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Chromatographic behavior and characterization of polydisperse surfactants using Ultra-High-Performance Liquid Chromatography hyphenated to High-Resolution Mass Spectrometry

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Highlights:

- Foundation of a HRMS database of polydisperse surfactants
- Chromatography behavior of surfactants with three separation modes: building of retention maps
- Limits of HRMS and one-dimension liquid chromatography-HRMS for the separation of polydisperse surfactants

Keywords: Surfactants, UHPLC, HILIC, mixed-mode chromatography, High Resolution Mass Spectrometry, petroleum industry,

ABSTRACT

Surfactants are widely used in various petrochemical applications. Thus, it is essential to have highly efficient analytical tools to monitor the different classes of surfactants commonly used. Three among the four known classes of surfactants were studied in the present work: anionic (i.e. alkylbenzene sulfonate, alkyl ether carboxylic acid), nonionic (i.e. alkylcyclohexyl alcohol ethoxylated, alkyl alcohol ethoxylated) and cationic (i.e. alkyl trimethylammonium). Thanks to high-resolution mass spectrometry (HRMS), a useful mass list was created including 119 m/z values (error in mass around 5 ppm). This list was the foundation of a HRMS database, which, for the sake of simplicity, will be further denoted only “database”. To avoid ion competition and streamline attribution of structural formulas for isobar molecules, a suitable chromatographic method was used before MS. The retention behavior of six surfactants (trimethyloctadecylammonium bromide,

myristyltrimethylammonium bromide, Brij®C10, Triton X-100 reduced, glycolic acid ethoxylate lauryl ether, 4-dodecylbenzenesulfonic acid) was evaluated under three separation modes: reversed-phase liquid chromatography (RPLC), hydrophilic interaction chromatography (HILIC) and mixed-mode chromatography (combination of anion-exchange and reversed-phase mechanisms). In RPLC mode, six columns were tested including C4, C18, C30, polar-embedded C18, PFP and phenyl chemistries. Two HILIC columns were also tested including bare silica and urea chemistries. An anion-exchange combined with RPLC mechanism was investigated as mixed-mode mechanism. Using ammonium formate at 10 mM as buffer provided the best signal in HRMS. In liquid chromatography, acid conditions (pH 3.5) were preferred, to avoid peak tailing due to residual silanols. The mixed-mode separation mode clearly appears as the best compromise for the characterization of the three surfactants classes. Nevertheless, the orthogonality observed for the separations obtained in HILIC and RPLC modes offers some possibilities for further multidimensional separations.

1. Introduction

Surfactants, also called surface-active agents, are amphiphilic molecules that are generally used to perform specific functions such as cleaning, softening and emulsifying. These molecules consist of a lipophilic chain together with a group having a hydrophilic character. They are classified in four categories according to the nature of the terminal group: anionic (i.e. sulfonates, sulfates, carboxylates etc.), cationic (i.e. quaternary ammoniums), nonionic (i.e. alcohol), and zwitterionic (i.e. amino acid). Due to their amphiphilic nature, surfactants partition into oil but also into the aqueous phase, while they are able to stabilize the dispersion of these two immiscible liquids, through emulsions.

Thanks to this particular feature, surfactants are widely used in different industries: textile [1–3], cosmetics [4], pharmaceuticals [5], surface treatment [6], agriculture [7], etc. At the end of the twentieth century, Bhardwaj *et al.* reported the place of surfactants in petroleum industry and especially in petroleum production [8], highlighting that surfactants have been ubiquitous in this process. Indeed, using surfactants is known to be a useful way to enhance oil recovery (EOR): they are supposed to reduce interfacial tension between oil and water and modify the wettability of the rock [9–12]. Surfactants were also used for hydraulic fracturing, to release the oil trapped in the rocks [13,14]. Finally, for crude oil transportation, surfactants are particularly useful to reduce fluid viscosity, as demonstrated by Al-Roomi *et al.* [15].

Despite all precautions to avoid surfactants release in the environment, traces can be found in sediments [16–19] and river waters [20–23]. As example, Lara-Martin *et al.* determined the concentration of two anionic surfactants: alkylbenzenesulfonate (LAS) and alkylethoxysulfate (AES) in the Guadalete estuary. The concentrations found in sediments (200 - 1100 ng g⁻¹), were higher than those determined in water (4.5 -

64.4 ng mL⁻¹) [17]. With the aim of controlling surfactants, it is therefore essential to have efficient analytical tools to monitor the different families of surfactants. According to the literature, many techniques were already investigated for these amphiphilic molecules, including Gas Chromatography (GC), Capillary Electrophoresis (CE), Thin-Layer Chromatography (TLC), Liquid Chromatography (LC), Mass spectrometry (MS), etc. Table 1 highlights the preferred techniques for the analysis of surfactants according to their nature (zwitterionic surfactants were not mentioned in this table). The assessment criteria are the establishment of a separation method, the separation mechanisms used, and the detection.

Table 1 : Advantages and drawbacks of available methods for surfactants analysis

Analytical techniques	Anionics	Cationics	Nonionics	References
GC	-	-	++	[185–187]
CE	++	++	-	[171,188,189]
TLC	+	+	++	[111,190]
LC	++	++	++	[70,73,80,82,84,100,103,106–108,130,132,172,191]
MS	++	++	+	[192]

(-) separation and detection impossible

(+) separation possible, but difficult detection

(++) separation and detection possible

Surfactant analysis using GC is difficult for two reasons. Firstly, when nonionic alkoxyated surfactants have a high degree of ethoxy (EO) or/and propoxy (PO) groups, their volatility is low. Second, ionic surfactants (anionic and cationic) are not volatile. To fill this gap, a derivatization procedure has to be included prior to their analysis. For example, Wulf *et al.* have successfully analyzed nonionic, anionic and several cationic surfactants by comprehensive 2D Gas Chromatography (GC×GC) with HRMS detection, after silylation [24]. Despite the efficient separation and detection of all classes of surfactants, the highest degree of ethoxylation was only eight.

CE was used for surfactant analysis until the beginning of the 21st century. However, despite the common use of surfactants for capillary coating, their separation was less attractive and thus less studied. Heinig and Vogt made an exhaustive review in 1999 [25] and highlighted the lack of CE reproducibility, as one of the main weaknesses of the technique. Moreover, nonionic surfactants were unsuitable for CE separation, because their electrophoretic mobility was null. To circumvent this limitation, a derivatization procedure can be implemented to obtain charged species, as shown by Wallingford for the separation of PEG 1000 [26].

However, a cleaning step was required to eliminate the reactants in excess and the reaction was incomplete, leading to possible loss of compounds and unreliable quantitative analysis.

Surfactant separation using TLC was reported by Bhawani *et al.* [27] and by Mohammad *et al.* [28]. They mainly highlighted the lack of adequate detection. Moreover, the methodology is not generic, and conditions have to be adapted according to the sample nature.

MS is also reported as an important technique for qualitative and quantitative surfactant analysis [7,22,29,30]. As it allows different ways to explore the samples, MS is an interesting tool for structural comprehension. Charged compounds are obviously easier to analyze than neutral ones, since they need less energy to be detected. For qualitative evaluation, simple samples can be studied by direct infusion. However, one must remember that as all compounds reach the ionization source at the same time, matrix effects (ion suppression or enhancement) can occur and bias MS results. Moreover, the size of the molecules introduce discrimination in the ionization process. Fenn *et al.* [31] reported that when compounds had a long alkyl chain, they were placed onto the surface of the droplets and are thus more easily ionized than small ones. Despite the efforts made by different authors to understand ionization mechanisms [32–34], much remains still not understood.

