, 75005 Paris, France

Figure S1: (A) Brij mass spectrum in (+)HESI-HRMS; (B-C) Carboxylate mass spectra in respectively (-)HESI-HRMS and (+)HESI-HRMS conditions.

Figure S2: UHPLC-(+)HESI-HRMS EIC of BrijC10 homolog (m/z =744.5836): in 1 QC injection (blue color) and in 12 blanks injections (black color)

Table S1: Retention times, peak areas and their RSDs for the study of THF/Toluene impact on Brij separation

BrijC10 [M+NH ₄] ⁺		QCs				Surfactants + THF/Toluene			
m/z	Nb EO	tr	RSD (tr) (n=5)	Peak Area ×10 ⁶	RSD (Area) (n=5)	tr	RSD (tr) (n=5)	Peak Area ×10 ⁶	RSD (Area) (n=5)
392.374	3	11.70	0.26%	0.95	36.15%	11.64	0.10%	1.82	1.50%
436.400	4	11.40	0.19%	1.48	39.21%	11.33	0.13%	2.92	1.28%
480.426	5	11.15	0.16%	1.77	37.61%	11.08	0.13%	3.63	2.15%
524.453	6	10.93	0.21%	2.16	36.77%	10.85	0.11%	4.40	2.87%
568.479	7	10.74	0.20%	2.49	33.54%	10.68	0.09%	5.00	2.17%
612.505	8	10.57	0.17%	2.61	30.45%	10.50	0.10%	5.28	4.78%
656.531	9	10.41	0.20%	2.61	27.95%	10.35	0.13%	5.15	3.45%
700.557	10	10.27	0.14%	2.52	27.05%	10.21	0.10%	4.80	3.31%
744.584	11	10.14	0.24%	2.29	22.98%	10.09	0.07%	4.20	3.41%
788.610	12	10.02	0.23%	2.00	20.10%	9.97	0.15%	3.54	2.39%
832.636	13	9.91	0.23%	1.66	17.72%	9.86	0.18%	2.85	3.47%
876.662	14	9.80	0.16%	1.21	15.17%	9.76	0.07%	2.00	1.66%
920.688	15	9.71	0.15%	1.22	11.98%	9.66	0.09%	3.99	2.96%
964.715	16	9.62	0.23%	1.18	11.27%	9.58	0.06%	3.81	6.17%
1008.741	17	9.54	0.13%	0.99	10.74%	9.50	0.16%	3.11	3.19%
1052.767	18	9.46	0.18%	0.77	8.65%	9.42	0.06%	2.53	13.52%
1096.793	19	9.38	0.14%	0.57	8.12%	9.34	0.08%	1.75	2.85%

579.427	20	9.30	0.16%	0.77	6.61%	9.27	0.06%	1.18	4.10%
601.440	21	9.23	0.24%	0.52	8.74%	9.21	0.17%	0.77	3.51%
623.453	22	9.17	0.18%	0.33	6.11%	9.16	0.10%	0.47	4.64%
645.466	23	9.10	0.18%	0.20	8.05%	9.09	0.09%	0.29	4.26%
667.479	24	9.05	0.17%	0.12	5.82%	9.02	0.12%	0.17	6.73%
689.492	25	8.97	0.13%	0.07	3.50%	8.97	0.21%	0.09	7.59%
711.506	26	8.93	0.14%	0.04	7.25%	8.93	0.09%	0.05	3.71%
733.519	27	8.87	0.26%	0.02	16.90%	8.86	0.38%	0.02	5.71%
755.532	28	8.81	0.29%	0.00	37.76%	8.81	0.24%	0.01	17.82%
Min		/	0.13%	/	3.50%	/	0.06%	/	1.28%
Max		/	0.29%	/	39.21%	/	0.38%	/	17.82%

Table S2: Retention times, peak areas and their RSDs for the study of THF/Toluene impact on Carboxylate separation

