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Development of an analytical method for the quantification of surfactants and its application to wastewater treatment plant effluents

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Development of an analytical method for the quantification of surfactants and its application to wastewater treatment plant effluents

by

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Abstract

A monitoring campaign was conducted which collected seven-day composite effluent samples (n=33) from 33 conventional wastewater treatment plants (WWTPs) across Germany to measure the concentrations of linear alkylbenzene sulfonates (LAS) and alkyl ethoxysulfates (AES). In addition, seven-day composite influent samples of four WWTPs were taken and analyzed for the same set of compounds, to determine the removal rates of the aforementioned surfactants during conventional wastewater treatment. This study encompasses the analysis of four LAS homologs (C_{10}–C_{13}) and two AES homologs with each 10 ethoxymers (C_{12} and C_{14} with 0–9 ethoxy units). Sample pretreatment was carried out by removing the aqueous phase using a rotational vacuum concentrator and reconstituting the analytes in a defined volume of ultra-pure water and acetonitrile. The identification and quantification of target compounds were performed by high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). The analytical performance of the methods was validated in tap water and effluent water, obtaining good trueness and precision for both matrices. Based on the estimated average effluent concentrations of individual LAS homologs, the average total LAS concentration in monitored WWTP effluents was 14.4 µg/L. Total AES effluent concentrations were lower compared to LAS, with an average total AES effluent concentration of 0.57 µg/L. No correlation between total LAS and AES effluent concentrations was found. Total LAS influent concentrations averaged at 3,200 µg/L, which translates to an average removal rate of 99.6%. The average total influent concentration of AES was 680 µg/L, indicating an average removal rate greater than 99.9%. Retrospective screening of 1,564 suspect list surfactants and their transformation products (TP) by a second laboratory was performed using ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS). The LAS-byproducts dialkyltetralin sulfonates (DATSs), the metabolites sulfophenyl alkyl carboxylic acids (SPACs) and sulfo-tetralin alkyl carboxylic acids (STACs) reached maximum concentration levels of 19 µg/L, 17 µg/L and 5.3 µg/L, respectively. It was shown that in many cases the sum of concentrations of all LAS-related byproducts and TPs surpassed the concentration of the four precursor LAS homologs (C_{10} – C_{13}) themselves. High concentrations of up to 7.4 µg/L for 41 polyethylenoglycols (PEGs), the longest homolog series so far reported for PEGs, were detected. All quantified surfactants and their TPs and by-products together accounted for concentrations of up to 82 µg/L in effluent wastewater

Kurzbeschreibung

Zur Bestimmung der Konzentrationen linearer Alkylbenzolsulfonate (LAS) und Alkylethersulfate (AES) in Kläranlagenabläufen wurden 7-Tagesmischproben (n=33) an Abläufe von 33 konventionellen Kläranlagen in Deutschland genommen. Zudem wurden an vier der untersuchten Kläranlagen die Zuläufe beprobt und ebenfalls auf LAS und AES untersucht, um Rückschlüsse auf die Entfernung dieser Tenside in konventionellen Kläranlagen ziehen zu können. Insgesamt umfasste die Studie die Analyse von vier LAS-Homologen (C_{10}-C_{13}) sowie von jeweils 10 Ethoxymeren zweier Homologe von AES (C_{12} und C_{14}, jeweils mit 0-9 Ethoxygruppen). Die Probenvorbereitung bestand aus der Entfernung der wässrigen Phase mit Hilfe eines Rotations-Vakuum-Konzentrators und anschließender Resolvatisierung des Trockenrückstandes in einer definierten Menge Reinstwasser und Acetonitril. Die Identifikation und Quantifizierung der Zielanalyten erfolgte mittels Hochleistungsflüssigkeitschromatographie mit Tandem-Massenspektrometrie-Kopplung (HPLC-MS/MS). Die Leistungsfähigkeit der analytischen Methoden wurde in Leitungswasser und Kläranlagenablauf evaluiert. Die Analysemethoden zeigten für beide Matrices eine allgemein gute Richtigkeit sowie Präzision. Basierend auf den geschätzten mittleren Konzentrationen einzelner LAS-Homologe wurde eine mittlere Gesamtkonzentration von 14,4 µg/L in Kläranlagenabläufen ermittelt. Verglichen mit