A second inconvenience of direct infusion for identification lies in the presence of isobaric ions. For example, nonylphenol ethoxylates with five ethylene oxide units $[\text{NP}(\text{EO})_5+\text{NH}_4]^+$ and nonylphenol ethoxylates with fifteen ethylene oxide units $[\text{NP}(\text{EO})_{15}+2\text{NH}_4]^{2+}$ have the same m/z using low resolution mass spectrometry (LRMS), because the former one is singly charged, while the latter is doubly-charged [1]. To limit such isobaric interferences, two solutions are available: use of HRMS and/or coupling LC with MS. As HRMS is relatively expensive and only ultra-high resolution mass spectrometers can sufficiently resolve isobars, an upstream efficient chromatographic system appears as the best solution. Indeed, isobaric compounds can possibly be separated when using an adequate column stationary phase. Another advantage with LC is that the derivatization is not required. In addition, separation of molecules with a high number of ethylene oxide (EO) units can also be achieved, as there is no more boiling point limitation due to EO number. Wang *et al.* reported the separation of alkylphenol ethoxylates in a textile matrix using normal phase liquid chromatography (NPLC) hyphenated to UV detection. In this work, more than 15 units of EO were identified [35]. Different works reported the use of derivatization for LC-MS/MS analysis. Zembruska *et al.* described a LC-MS/MS method for the separation of dodecanol and homogenous fatty alcohols ethoxylated containing a dodecyl moiety and ethoxy groups. These molecules were derivatized before analysis with phenyl isocyanate [36]. Zgoła-Grzeskowiak *et al.* reported the development of a new method for preconcentration, derivatization and analysis of short-chained dodecyl alcohol ethoxylates and dodecyl alcohol. In this work, the derivatization procedure was carried out with 1-naphthoyl chloride [37]. However, the major limitation on UV detection lies in the lack of chromophore groups for many usual surfactants.

In the literature, most of the modern LC methods dedicated to surfactants analysis involved RPLC with an octadecylsilica column (C18) hyphenated to MS [38–40]. Luo *et al.* reported the comparison between four C18 columns from three suppliers to simultaneously analyze a high number of nonionic surfactants in textile matrices [1]. Lara Martin *et al.* reported a novel methodology capable of analyzing at the same time surfactant and their carboxylates metabolites. García *et al.* also reported an analytical workflow using UHPLC-HRMS to detect aqueous film-forming foams containing nine classes of hydrocarbon surfactants in contaminated groundwater [41]. However, the C18 column was not able to provide a complete separation of all the homologs [30]. Only a few studies reported the use of shorter alkyl chains, such as butylsilica (C4), octylsilica (C8) or dodecylsilica (C12) columns [4,6,42–45]. Separations in HILIC mode were also investigated as nonionic surfactants could be separated according to the number of EO or propylene oxide (PO) units. Zembruska *et al.* reported a performant analytical method using a TSKgel Amide-80 to quantify non-ionic surfactants, alcohol ethoxylates by LC-MS/MS. They investigated the fragmentation pattern and showed some common fragments [21,46]. Using the MRM mode, the non-ionic concentration found in River Warta was between 1.4 to 11.2 $\mu\text{g L}^{-1}$ over the period October 2011 – September 2012.

Vanhoenacker *et al.* developed a comprehensive two-dimensional liquid chromatography (2D-LC) approach to detail the composition of nonionic ethoxylated surfactants in pharmaceutical formulations [5]. The setup includes an HILIC column in the first dimension, combined with RPLC in the second one. Groeneveld *et al.* successfully characterized three types of glycerol using 2D-LC: glycerol ethoxylate, glycerol propoxylate and glycerol ethoxylate-random-propoxylate copolymer using the same setup [47]. The HILIC x RPLC configuration separated the surfactants according to their degree of ethoxylation (EO units) in the first dimension and by the length of the alkyl chain and degree of ethoxylation (PO units) in the second dimension.

To avoid complex 2D-LC assembly, an alternative solution consists in using a mixed-mode column, such as the Acclaim™ Surfactant Plus developed by Thermofisher Scientific. This column combines RPLC and ion exchange modes. Liu *et al.* [44,48] demonstrated that a mixed-mode column was able to simultaneously analyze different types of surfactants: cationic, anionic and nonionic. However, only monodispersed surfactants were analyzed.

The main purpose of this study was to investigate separation capabilities of RPLC, HILIC and mixed mode chromatography for analyzing some mixtures of three classes of surfactants including polydisperse ones. For this purpose, six columns were tested in RPLC mode, including C4, C18, C30, but also PFP, polar-embedded C18 and phenyl chemistries. Two HILIC columns, namely bare silica and urea, were also tested. Finally, the mixed-mode mechanism was investigated using an Acclaim™ Surfactant Plus column. This column allows both reverse phase mechanism and anion-exchange. Since they are representative of the petroleum industry, three classes of surfactants were considered: anionic (i.e. alkybenzene sulfonates, carboxylates,

alkyl ethoxycarboxylates), cationic (i.e. alkylquaternary ammoniums) and nonionic (i.e. alcohol ethylates, alkyl phenoethoxylated reduced). The final aim of this work is to propose method able to provide a full characterization of surfactants in real samples, according to alkyl chain lengths and EO number. In addition, the developed method should provide reliable quantification and mass balance in petroleum samples.

2. Materials and methods

2.1. Chemicals

LC-MS grade acetonitrile (ACN) and water (W), isopropanol (HPLC Plus gradient grade) 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. Ammonium acetate (96%) and acetic acid (100%) were provided by MERCK (Lyon, 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. Triton™ X-100 reduced (Triton), Brij®C10 (Brij, Mn 683), pentadecanoic acid (analytical standard), myristic acid (analytical standard), glycolic acid ethoxylate lauryl ether (C₁₁EO_nCO₂H and C₁₃EO_nCO₂H, Mn 360), trimethyloctadecylammonium bromide (N_{VIII}, purity 98%), myristyltrimethylammonium bromide (N_{IV}, purity 99%) and 4-dodecylbenzenesulfonic acid (DBSA, purity 95%) were purchased from Sigma Aldrich (Saint-Quentin-Fallavier, France). When DBSA is mentioned in the text, it referred to a mixture of four homologs.

All surfactants were characterized by infusion in HRMS. For retention measurement, only the following surfactants were used: DBSA, Triton, Brij, C₁₁EO_nCO₂H, C₁₃EO_nCO₂H, N_{VIII} and N_{IV}. All standards were diluted in ultrapure water or ACN to obtain a solution at the desired concentration. All solutions were stored at 4°C.

2.1. MS conditions

Direct infusions were carried out to optimize the MS parameters and create the database of the characteristic ions for each standard. For this purpose, the Vanquish Ultra-High-Performance Liquid Chromatography system (UHPLC - ThermoFisher, Scientific), a syringe pump with a glass 1 mL-syringe (Hamilton - Chromoptic) and the Exactive Plus (ThermoFisher, Scientific) orbitrap mass spectrometer with a Heated ElectroSpray II (HESI-II) were used. The syringe pump infused the standard solution (flow rate between 10 and 20 µL min⁻¹) into the mobile phase delivered by the UHPLC pump (flow rate was 400 µL min⁻¹) via a T-fitting, to mimic real LC conditions. For ionization source optimization, the criteria to select the best ionization parameters were the spray stability (Total Ion Chromatogram (TIC) variation below 10%) and the spectrum quality (current and signal intensity). The resulting ionization parameters for each surfactant are presented in Table 2. Xcalibur 4.2 software (Thermo Scientific) was used for instrument control and data acquisition. FreeStyle (Thermo Scientific) was used for data processing.

Table 1 : Optimized MS parameters for direct infusion MS and LC-MS analysis

	Sulfonate	Carboxylate	BrijC10	Triton	Ammonium
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Spray Voltage (kV)	4.0	3.0	3.0	4.0	3.5
Capillary Temp. (°C)	300	350	300	300	350
Sheath gas (psi)	30	40	40	45	35
Aux Gas (arbitrary unit)	10	20	5	15	5
Spare Gas (arbitrary unit)	0	10	0	0	0
Probe Heater Temp. (°C)	250	350	250	250	350
S-Lens	50	50	50	50	50
m/z	200-500	200-1000	125-1100	125-1100	200-600
Resolution	R = 70 000 @m/z 200 (LC-MS) R = 140 000 @m/z 200 (Direct Infusion-MS)				
AGC target / Injected time (ms)	1E6 (Direct Infusion-MS and LC-MS) 200 (Direct Infusion-MS and LC-MS)				

2.2. LC-MS conditions

The Vanquish UHPLC system with a binary pump and autosampler was coupled with an Exactive Plus Orbitrap HRMS equipped with a heated electrospray ionization source (HESI-II).