Carboxylate (+/-)		QCs				Surfactants + THF/Toluene			
m/z	Nb EO	tr	RSD (tr) (n=5)	Peak Area ×10 ⁶	RSD (Area) (n=5)	tr	RSD (tr) (n=5)	Peak Area ×10 ⁶	RSD (Area) (n=5)
Carboxylate C11 and C13 (+) HESI									
350.291	3	15.00	0.16%	1.24	3.64%	14.92	0.14%	1.33	0.77%
394.317	4	14.20	0.18%	5.26	3.66%	14.12	0.05%	5.71	0.97%
438.343	5	13.58	0.18%	5.91	4.25%	13.50	0.09%	6.27	1.19%
482.369	6	13.04	0.20%	4.48	3.90%	12.98	0.05%	4.66	1.69%
526.395	7	12.58	0.24%	3.26	4.82%	12.52	0.08%	3.26	1.39%
570.422	8	12.19	0.14%	2.28	5.00%	12.14	0.08%	2.11	2.39%
614.448	9	11.84	0.22%	1.49	4.22%	11.79	0.08%	1.24	1.91%
658.474	10	11.53	0.25%	0.92	4.94%	11.47	0.18%	0.67	1.39%
702.500	11	11.25	0.16%	0.51	4.23%	11.20	0.19%	0.32	2.80%
746.527	12	11.00	0.17%	0.28	5.13%	10.95	0.23%	0.14	3.36%
378.322	3	17.15	0.14%	0.42	2.44%	17.06	0.11%	0.47	0.95%
422.348	4	16.29	0.18%	1.64	3.54%	16.18	0.10%	1.79	1.24%
466.374	5	15.60	0.14%	1.88	3.78%	15.52	0.16%	2.05	1.24%
510.401	6	15.02	0.14%	1.43	3.80%	14.96	0.05%	1.54	1.38%
554.427	7	14.54	0.20%	1.06	4.71%	14.46	0.12%	1.10	1.77%
598.453	8	14.10	0.16%	0.75	3.57%	14.03	0.09%	0.74	0.90%
642.479	9	13.71	0.25%	0.47	5.00%	13.65	0.12%	0.43	1.82%
686.505	10	13.39	0.22%	0.30	4.75%	13.33	0.16%	0.24	1.82%
730.532	11	13.08	0.18%	0.17	3.87%	13.01	0.17%	0.12	1.02%
774.558	12	12.79	0.19%	0.09	5.92%	12.73	0.18%	0.05	1.19%
Carboxylate C11 and C13 (-) HESI									
243.196	1	15.30	0.19%	0.02	6.28%	15.21	0.11%	0.02	12.42%
287.222	2	15.76	0.20%	0.13	1.52%	15.65	0.23%	0.13	2.46%
331.248	3	15.03	0.28%	0.19	1.94%	14.90	0.14%	0.20	3.06%
375.275	4	14.21	0.18%	0.18	2.64%	14.13	0.17%	0.19	1.12%
419.301	5	13.56	0.21%	0.14	3.57%	13.51	0.28%	0.15	1.53%
463.327	6	13.08	0.15%	0.10	4.12%	12.97	0.13%	0.10	3.03%
507.353	7	12.58	0.16%	0.07	3.79%	12.54	0.17%	0.07	4.32%
551.380	8	12.17	0.27%	0.05	4.85%	12.13	0.23%	0.05	3.30%
595.406	9	11.86	0.17%	0.03	12.02%	11.78	0.29%	0.03	5.42%
639.432	10	11.56	0.29%	0.02	8.84%	11.47	0.32%	0.01	18.97%
359.280	3	17.16	0.30%	0.09	3.87%	17.04	0.13%	0.09	2.25%
403.306	4	16.30	0.33%	0.07	2.56%	16.18	0.31%	0.07	1.08%

447.332	5	15.61	0.24%	0.05	2.96%	15.52	0.27%	0.06	2.71%
491.358	6	15.04	0.20%	0.03	5.69%	14.94	0.19%	0.04	5.15%
535.385	7	14.51	0.21%	0.03	9.09%	14.47	0.23%	0.03	6.77%
579.411	8	14.12	0.25%	0.02	10.35%	14.02	0.18%	0.01	9.69%
623.437	9	13.71	0.34%	0.01	8.82%	13.68	0.17%	0.01	11.51%
Min		/	0.14%	/	1.52%	/	0.05%	/	0.77%
Max		/	0.34%	/	12.02%	/	0.32%	/	18.97%