LAS, wurden für AES stets geringere Gesamtkonzentrationen im Ablauf gemessen: Die mittlere AES-Gesamtkonzentration in den Abläufen betrug 0,57 µg/L. Zwischen den Gesamtkonzentrationen von AES und LAS bestand keine Korrelation. In den Zuläufen beprobter Kläranlagen wurden im Mittel 3.200 µg/L LAS detektiert. Damit betrug die mittlere Entfernung für LAS 99,6 %. Die mittlere AES-Konzentration im Kläranlagenzulauf belief sich auf 680 µg/L, was einer mittleren AES-Entfernung von >99.9% entspricht. Retrospektives Screening von 1.564 Tensiden und deren Transformationsprodukte (TPs) erfolgte durch ein zweites Labor unter Anwendung der Ultrahochleistungsflüssigkeitschromatographie mit Flugzeitmassenspektrometer-Kopplung (UHPLC-QTOF-MS). In vielen Fällen wurde die Konzentration von LAS von der Summe der Konzentrationen der Neben- und Transformationsprodukte von LAS überstiegen. Für die LAS-Nebenprodukte Dialkyltetralinsulfonate (DATS) lag die maximale Summenkonzentration bei 19 µg/L, für die Sulfophenylalkylcarbonsäuren (SPACs) bei 17 µg/L und für die Sulfotetralinalkylcarbonsäuren (STACs) bei 5,3 µg/L. Hohe Konzentrationen von bis zu 7,4 µg/L wurden für Polyethylenoglycole in den Abwasserproben bestimmt. Die Gesamtkonzentration aller quantifizierten Tenside, TPs und Nebenprodukte in einer einzelnen Probe betrug bis zu 82 µg/L.

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List of Abbreviations

ABS	Branched alkylbenzene sulfonate
ACN	Acetonitrile
AES	Alkyl ethoxysulfate
AS	Alkyl sulfate
DATS	Dialkyltetralin sulfonate
DCT	Data collection template
DSFP	Digital sample freezing platform
EO	Ethoxy
EIC	Extracted ion chromatogram
ESI	Electrospray ionization
FoA	Frequency of appearance
FLD	Fluorescence detection
GC	Gas chromatography
HPLC	High-performance liquid chromatography
IS	Internal standard
LAS	Linear alkylbenzene sulfonates
LOQ	Limit of quantification
MCA	Multi-channel aquisition
MeOH	Methanol
MLE	Maximum likelihood estimation
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MW	Molecular weight
PE	Population equivalent
PEG	Polyethylenoglycol
PNEC	Predicted no effect concentration
ROS	Regression on order statistics
RSD	Relative standard deviation
SLES	Sodium lauryl ether sulfate
SPAC	Sulfophenyl alkyl carboxylic acids
SPADC	Sulfophenyl alkyl di-carboxylic acids
SPE	Solid phase extraction
STAC	Sulfo-tetralin alkyl carboxylic acids
STADC	Sulfo-tetralin alkyl di-carboxylic acids
TOF-MS	Time-of-flight mass spectrometry
TP	Transformation product

ABS	Branched alkylbenzene sulfonate
UV	Ultraviolet
WWTP	Wastewater treatment plant

Summary

Synthetic surfactants are globally used in large volumes as active ingredients for both industrial and domestic purposes. Approximately three million tons of surfactants were manufactured in Western Europe alone in 2016. Linear alkylbenzene sulfonates (LAS) and alkyl ethoxysulfates (AES) have broad application in laundry and cleaning products and are the most commonly used anionic surfactants in Europe. Once used, surfactants enter aquatic environments via discharges of treated or untreated wastewater. Several studies have already reported high µg/L-concentrations of LAS in various surface waters, while generally lower environmental concentrations have been found for AES. However, there are significantly fewer studies assessing the presence of AES in aquatic systems, in comparison to LAS.

Several studies conducted on the occurrence and behavior of surfactants during conventional wastewater treatment showed that modern surfactants are extensively removed by a combination of biodegradation and sorption/settling processes. As the high removal rates of surfactants are compensated for their exceptionally high consumption volumes, surfactant residues and their transformation products are continuously discharged via wastewater treatment plants (WWTPs) into aquatic ecosystems. Most studies on the fate of modern surfactants during wastewater treatment were conducted from the late 1980s to the early 2000s and usually encompassed only a small number of WWTPs.

This study evaluates present-day effluent concentrations of two common groups of anionic surfactants, LAS and AES, from multiple WWTPs (n=33) in Germany. A national monitoring campaign was conducted which collected seven-day composite effluent samples (n=33) over the time course of three months. Additionally, seven-day composite influent samples of four WWTPs were taken to determine the removal rates of studied surfactants during conventional wastewater treatment.

Since no standards of individual LAS and AES homologs/ethoxymers were available, the concentrations of individual LAS and AES homologs/ethoxymers in two mixture standards were experimentally determined using single mass spectrometry (MS) measurements. Four LAS homologs (C_{10}–C_{13}) and two AES homologs each with 10 ethoxymers (C_{12} and C_{14}, with 0–9 ethoxy units (EO)) were identified in the respective mixture standards and the mass spectrometric conditions in negative ionization mode were individually optimized for each analyte.

Two analytical methods based on high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) were developed for the identification and quantification of both LAS and AES. For the determination of LAS homologs, a C_8 HPLC column was chosen, as it enabled sufficient chromatographic separation, as well as good peak shapes. For the analysis of AES homologs/ethoxymers, a polar modified C_{18} HPLC column was employed, as it allowed generally good peak shapes and intensities as well as a short runtime. As the contamination of solvents and equipment with surfactants is a common issue in laboratories, a rotational vacuum concentrator was chosen for sample pretreatment. This technique was found preferable compared to solid phase extraction (SPE) as the determined LAS and AES sample concentrations were not altered by any potential background contamination from solvents or laboratory equipment.