2.2.1. LC conditions

Nine columns were tested: The detailed characteristics of each column and LC conditions (mobile phase composition, gradient) are summarized in Table 3. In the literature, only limited information can be found about surfactants retention on the selected columns. Consequently, a wide mobile phase composition was employed to ensure that all surfactants were eluted. Whatever the separation mode, an isocratic plateau of 10 min was systematically applied at the end of the gradient to elute the most retained compounds. This isocratic will be necessary when real samples are analyzed. To compare retention on columns having different lengths (250 and 150 mm), the gradient was geometrically scaled [49]. For all chromatographic conditions, the injection volume was 3 μL and flow rate was 400 $\mu\text{L min}^{-1}$. To evaluate the effect of column temperature, experiments were carried out both at room temperature (21°C) and 40°C. Peak shapes and retention times were similar in both cases. So, the column oven and preheater temperature were set at 40°C to limit pressure drop. Surfactants were injected individually.

Despite end capping processes developed by column providers, unreacted silanol groups are always present at the surface of the stationary phases [50]. These residual silanols are able to interact with cationic compounds through cation exchange mechanism, and are responsible for peak tailing or possible adsorption of basic compounds, such as quaternary ammoniums. The actual pKa of silanol groups on column packings

ranges between 3.8-4.5 depending on their location [51]. Therefore, the mobile phase was acidified at pH 3.5, to limit these ionic interactions, since residual silanols are mostly protonated under these conditions. The use of ammonium formate can also create ion pairs between ammonia and silanols, thus limiting the strength of ionic interactions. Three levels of concentrations: 5mM, 10mM and 20mM were tested. Indeed, an impact on the ionization was observed, the signal was better at 20 mM of ammonium formate. But, at 20mM of salt, the system got dirty quickly and required cleaning too frequently. 10mM was a compromise between the fouling and the signal intensity. Ammonium formate buffer at 10 mM, pH 3.5, was systematically used in the aqueous phase.

Table 2 : Stationary phase and mobile phase conditions for LC-MS analysis of surfactants

	length (mm) id* 2.1mm	d _p * (μm)	Pore Size (Å)	Specific Area (m ² .g ⁻¹)	Carbon (%)	Mobile Phase	Gradient program
Accucore™ C4	150	2.6	150	80	2	A : 10mM ammonium formate pH 3.5 B : ACN	
Accucore™ C18	150	2.6	80	130	9		-5-0 min 30% (equilibration step)
Accucore™ C30	150	2.6	150	90	5		0-12 min 30%-100%, 6 min at 100%
Accucore™ PFP*	150	2.6	80	130	5		
Accucore™ Phenyl-X	250	2.6	80	130	6		-5-0 min 30% (equilibration step)
Accucore™ Polar Premium	250	2.6	150	90	9		0-20 min 30%-100%, 10 min at 100%

To compare retention of surfactants, some fundamental values were calculated. As gradient elution was used for the separation, the apparent retention factors (k^*) were calculated using the dead time of each column. The dwell time was calculated thanks to fluoroglycynol for RPLC columns. The peak width value ($w_{50\%}$), in time units, was calculated at 50% of the peak height. The asymmetry factor (A_s) was calculated according to American Society for Testing and Materials (ASTM-E682) [52].

3. Results and discussion

3.1. Characterization of surfactants by infusion HRMS experiments

The model surfactants selected in this work were infused in both negative and positive ionization modes to characterize the ions distribution and build a database. Figure 1 shows the corresponding mass spectra under MS optimized conditions. A few standards were monodispersed such as quaternary ammoniums and therefore only one ion was present. For polydispersed surfactants having different alkyl chain lengths and different EO units (i.e. Triton, Brij, DBSA, glycolic acid ethoxylated lauryl ether), several ions were detected. Thanks to HRMS, the number of carbon atoms in the alkyl chain, the number of EO units and the nature of the protonated/deprotonated molecules were determined with a mass accuracy below 5 ppm. The list of ions observed for all the studied surfactants is provided in Table 4.

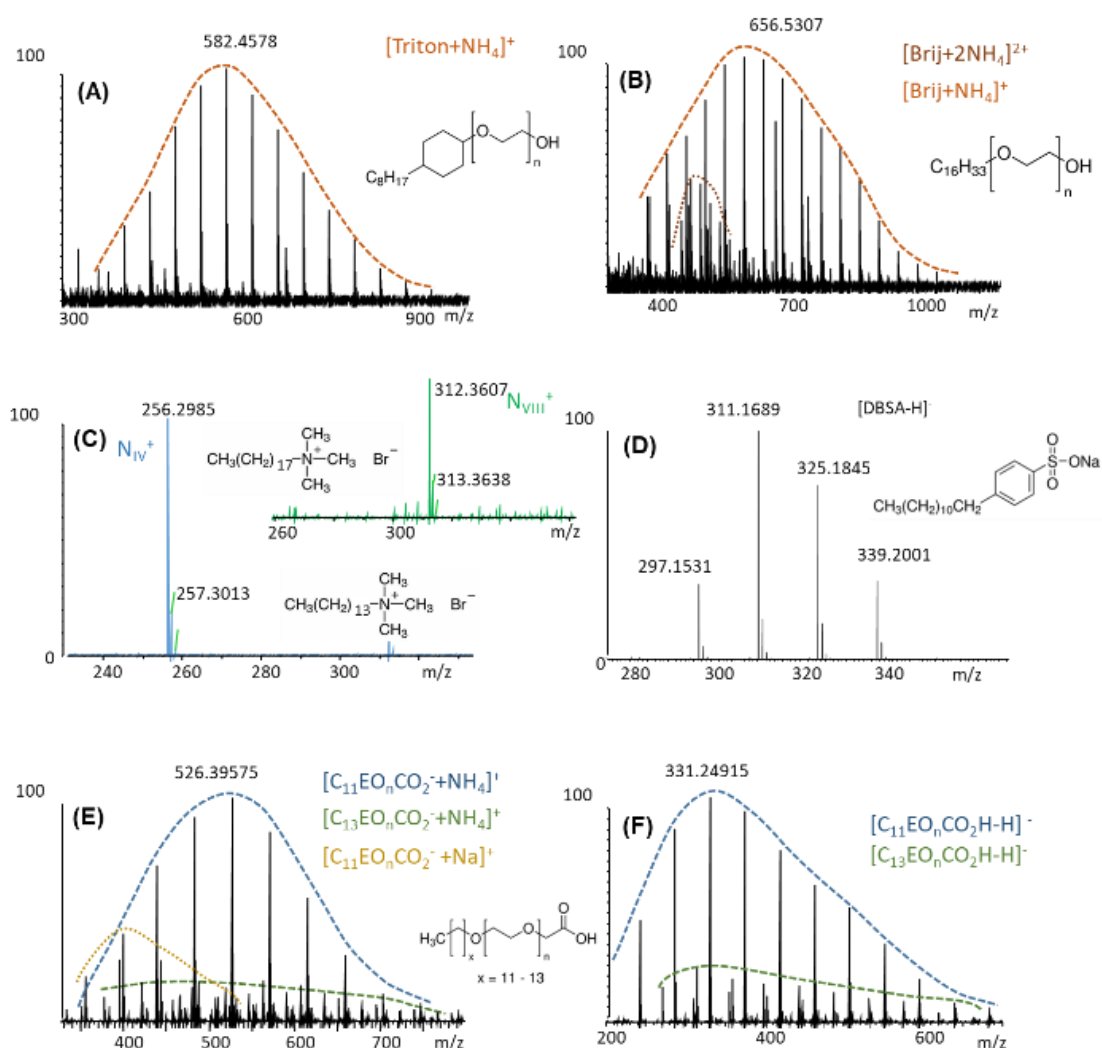


Figure 1: Infusion-MS experiments of (A) Triton X-100 reduced , (B) BrijC10, (C) triméthylheptadécylammonium bromide and myristyltrimethylammonium bromide, (D) DBSA, glycolic acid ethoxylated lauryl ether in positive (E) and negative (F) ionization mode; concentration 1 mg.L⁻¹ each; mobile phase Water/ACN 50/50

Table 1 : list of ions observed in MS for the studied surfactants in both electrospray positive and negative ionization modes