Table S3: Retention times, peak areas and their RSDs of Brij homologues injected in THF/Toluene containing 1% of Crude oil

BrijC10 [M+NH ₄] ⁺		QCs				Crude1%			
m/z	Nb EO	tr	RSD (tr) (n=3)	Peak Area ×10 ⁶	RSD (Area) (n=3)	tr	RSD (tr) (n=6)	Peak Area ×10 ⁶	RSD (Area) (n=6)
392.374	3	11.70	0.25%	0.25	18.51%	11.63	0.07%	1.95	1.82%
436.400	4	11.40	0.21%	0.38	14.65%	11.33	0.11%	2.80	2.88%
480.426	5	11.15	0.30%	0.51	16.81%	11.09	0.07%	3.88	1.68%
524.453	6	10.94	0.30%	0.68	17.07%	10.88	0.07%	4.77	1.33%
568.479	7	10.74	0.09%	0.89	14.50%	10.69	0.10%	5.18	1.89%
612.505	8	10.57	0.18%	1.08	14.07%	10.51	0.07%	5.38	1.67%
656.531	9	10.42	0.09%	1.22	12.97%	10.36	0.08%	5.16	1.73%
700.557	10	10.27	0.09%	1.29	10.68%	10.22	0.12%	4.74	1.96%
744.584	11	10.14	0.21%	1.31	13.43%	10.10	0.11%	4.14	2.08%
788.610	12	10.03	0.09%	1.24	9.05%	9.97	0.13%	3.48	1.88%
832.636	13	9.92	0.09%	1.09	11.34%	9.86	0.13%	2.79	1.94%
876.662	14	9.82	0.09%	0.84	10.76%	9.77	0.12%	1.94	1.73%
920.688	15	9.72	0.07%	0.89	9.86%	9.67	0.11%	4.29	1.49%
964.715	16	9.63	0.09%	0.89	9.20%	9.59	0.11%	3.98	1.40%
1008.741	17	9.55	0.15%	0.78	6.81%	9.50	0.04%	3.27	1.59%
1052.767	18	9.47	0.02%	0.61	8.31%	9.42	0.12%	2.49	1.59%
1096.793	19	9.38	0.14%	0.46	7.00%	9.34	0.09%	1.81	2.25%
579.427	20	9.32	0.09%	0.64	3.78%	9.27	0.14%	1.21	2.54%
601.440	21	9.24	0.09%	0.44	7.16%	9.20	0.09%	0.76	4.10%
623.453	22	9.18	0.30%	0.28	3.06%	9.14	0.08%	0.49	3.40%
645.466	23	9.12	0.09%	0.17	1.97%	9.09	0.23%	0.29	5.28%
667.479	24	9.06	0.02%	0.10	9.32%	9.03	0.25%	0.16	7.97%
689.492	25	9.01	0.17%	0.05	3.05%	8.97	0.24%	0.10	4.80%
711.506	26	8.94	0.28%	0.03	53.37%	8.92	0.28%	0.05	9.76%
733.519	27	8.90	0.29%	0.02	15.53%	8.86	0.16%	0.02	8.48%
755.532	28	8.85	0.09%	0.01	29.83%	8.80	0.49%	0.01	24.91%
Min		/	0.02%	/	1.97%	/	0.04%	/	1.33%
Max		/	0.30%	/	53.37%	/	0.49%	/	24.91%

Table S4: Retention times, peak areas and their RSDs of Carboxylate homologues injected in THF/Toluene containing 1% of Crude oil