Compounds	[M] ⁻		[M] ⁺		Non-ionic	[M+NH ₄] ⁺		[M+2NH ₄] ²⁺	
DBSA			Ammonium		Compounds	Triton	BrijC10	Triton	BrijC10
C ₁₀	297.1531		C ₁₄ N(CH ₃) ₃	256.2985	n _{EO} =1	/	304.3215		
C ₁₁	311.1689		C ₁₈ N(CH ₃) ₃	312.3607	n _{EO} =2	318.3008	348.3477		
C ₁₂	325.1845				n _{EO} =3	362.3270	392.3739	/	/
C ₁₃	339.2001				n _{EO} =4	406.3532	436.4001		
					n _{EO} =5	450.3795	480.4263		
					n _{EO} =6	494.4057	524.4526		
					n _{EO} =7	538.4319	568.4788		
					n _{EO} =8	582.4581	612.5050		
					n _{EO} =9	626.4843	656.5312		
					n _{EO} =10	670.5105	700.5574		
					n _{EO} =11	714.5368	744.5836		
					n _{EO} =12	758.5630	788.6098		
					n _{EO} =13	802.5892	832.6361		
					n _{EO} =14	846.6154	876.6623		447.3483
					n _{EO} =15	890.6416	920.6885		469.3614
					n _{EO} =16	934.6678	964.7147		491.3745
					n _{EO} =17		1008.7409		513.3876
					n _{EO} =18		1052.7671		535.4007
					n _{EO} =19		1096.7934		557.4138
					n _{EO} =20				579.4269
					n _{EO} =21				601.4400
					n _{EO} =22	/			623.4531
					n _{EO} =23				645.4663
					n _{EO} =24			/	667.4794
					n _{EO} =25				689.4925
					n _{EO} =26				711.5056
					n _{EO} =27				733.5187
					n _{EO} =28				755.5318

Compounds	[M] ⁻		[M+NH ₄] ⁺		[M+2NH ₄] ²⁺	
Carboxylate	C ₁₁ H ₂₃ EO _n COOH	C ₁₃ H ₂₇ EO _n COOH	C ₁₁ H ₂₃ EO _n COOH	C ₁₃ H ₂₇ EO _n COOH	C ₁₁ H ₂₃ EO _n COOH	C ₁₃ H ₂₇ EO _n COOH
n _{EO} =1	243.1960	271.2273	262.2381	290.2694		
n _{EO} =2	287.2222	315.2535	306.2644	334.2957		
n _{EO} =3	331.2485	359.2798	350.2906	378.3219		
n _{EO} =4	375.2747	403.3060	394.3168	422.3481		
n _{EO} =5	419.3009	447.3322	438.3430	466.3743		
n _{EO} =6	463.3271	491.3584	482.3692	510.4005	/	
n _{EO} =7	507.3533	535.3846	526.3954	554.4267		
n _{EO} =8	551.3795	579.4108	570.4217	598.4530		
n _{EO} =9	595.4057	623.4370	614.4479	642.4792		/
n _{EO} =10	639.4320	667.4633	658.4741	686.5054		
n _{EO} =11	683.4582	711.4895	702.5003	730.5316		
n _{EO} =12	727.4844	755.5157	746.5265	774.5578		
n _{EO} =13	771.5106		790.5527	818.5840	404.2935	
n _{EO} =14	815.5368		834.5789	862.6102	426.3066	
n _{EO} =15		/	878.6052	906.6365	448.3197	
n _{EO} =16	/	/	922.6314	950.6627	470.3328	
n _{EO} =17	/				492.3459	
n _{EO} =18			/	/	514.3591	

3.1.1. Cationic surfactants: quaternary ammoniums

Two quaternary ammonium surfactants, namely myristyltrimethylammonium bromide (m/z at 256.3047) and trimethyloctadecylammonium (m/z at 312.3682) were studied, as reported in Figure 1C. As expected, quaternary ammoniums were ionized only in the positive ionization mode. The fragmentation pattern of trimethyloctadecylammonium was studied using HCD (Higher Energy Collisional Dissociation), and three voltage levels were tested (15, 35 and 65V). When the energy increased, the intensity of the parent ion decreased and a specific fragment ion at $m/z=60.0816$ appeared, corresponding to $(\text{CH}_3)_3\text{NH}^+$. This fragmentation pattern is specific to quaternary ammonium [53]. This specific transition could be used for further quantification purposes. Indeed, multiple reaction monitoring (MRM) can be a way to selectively quantify all quaternary ammoniums surfactants at the same time.

3.1.2. Anionic surfactants

There are three classes of anionic surfactants: sulfonates, sulfates and carboxylates. In this study, only sulfonates and carboxylates were studied. The mixture of DBSA homologs was ionized only in the negative ionization mode. Figure 1D shows four homologs differing only by their methyl units: decylbenzenesulfonate ($\text{C}_{16}\text{H}_{25}\text{O}_3\text{S}^-$, $m/z = 297.1531$), undecylbenzenesulfonate ($\text{C}_{17}\text{H}_{27}\text{O}_3\text{S}^-$, $m/z = 311.1689$), dodecylbenzenesulfonate, ($\text{C}_{18}\text{H}_{29}\text{O}_3\text{S}^-$, $m/z = 325.1845$) and tridecylbenzenesulfonate ($\text{C}_{19}\text{H}_{31}\text{O}_3\text{S}^-$, $m/z = 339.2001$). The fragmentation pattern was studied on undecylbenzenesulfonate. In addition to the molecular ion, three product ions were identified at m/z 183.0115 ($\text{C}_8\text{H}_7\text{O}_3\text{S}^-$), 119.0489 ($\text{C}_8\text{H}_7\text{O}^-$) and 79.9557 (SO_3^-). These product ions were also observed in other studies [3,17,53].

For carboxylate surfactants, the glycolic acid ethoxylated lauryl ether ($\text{C}_{11}\text{EO}_n\text{CO}_2\text{H}$, $\text{C}_{13}\text{EO}_n\text{CO}_2\text{H}$) was studied in both ionization modes. In negative mode, two distributions were observed (Figure 1F): $\text{C}_{11}\text{H}_{23}(\text{OC}_2\text{H}_4)_n\text{COO}^-$ with n comprised between 1 to 13 and $\text{C}_{13}\text{H}_{27}(\text{OC}_2\text{H}_4)_n\text{COO}^-$, with $n = 1$ to 13. These two distributions consisted of peaks separated by 28 m/z units, corresponding to ethyl units. In positive mode, carboxylate surfactants were protonated. The polyethylene oxide chain formed the following adducts: $[\text{M}+\text{NH}_4]^+$, $[\text{M}+\text{Na}]^+$ and $[\text{M}+\text{K}]^+$. The abundance of each adduct could be influenced by the solvent nature and the glassware employed to store mobile phases [54]. Figure 1E shows the distribution of carboxylates ammonium adducts, $[\text{C}_{11}\text{H}_{23}(\text{OC}_2\text{H}_4)_n\text{COOH},\text{NH}_4]^+$. The number of EO units ranged from 3 to 12. In positive ionization mode, carboxylates having less than three EO units were not detected. Luo *et al.* showed that signal intensity of short EO chain carboxylates ($n < 3$) can be improved, by optimizing MS parameters such as S-Lens, RF amplitude and temperature [1].

However, under such optimal conditions for short EO chain carboxylates, the signal of long EO chain carboxylates was decreased. Thus, a compromise between long and short EO chain carboxylates signals had to be found, so the S-Lens was set to 50.

Finally, pentanoic acid and myristic acid do not ionize in positive mode, probably because the cationic affinity is too weak in comparison to EO groups. These two acids were not used for retention measurements.

3.1.3. Non-ionic surfactants

Triton X-100 reduced and BrijC10 are presented in Figure 1A and 1B, respectively and are only ionized in positive ionization mode, as ammonium adducts. The molecular formula of Triton was $[C_{14}H_{27}(OC_2H_4)_nOH, NH_4]^+$, and the number of EO units, n , was between 3 and 14. Mass spectrum of BrijC10 was more complex than Triton, and was composed of two major distributions. The first distribution corresponded to singly charged ions with molecular formula $[C_{16}H_{33}(OC_2H_4)_nOH, NH_4]^+$. In this case, the number of EO units ranged from 3 to 19. On the other hand, the second distribution was located between $m/z = 400$ and 600 g/mol, because the EO number increases the stability of double ammonium adduct. So the doubly charged ions correspond to a molecular formula $[C_{16}H_{33}(OC_2H_4)_nOH, 2NH_4]^{2+}$ with n ranging from 14 to 25.

3.2. Analytical characterization of surfactants by chromatographic techniques

The retention of surfactant standards was studied using nine different stationary phases involving three different chromatographic modes: RPLC, HILIC and mixed-mode chromatography.

For each chromatographic mode, retention maps were obtained: They represent the elution profile of the surfactants according under the selected stationary and mobile phase conditions.

3.2.1. Retention map in RPLC conditions

The retention map of the model surfactants obtained in RPLC mode is shown in Figure 2. In this representation, the elution windows of the different surfactants under the selected conditions (six different stationary phases were tested) were represented as arrows and diamonds for polydispersed and monodispersed surfactants, respectively. In addition, k^* was also systematically indicated as a single value for monodispersed surfactants, and as a range for polydispersed surfactants. Anionic – DBSA (mixture of four homologs) & carboxylate acid ($C_{11}EO_nCO_2H$, $C_{13}EO_nCO_2H$) – surfactants were detected in negative ionization mode (red arrows), whereas cationic – N_{IV} & N_{VIII} – and non-ionic – Triton & BrijC10 – surfactants were detected in positive ionization mode (green arrows). According to this representation, the separations were in good agreement with RP mechanism. As example, the

longer the alkyl chain, the higher the retention of quaternary ammonium. In the case of anionic surfactants, sulfonates (DBSA) were less retained than carboxylates ($C_{11}EO_nCO_2H$, $C_{13}EO_nCO_2H$), due to the higher polarity of sulfonic acid moiety vs. carboxylic acid. This retention order was consistent with previously published works [4,55]. The retention between these two surfactants (DBSA and ($C_{11}EO_nCO_2H$, $C_{13}EO_nCO_2H$)) was similar for Polar embedded C18 and Phenyl columns. In the case of these two columns (Phenyl and Polar embedded C18), the polarity and the structure of anionic surfactants (DBSA and $C_{11}EO_nCO_2H$, $C_{13}EO_nCO_2H$) have a strong impact on the retention. This difference is explained later section 3.2.1.2.

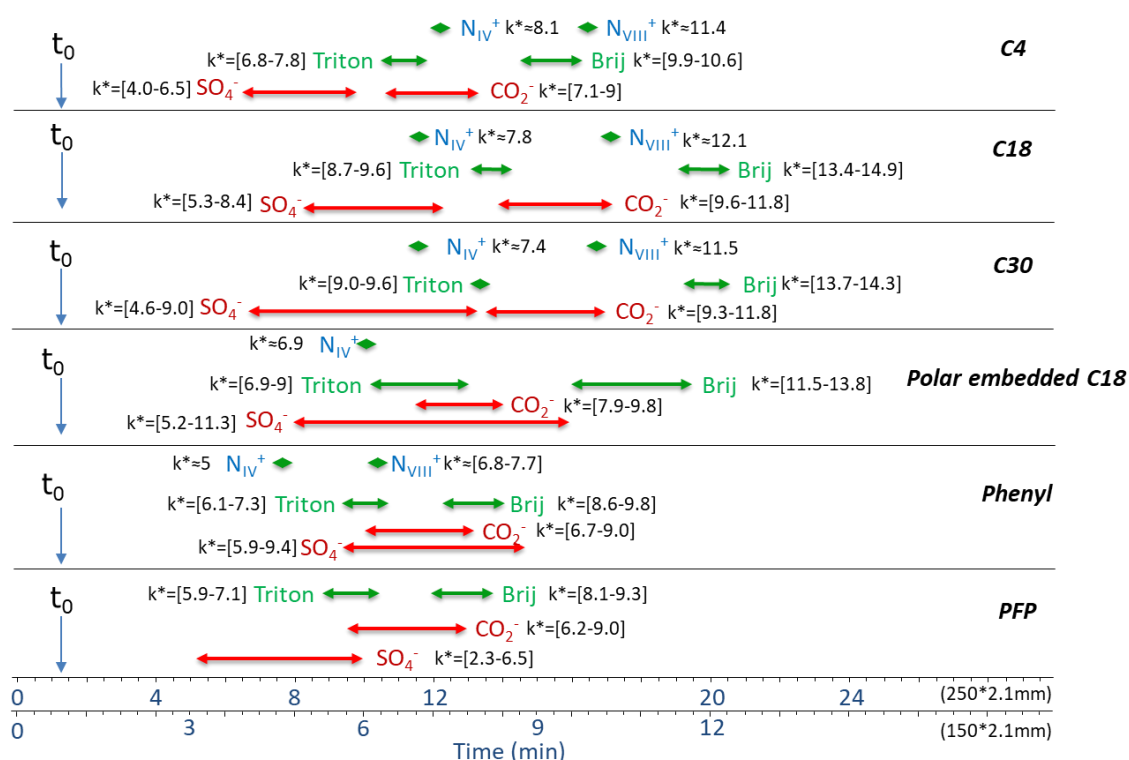


Figure 2 : Retention maps of studied surfactants in RPLC mode

3.2.1.1. Cationic surfactants

Whatever the RPLC column used, the retention order of quaternary ammonium surfactants was the same: myristyltrimethylammonium (N_{IV}^+) eluted before trimethyloctadecylammonium (N_{VIII}^+), which is in agreement with the lipophilicity of these two surfactants (log P of 1.8 vs. 3.6, according to Chemicalize). Despite the acidified mobile phase, ammonium surfactants were not eluted on PFP columns, which is logical since PFP moiety is a strong Lewis acid, which should therefore strongly interact with ammonium ions. On this column, the addition of 0.1% TFA to the mobile phase could be of interest to further decrease mobile phase pH below 2, and also obtain ion pairing between quaternary ammonium of the surfactant and carboxylic acid of the TFA. This was not tested because

at pH below 2, the bonded phase would be probably unstable (specifications of the column – ThermoScientific).

The alkyl chain length of the RPLC stationary phase also had a strong influence on peak asymmetry and more limited impact on retention. Indeed, for these three columns two mechanisms were probably engaged, based on ionic interactions with residual silanols and hydrophobic interaction with alkyl chain. Depending on the alkyl chain length, one of these mechanisms was stronger. For example, on C4 column, the mechanism of retention was mostly based on ionic interaction. The accessibility of residual silanols was easier than other columns (C18/C30). Oppositely, on C18 and C30 columns the retention mechanism was mostly based on hydrophobic interaction. This hypothesis can explain the similar retention of quaternary ammoniums but also the difference of asymmetry obtained. For example, the myristyltrimethylammonium apparent retention factor was 7.4, 7.8 and 8.1 for C30, C18 and C4 columns respectively. The asymmetry factor for this ammonium was 10 on C4 and only 1.75 on C30 column.

The retention of ammonium surfactants was lower on Phenyl column than on alkyl grafted ones. This behavior is consistent, since the Phenyl column is less hydrophobic than C18 or C30, and has less accessibility to silanols than the C4, due to steric hindrance. In the end, both hydrophobic and ionic interactions are more limited, leading to an apparent retention factor of only 5 vs. 7.4 on the C30 column. On the polar embedded C18 phase, the retention was slightly reduced compared to the other alkyl grafted columns, probably due to the presence of an amide group in the alkyl chain, able to provide electrostatic repulsion, thus limiting accessibility to silanols. For the ammonium N_{IV}^+ , the Phenyl column presented an asymmetry of 4, whereas it was only 2.5 with the polar embedded C18. In addition, when using Phenyl or polar embedded columns, the ammonium surfactant peak widths increased. The $w_{50\%}$ was equal to 0.5, 0.25 and 0.07 min for the Polar Premium, Phenyl-X and C30 columns, respectively.

3.2.1.2. *Anionic surfactants*

The glycolic acid ethoxylated lauryl ether (carboxylate surfactant) was composed of two families with two different alkyl chain lengths (C_{11} and C_{13}). It is important to notice that in these two families, homologs (i.e. different EO, identical alkyl chain length) were partly separated with only PFP, Phenyl, and polar embedded C18 phases ($R_s \approx 0.8$). On alkyl stationary phases (C4, C18 and C30 columns), the resolution was null ($R_s \approx 0.1$). These separations were likely to originate from the presence of polar interactions (i.e. H-bond, dipole-dipole, π - π) between the EO chain and the stationary phase

bonding. Obviously, such polar interactions are not available on alkyl bonded stationary phases, leading to very low resolution between homologs.