Carboxylate (+/-)		QCs				Crude1%			
m/z	Nb EO	tr	RSD (tr) (n=3)	Peak Area ×10 ⁶	RSD (Area) (n=3)	tr	RSD (tr) (n=6)	Peak Area ×10 ⁶	RSD (Area) (n=6)
Carboxylate C11 and C13 (+) HESI									
350.291	3	15.01	0.10%	1.30	0.89%	<i>nd</i>	<i>nd</i>	<i>nd</i>	<i>nd</i>
394.317	4	14.18	0.10%	5.57	0.36%	14.12	0.11%	4.86	2.67%
438.343	5	13.55	0.08%	6.26	1.05%	13.50	0.09%	4.76	2.76%
482.369	6	13.03	0.10%	4.73	1.04%	12.98	0.08%	2.70	3.36%
526.395	7	12.59	0.30%	3.41	1.45%	12.53	0.17%	2.12	3.73%
570.422	8	12.19	0.17%	2.37	1.11%	12.14	0.12%	1.21	4.50%
614.448	9	11.82	0.08%	1.53	2.13%	11.79	0.13%	0.76	4.16%
658.474	10	11.51	0.21%	0.95	0.42%	11.49	0.19%	0.42	4.39%
702.500	11	11.23	0.16%	0.55	2.12%	11.20	0.26%	0.23	5.88%
746.527	12	10.99	0.19%	0.29	1.06%	10.96	0.16%	0.09	6.68%
378.322	3	17.18	0.08%	0.45	1.93%	<i>nd</i>	<i>nd</i>	<i>nd</i>	<i>nd</i>
422.348	4	16.27	0.17%	1.72	1.64%	16.19	0.09%	1.73	2.47%
466.374	5	15.61	0.13%	1.97	1.75%	15.51	0.04%	1.64	3.13%
510.401	6	15.01	0.07%	1.50	1.31%	14.95	0.15%	0.59	11.54%
554.427	7	14.53	0.19%	1.10	0.19%	14.45	0.15%	0.70	3.89%
598.453	8	14.11	0.32%	0.79	1.11%	14.03	0.18%	0.39	5.90%
642.479	9	13.72	0.15%	0.50	1.41%	13.67	0.21%	0.25	4.39%
686.505	10	13.39	0.24%	0.32	1.56%	13.33	0.13%	0.17	4.45%
730.532	11	13.05	0.22%	0.19	0.61%	13.02	0.29%	0.09	6.25%
774.558	12	12.82	0.02%	0.10	2.18%	12.74	0.33%	0.02	25.27%
Carboxylate C11 and C13 (-) HESI									
243.196	1	15.27	0.15%	0.02	7.14%	15.14	0.28%	0.05	9.72%
287.222	2	15.69	0.28%	0.13	3.25%	15.65	0.19%	0.30	3.45%
331.248	3	15.01	0.12%	0.19	2.46%	14.91	0.16%	0.21	3.31%
375.275	4	14.18	0.15%	0.19	1.01%	14.11	0.16%	0.16	4.56%
419.301	5	13.58	0.21%	0.15	2.11%	13.52	0.17%	0.11	3.24%
463.327	6	13.05	0.23%	0.11	1.73%	13.00	0.16%	0.07	7.30%
507.353	7	12.61	0.09%	0.08	1.83%	12.50	0.23%	0.05	5.09%
551.380	8	12.24	0.08%	0.05	5.50%	12.13	0.27%	0.03	6.93%
595.406	9	11.87	0.20%	0.03	5.48%	11.77	0.30%	0.02	13.63%
639.432	10	11.52	0.41%	0.02	1.37%	11.51	0.23%	0.00	38.39%
359.280	3	17.17	0.34%	0.09	6.10%	17.06	0.13%	0.65	5.53%
403.306	4	16.27	0.31%	0.07	1.16%	16.19	0.11%	0.44	3.68%

447.332	5	15.60	0.09%	0.06	3.98%	15.52	0.24%	0.12	2.71%
491.358	6	15.01	0.15%	0.04	6.91%	14.95	0.19%	0.04	7.77%
535.385	7	14.56	0.15%	0.02	9.20%	14.48	0.25%	0.02	12.76%
579.411	8	14.10	0.30%	0.02	1.81%	14.06	0.39%	0.01	21.31%
623.437	9	13.75	0.11%	0.01	15.20%	13.66	0.29%	0.00	33.49%
Min		/	0.02%	/	0.19%	/	0.04%	/	2.47%
Max		/	0.41%	/	15.20%	/	0.39%	/	38.39%