In the case of sulfonate surfactants, DBSA was composed of four homologs, as observed in direct infusion experiments; alkyl phases (i.e. C4, C18 and C30) were able to separate these homologs ($R_s > 1.5$). When the length of the alkyl chain of the stationary phase increased, more than one peak was observed for each homolog, suggesting the presence of positional isomers. These results are in good agreement with those of Motteran *et al.* who showed the presence of isomers for each DBSA homolog [3]. Sulfonate surfactants such as DBSA have an aromatic ring with sulfonate group in para position, thus the aromatic ring is π -attractor. On the Phenyl column, the aromatic ring is π -donor, while the PFP column includes a phenyl group with five fluorines, thus making the aromatic ring π -attractor. Based on these features, it is therefore logical that the DBSA was more retained ($k^* > 6$) on Phenyl column, compared to the PFP column ($k^* < 6$).

3.2.1.3. *Non-ionic surfactants*

Unlike MS, LC can separate isomers, as observed with the reduced Triton X-100. Whatever the column used, two distributions of peaks were observed when the extracted ion chromatograms (EIC) were taken at the same m/z . The presence of two distributions at the same m/z could be explained by two possible positions of the alkyl chain on the aromatic ring (para and ortho), or/and by the ramification of the alkyl chain.

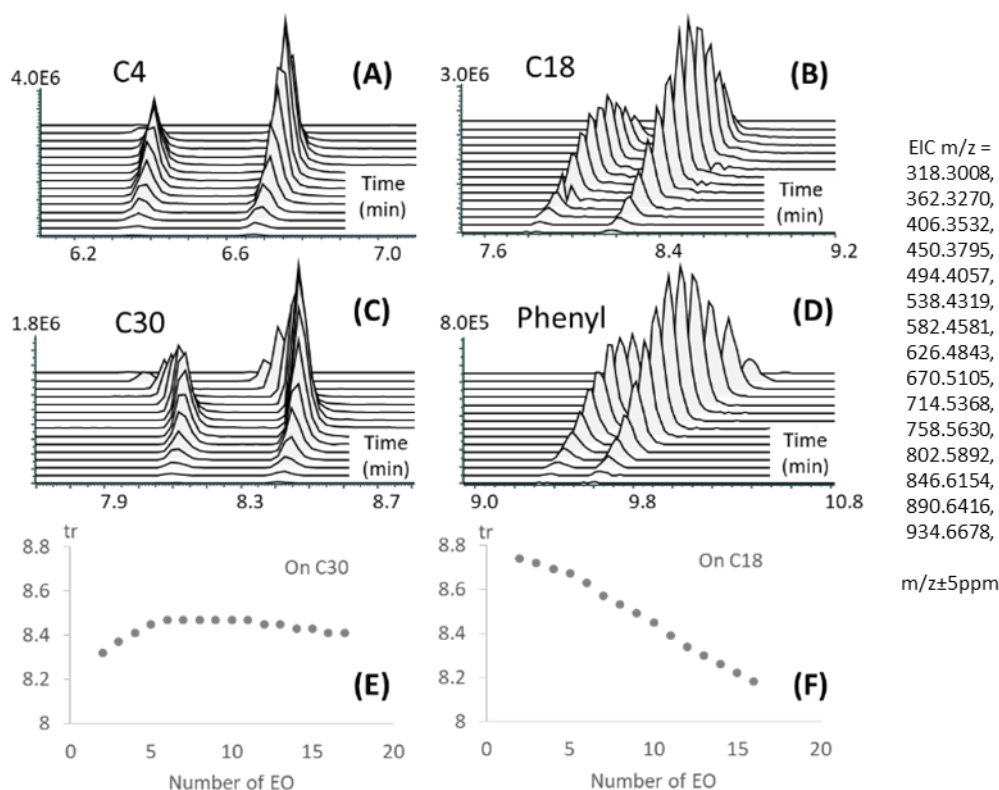


Figure 3 : (A-D) EICs of Triton X100 reduced ($2 < n_{EO} < 16$) at 200 mg.L^{-1} on Accucore™ C4, Accucore™ C18, Accucore™ C30 (0-12 min 30%-100%B, 6 min at 100%B) and Accucore™ Phenyl (0-20 min 30%-100%B, 10 min at 100%B) – (E-F) representative retention of Triton X100 reduced according number of EO on C30 and C18 columns, respectively

Using C18, polar embedded C18, Phenyl and PFP columns retention decreased linearly when number of EO increase. As the diminution was the same for these four columns, only C18 and Phenyl were shown in Figure 3B and 3D, respectively. The elution order of oligomers of Triton and Brij was identical: the higher the number of EO units, the lower the retention [29]. The explanation for this behavior was that the EO unit increased the hydrophilic character of the compounds, thus decreased their retention. However, this phenomenon was not observed on only C4 (Figure 3A) and C30 (Figure 3C) columns. For EO number below 6, oligomers retention seems slightly increased, and then stabilized. Actually, this phenomenon was not really explained. Only assumptions could be done about the effect of the stationary phase on the protection of residuals silanols.

The other non-ionic surfactant, namely Brij eluted as a wide peak corresponding to two distributions slightly superimposed. Figure 4 shows the corresponding chromatogram obtained on the C18 column. The first distribution corresponded to molecules with long EO chains detected as double charged ions ($M+2\text{NH}_4^{2+}$), while the second one corresponded to molecules with shorter EO chains detected as singly charged ions ($M+\text{NH}_4^+$). This figure illustrates the elution of Brij oligomers: in green is the Base Peak Chromatogram, in blue is EIC of n_{EO} ranging from 3 to 17 detected as monocharged

homologs, while EIC of n_{EO} ranging from 14 to 26 detected as doubly-charged homologs is shown in orange. Oligomers $n=14$ to 17 were detected both as a singly and doubly charged ion.

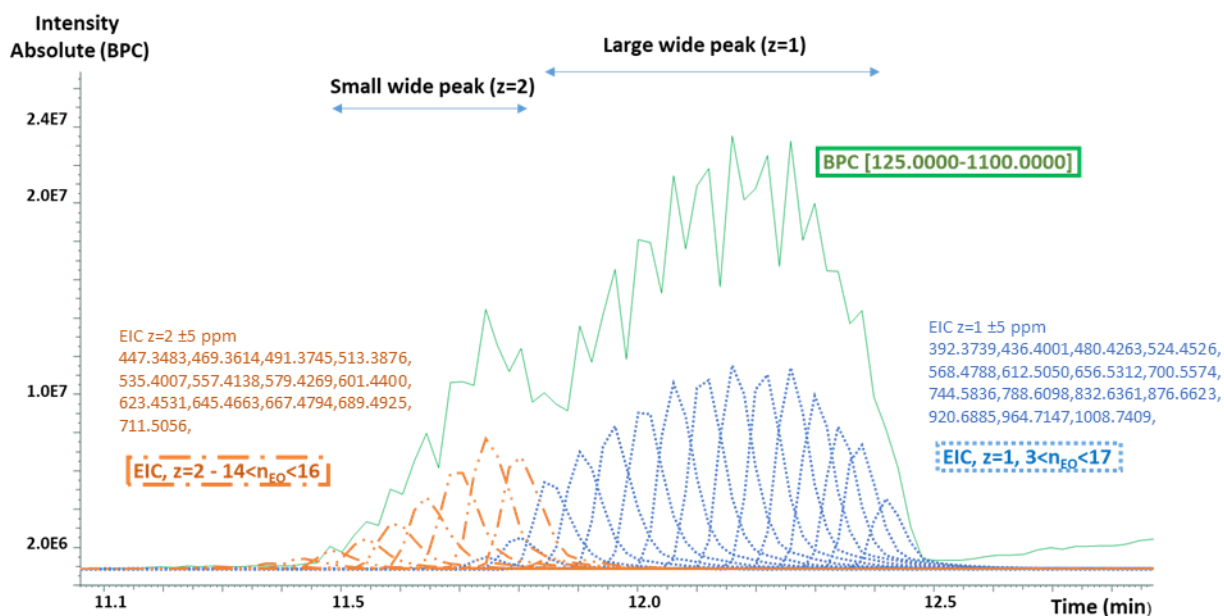


Figure 4 : UHPLC-MS chromatograms in positive ion mode of BrijC10 at 1 mg.L⁻¹ on C18 column (150 mm × 2.6 mm, 2.1 μm)

Whatever the stationary phase chemistry in RPLC conditions, some co-elutions are still present, which represented the limitation of this chromatographic mode for the analytical characterization of surfactants.