Table S5: Retention times, peak areas and their RSDs of Brij homologues injected in THF/Toluene containing 10% of Crude oil

BrijC10 [M+NH ₄] ⁺		QCs				Crude10%			
m/z	Nb EO	tr	RSD (tr) (n=3)	Peak Area ×10 ⁶	RSD (Area) (n=3)	tr	RSD (tr) (n=6)	Peak Area ×10 ⁶	RSD (Area) (n=6)
392.374	3	11.73	0.04%	0.12	59.50%	11.65	0.13%	1.66	6.93%
436.400	4	11.42	0.03%	0.23	20.13%	<i>nd</i>	<i>nd</i>	<i>nd</i>	<i>nd</i>
480.426	5	11.17	0.08%	0.31	18.27%	11.09	0.07%	3.59	5.46%
524.453	6	10.95	0.03%	0.43	18.77%	10.88	0.07%	4.42	6.05%
568.479	7	10.75	0.12%	0.58	17.49%	10.69	0.10%	4.69	6.04%
612.505	8	10.58	0.14%	0.71	16.91%	10.52	0.15%	5.49	4.07%
656.531	9	10.44	0.11%	0.80	14.31%	10.37	0.07%	4.97	4.33%
700.557	10	10.29	0.03%	0.87	15.04%	10.22	0.14%	4.85	4.96%
744.584	11	10.16	0.03%	0.89	14.60%	10.10	0.07%	4.19	5.23%
788.610	12	10.03	0.14%	0.85	13.23%	9.98	0.08%	3.50	5.57%
832.636	13	9.93	0.09%	0.76	12.68%	9.87	0.22%	2.96	3.85%
876.662	14	9.83	0.03%	0.60	11.53%	9.78	0.14%	2.05	4.41%
920.688	15	9.73	0.03%	0.66	10.84%	9.68	0.06%	4.33	3.65%
964.715	16	9.64	0.15%	0.68	12.79%	9.59	0.06%	3.77	5.24%
1008.741	17	9.55	0.08%	0.60	9.73%	9.51	0.13%	2.97	3.32%
1052.767	18	9.47	0.13%	0.49	8.73%	9.44	0.17%	2.21	5.02%
1096.793	19	9.39	0.03%	0.36	8.82%	9.36	0.19%	1.61	7.90%
579.427	20	9.32	0.23%	0.49	7.28%	9.29	0.15%	1.14	5.73%
601.440	21	9.26	0.04%	0.34	4.21%	9.24	0.37%	0.62	11.14%
623.453	22	9.18	0.17%	0.22	5.07%	9.17	0.29%	0.45	12.38%
645.466	23	9.13	0.16%	0.15	6.85%	9.09	0.12%	0.38	15.79%
667.479	24	9.06	0.17%	0.08	10.57%	9.06	0.41%	0.12	29.06%
689.492	25	8.99	0.13%	0.05	9.94%	9.00	0.29%	0.08	25.27%
711.506	26	8.96	0.16%	0.03	18.85%	8.95	0.22%	0.02	60.75%
733.519	27	8.91	0.05%	0.01	7.57%	8.88	0.67%	0.01	7.49%
755.532	28	8.84	0.24%	0.00	20.06%	<i>nd</i>	<i>nd</i>	<i>nd</i>	<i>nd</i>
Min		/	0.03%	/	4.21%	/	0.06%	/	3.32%
Max		/	0.24%	/	59.50%	/	0.67%	/	60.75%

Table S6: Retention times, peak areas and their RSDs of Carboxylate homologues injected in THF/Toluene containing 10% of Crude oil