3.2.2. Separation of surfactants in HILIC mode

Initially, two different stationary phases were considered, namely bare silica and silica bonded with urea. Unfortunately, urea column was not adapted to the separation of surfactants, because the mechanism of retention involved with this column did not allow to retain these molecules and all surfactant families were eluted before 2 min. At this step, this column was discarded. Figure 5 shows EICs of surfactants obtained using the bare silica column.

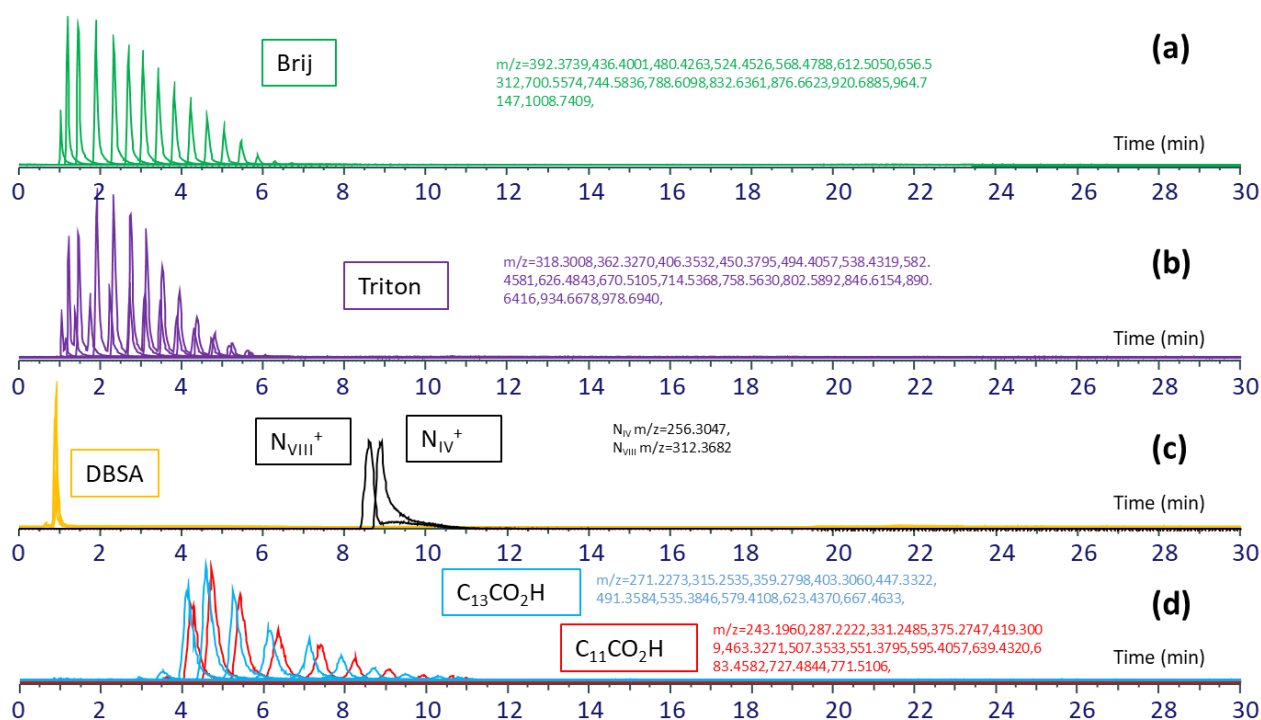


Figure 5 : EICs of surfactants on bare silica-HILIC column – (a) BrijC10, (b) Triton, (c) DBSA and quaternary ammonium, and (d) the glycolic acid ethoxylated lauryl ether

Besides the hydrophilic interaction mechanism observed under HILIC conditions with a large proportion of aprotic solvent, bare silica columns also present an ability for cation exchange. As the pH of the mobile phase was low (pH=3.5), a large proportion of silanols should be neutral, but it is hard to know the real amount of negatively charged deprotonated silanols, since the mobile phase is composed of a very high proportion of ACN and therefore, the pH scales are different from purely aqueous media. Under these selected HILIC conditions, positively charged quaternary ammoniums were well retained ($k^* > 10$), mostly through ionic interactions. Sulfonate surfactants (pK_a lower than 0), were negatively charged at this pH and because of electrostatic repulsion with silanulates, they were not retained (k^* around 0). Unlike sulfonates, carboxylate surfactants (pK_a of around 4) were probably protonated and therefore retained under HILIC conditions. However, both families of carboxylates (C_{11} and C_{13}) were eluted at the same retention times, which is expected since the separation of their homologs was affected only by the number of EO (polar groups). Obviously, retention increased when the number of EO increased. The same behavior was observed for non-ionic compounds, Brij and Triton, which also contains some EO units. Moreover, each homolog of Triton was eluted under the form of two peaks, similarly to what was shown in RPLC mode, thus confirming the presence of isomers. HILIC separation showed a real complementarity to RPLC mode, since surfactants were separated by families in RPLC, whereas HILIC separated homologs.

So, in HILIC, for Brij homologs $n=11$ to 17 , the retention increased with the number of EO. If a RPLC column was used, the maximum resolution (R_s) between these homologs ($11 < n < 17$) was 0.8 for the C4 column, whereas in HILIC mode with the bare silica column, the R_s was above 1.5 for the same homologs. Figure 6 clearly illustrates this difference.

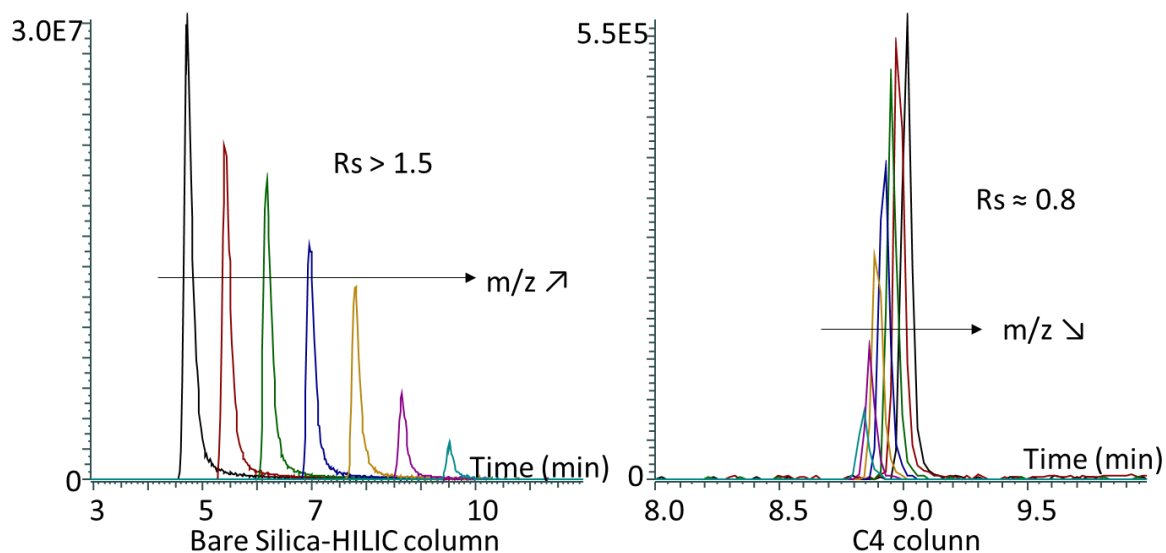


Figure 6 : EIC of BrijC10's homologs $n=11$ to 17 on C4 column (on the right) and on Bare silica column (on the left).

3.2.3. Separation of surfactants using mixed-mode chromatography

The surface chemistry of the selected mixed-mode column, namely Acclaim Surfactant Plus column, provides both reversed-phase and anion-exchange retention mechanisms. Figure 7 shows the separation of all surfactants employed in this work: anionic, cationic, and non-ionic. Cationic and non-ionic surfactants were detected in positive ionization mode (ESI+), whereas anionic surfactants were detected in negative ionization mode (ESI-). Cationic species were eluted first, followed by nonionic and finally anionic surfactants. This order confirmed the presence of an anion-exchange mechanism.