Carboxylate (+/-)		QCs				Crude10%			
m/z	Nb EO	tr	RSD (tr) (n=3)	Peak Area ×10 ⁶	RSD (Area) (n=3)	TR	RSD (tr) (n=6)	Peak Area ×10 ⁶	RSD (Area) (n=6)
Carboxylate C11 and C13 (+) HESI									
350.291	3	15.02	0.22%	1.27	2.50%	<i>nd</i>	<i>nd</i>	<i>nd</i>	<i>nd</i>
394.317	4	14.20	0.10%	5.45	0.54%	14.13	0.10%	3.39	5.93%
438.343	5	13.57	0.12%	6.19	0.37%	13.51	0.08%	5.17	3.05%
482.369	6	13.03	0.13%	4.66	0.37%	12.99	0.11%	1.31	2.83%
526.395	7	12.59	0.27%	3.40	1.25%	12.54	0.20%	2.91	3.02%
570.422	8	12.19	0.18%	2.37	0.56%	12.14	0.18%	1.63	5.66%
614.448	9	11.87	0.20%	1.54	2.13%	11.80	0.25%	1.27	4.65%
658.474	10	11.54	0.21%	0.96	1.83%	11.49	0.09%	0.94	4.93%
702.500	11	11.25	0.19%	0.55	0.97%	11.23	0.18%	0.55	8.88%
746.527	12	11.00	0.21%	0.29	0.85%	10.99	0.47%	0.20	20.91%
378.322	3	17.16	0.26%	0.42	6.98%	<i>nd</i>	<i>nd</i>	<i>nd</i>	<i>nd</i>
422.348	4	16.28	0.16%	1.63	1.99%	16.19	0.13%	0.40	31.13%
466.374	5	15.61	0.18%	1.90	0.11%	15.53	0.17%	1.40	5.84%
510.401	6	15.01	0.12%	1.45	1.93%	<i>nd</i>	<i>nd</i>	<i>nd</i>	<i>nd</i>
554.427	7	14.55	0.29%	1.07	0.58%	14.47	0.17%	0.71	8.98%
598.453	8	14.13	0.16%	0.77	1.50%	14.06	0.20%	0.16	43.53%
642.479	9	13.71	0.08%	0.49	0.85%	13.66	0.16%	0.36	5.33%
686.505	10	13.37	0.12%	0.31	1.92%	13.35	0.17%	0.42	13.76%
730.532	11	13.12	0.08%	0.18	1.71%	13.06	0.32%	0.19	22.07%
774.558	12	12.81	0.22%	0.10	2.03%	12.80	0.43%	0.11	23.78%
Carboxylate C11 and C13 (-) HESI									
243.196	1	15.26	0.30%	0.02	5.24%	15.17	0.34%	0.17	10.07%
287.222	2	15.74	0.56%	0.13	2.66%	15.63	0.12%	1.03	4.21%
331.248	3	15.00	0.25%	0.19	2.90%	14.95	0.17%	1.59	3.03%
375.275	4	14.21	0.14%	0.18	2.47%	14.14	0.07%	0.80	1.55%
419.301	5	13.60	0.19%	0.15	1.47%	13.53	0.13%	0.47	3.47%
463.327	6	13.02	0.08%	0.10	1.55%	13.03	0.22%	0.25	5.75%
507.353	7	12.59	0.36%	0.07	2.99%	12.54	0.17%	0.12	4.14%
551.380	8	12.21	0.29%	0.05	5.93%	12.15	0.22%	0.05	7.36%
595.406	9	11.83	0.12%	0.03	2.53%	11.81	0.29%	0.03	3.39%
639.432	10	11.57	0.43%	0.02	4.44%	11.50	0.33%	0.01	10.27%
359.280	3	17.17	0.21%	0.09	5.63%	17.09	0.65%	0.68	31.15%
403.306	4	16.28	0.17%	0.07	2.31%	16.16	0.14%	0.67	5.16%

447.332	5	15.64	0.15%	0.05	2.98%	15.53	0.14%	0.37	14.12%
491.358	6	15.00	0.12%	0.03	7.35%	14.97	0.27%	0.42	5.00%
535.385	7	14.52	0.18%	0.03	2.92%	14.49	0.31%	0.20	5.05%
579.411	8	14.12	0.19%	0.02	15.23%	14.05	0.30%	0.07	5.64%
623.437	9	13.74	0.13%	0.01	16.83%	13.67	0.16%	0.05	14.18%
Min		/	0.08%	/	0.11%	/	0.07%	/	1.55%
Max		/	0.56%	/	16.83%	/	0.65%	/	43.53%