For the two quaternary ammoniums, mainly RP mechanism was involved; they had a limited retention owing to the absence of ionic interactions with the proprietary positively charged group at the surface of the stationary phase. Indeed, myristyltrimethylammonium ($k^* \approx 1.74$), which had the shorter alkyl chain, was eluted before trimethyloctadecylammonium ($k^* \approx 3.65$).

Then, nonionic surfactants were eluted ($3.67 < k^* < 8.15$). Triton had a shorter alkyl chain compared to Brij, thus Triton eluted first. Within the same family (Triton or Brij), the separation of homologs was similar to what was observed under RPLC conditions with alkyl bonded columns. Homologs were

separated according to their number of EO units, with a retention decrease when the number of EO units increased.

Problems were observed for the mixture of DBSA homologs: they were not detected. As they were detected for all other columns and conditions, it seemed that they were not eluted. Liu et al. reported an analytical method for surfactants separation on Acclaim Surfactant Plus column [56]. The same alkylbenzene sulfonates ($C_{16}H_{25}O_3S^-$, $C_{17}H_{27}O_3S^-$, $C_{18}H_{29}O_3S^-$, $C_{19}H_{31}O_3S^-$) were eluted but using ammonium acetate buffer 0.1M at pH 5.2. Their elution was obtained during the last isocratic plateau at 85% of ACN. In the present study, the buffer pH was set at 3.5 and the salt concentration was 10 mM. In these conditions, alkylbenzene sulfonates were not eluted. As the salt concentration was lower (10mM) than those used in Liu study (100 mM), the anion exchange interaction mechanism had a much stronger intensity in our case and hindered the elution of these molecules.

As expected from RP mechanism, the family carboxylates in $C_{11}EO_nCO_2H$ eluted before the family in $C_{13}EO_nCO_2H$. The selectivity observed for nonionic surfactant, was also observed with carboxylates surfactants ($C_{11}EO_nCO_2H$, $C_{13}EO_nCO_2H$). In addition, as for Triton and Brij, homologs were separated according to the number of EO units. The resolution between carboxylates homologs was clearly superior (around 1.5 whereas for Brij homologs it was around 0.8).

Separation by surfactants families, namely cationic, anionic and nonionic, was partly obtained using this mixed-mode column. However, due to the polydispersity of surfactants, overlapping between families and consequently co-elution persisted. Indeed, Brij, $C_{11}COOH$ and $C_{13}COOH$ were partly superposed as shown in Figure 7.

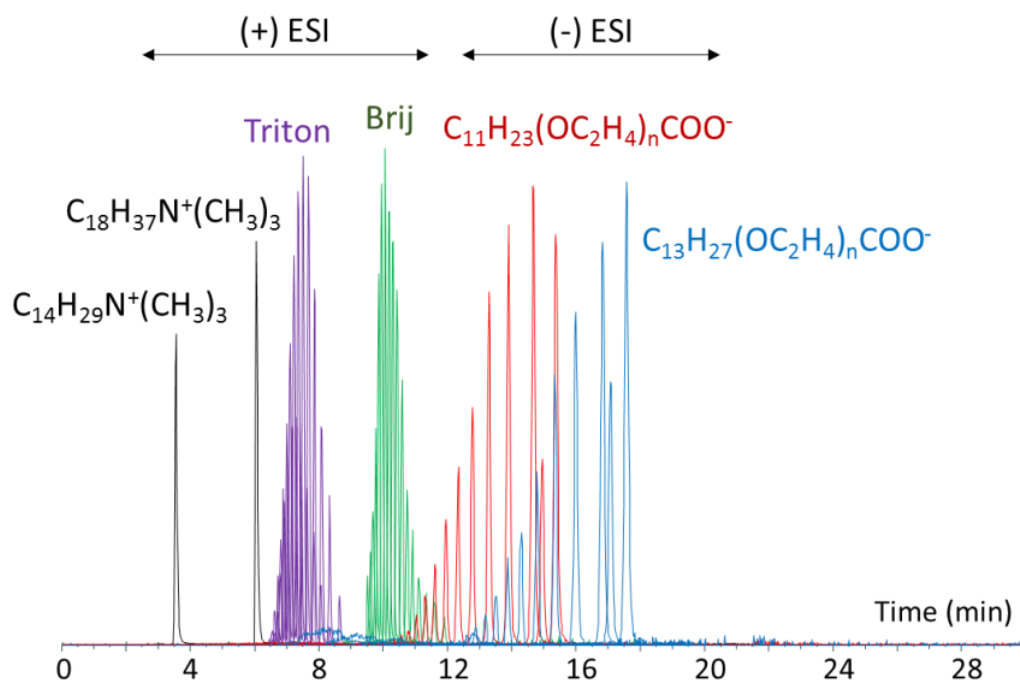


Figure 7 : Analysis of mixture surfactants with Acclaim Surfactant Plus

4. Conclusion

The aim of this study was to evaluate the possibilities offered by different chromatographic modes (i.e. RPLC, HILIC and mixed-mode chromatography) for the analysis of various surfactants using a wide range of stationary phases and HRMS detection. HRMS provided the information on the number of carbon atoms in the alkyl chain, the number of EO units present and adducts nature. This part was investigated by direct infusion and contributed to the foundation of an HRMS database. Then, owing to the lack of literature on the separation of surfactants in petroleum samples, a study of model surfactants retention on different stationary phases was investigated. Some representative elution patterns were observed showing the elution behavior of surfactants with the three different chromatographic modes, and co-elutions were systematically observed. This study showed that surfactant analysis was not easy, and depending on the nature of the surfactants, their polydispersity, their charge, the presence of polar groups in their structure, the investigated stationary phase could lead to more or less efficient separations. The RPLC mode provided separation according to the hydrophobic part of surfactants, whereas HILIC separated according to the number of EO units. These two modes of chromatography offer an excellent complementarity. The mixed-mode combined different retention mechanisms (hydrophobic and anion-exchange) and represented a good compromise for a one-dimension separation. However, for polydisperse surfactants, co-elution remained as observed for example with carboxylates. In the case of the analysis of more

complex samples consisting of several surfactants, distributions would probably overlap more. This limitation could be removed elegantly thanks to a multidimensional approach. Thus, the present study could be considered as a basis for the development of a multidimensional analytical strategy.

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Figures captions

Table 1: Advantages and drawbacks of available methods for surfactants analysis

Table 2: Optimized MS parameters for direct infusion MS and LC-MS analysis

Table 3: Stationary phase and mobile phase conditions for LC-MS analysis of surfactants

Table 4: list of ions observed in MS for the studied surfactants in both electrospray positive and negative ionization modes

Figure 1: Infusion-MS experiments of (A) Triton X-100 reduced, (B) BrijC10, (C) triméthyl-octadécylammonium bromide and myristyltriméthylammonium bromide, (D) DBSA, glycolic acid ethoxylated lauryl ether in positive (E) and negative (F) ionization mode; concentration 1 mg/L each; mobile phase Water/ACN 50/50.

Figure 2: Retention maps of studied surfactants in RPLC mode

Figure 3: (A-D) EICs of Triton X100 reduced ($2 < n_{EO} < 16$) at 200 mg/L on Accucore™ C4, Accucore™ C18, Accucore™ C30 (0-12 min 30%-100%B, 6 min at 100%B) and Accucore™ Phenyl (0-20 min 30%-100%B, 10 min at 100%B) – (E-F) representative retention of Triton X100 reduced according number of EO on C30 and C18 columns, respectively

Figure 4: UHPLC-MS chromatograms in positive ion mode of BrijC10 at 1 mg/L on C18 column (150 mm × 2.6 mm, 2.1 μm)

Figure 5: EICs ($\Delta 5$ ppm) of surfactants on bare silica-HILIC column – (A) BrijC10 ($3 < n_{EO} < 17$), (B) Triton ($2 < n_{EO} < 17$), (C) DBSA and quaternary ammonium, and (D) the glycolic acid ethoxylated lauryl ether ($1 < n_{EO} < 10$),

Figure 6: EIC ($\Delta 5$ ppm) of BrijC10's homologs $n=11$ to 17 on C4 column (on the right) and on Bare silica column (on the left).

Figure 7: Analysis of mixture surfactants with Acclaim Surfactant Plus