The precision and trueness of the analytical methods were determined by extracting six aliquots of a tap water and a WWTP effluent sample, respectively. Recovery rates of analytes ranged from 91% to 114% for tap water samples and from 90% to 120% for effluent water samples. Relative standard deviations (RSDs) ranged from 3% to 10% for both, tap and effluent water samples.

Total LAS concentrations in the monitored WWTP effluents ranged from below the limit of quantification (LOQ) to 47.7 µg/L. Based on the estimated average effluent concentrations of individual LAS homologs, the average total LAS effluent concentration in monitored WWTP effluents was 14.4 µg/L. The average LAS chain length of effluent samples was 11.2. Total AES effluent concentrations were lower compared to LAS and ranged from <LOQ to 1.9 µg/L, with an average total AES effluent concentration of 0.57 µg/L. For both AES homologs, ethoxymers with zero to three EO units showed the highest estimated average concentrations in effluents, resulting in an average number of 2.65 EO and 1.85 EO for AES-C_{12} and AES-C_{14}, respectively. No correlation between total LAS and AES effluent concentrations was found for the WWTPs monitored in the present study. Total LAS influent concentrations were between 2,600 µg/L and 3,500 µg/L, which translated to very high removal rates between 99.2% and 99.9%. Total influent concentrations of AES varied from 400 µg/L to 1,000 µg/L, indicating very high removal rates >99.8%. Both removal rates suggest a successful implementation of the detergent regulation that requires the readily degradation of surfactants used in detergents.

Sample aliquots were sent to the project partner where they were subjected to ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) analyses. A novel approach of screening selected suspect surfactants and their transformation products (TPs) for their presence in 'digitally archived' samples using Digital Sample Freezing Platform (DSFP) developed by the NORMAN Association (www.norman-network.net) was applied. A screening of 1,564 surfactants and their metabolites in the effluent samples was performed. The analysis included target screening of the same set of LAS and AES homologs/ethoxymers using the identical analytical standards provided by the other laboratory (TZW) to compare the results of the different analytical methods. The comparison indicated a very good agreement of the results of LAS considering the variability of the subsample, the variability introduced by the different sample preparation techniques applied and the different instrumental facilities. For AES, deviations between the results were higher. However, in all cases, concentration levels were at the same order of magnitude.

Known TPs of LAS (i.e. sulfophenyl alkyl carboxylic acids (SPACs) & sulfophenyl lkyl di-carboxylic acids (SPADCs), the LAS-byproducts dialkyltetralin sulfonates (DATSs) and its TPs sulfophenyl alkyl carboxylic acids (SPACs) and sulfo-tetralin alkyl carboxylic acids (STACs) were semi-quantified based on the comparison of their signals to the parent LAS molecules. The suspect screening showed that in many cases the sum of concentration of all LAS-related byproducts and TPs surpassed the concentration of LAS themselves. DATs, SPACs and STACs reached concentration levels of 19 µg/L, 17 µg/L and 5.3 µg/L, respectively. Additional to the target substances and LAS-related byproducts and TPs, occurrence of other surfactants was investigated. High concentrations of polyethylene glycols (PEGs) up to 7.4 µg/L were detected. All quantified surfactants and their TPs and by-products together accounted for concentrations up to 82 µg/L in effluent wastewater.

Several other surfactants were screened for without a possibility to estimate their concentrations due to unavailability of structurally similar reference standards. High frequency of appearance was observed for secondary alkane sulfonates (SAS), NP1ethoxycarboxylate, naphthalene-1-sulfonate and dihexyl sulfosuccinate.

Zusammenfassung

Synthetisch Tenside werden global in großen Mengen als Wirksubstanzen für unterschiedlichste industrielle und häusliche Anwendungen eingesetzt. So wurden allein in Westeuropa im Jahr 2016 ca. 3 Millionen Tonnen an Tensiden produziert. Lineare Alkylbenzolsulfonate (LAS) und Alkylethersulfate (AES) finden breite Verwendung in Wasch- und Reinigungsmitteln und stellen die mengenmäßig wichtigsten Vertreter aus der Gruppe der anionischen Tenside dar. Nach erfolgter Anwendung werden Tenside, im Zuge der Einleitung von geklärtem oder ungeklärtem Abwasser in den Vorfluter, in die aquatische Umwelt eingetragen. Diverse Studien konnten bereits LAS in Konzentrationen im hohen µg/L-Bereich in Oberflächengewässern nachweisen. Verglichen mit LAS, liegen die in der Literatur beschriebenen Konzentrationen für AES in Oberflächengewässern auf einem allgemein niedrigeren Niveau, wenngleich für AES nur wenige Studien existieren.

Zahlreiche Forschungsarbeiten zeigten bereits, dass moderne Tenside in konventionellen Kläranlagen durch eine Kombination aus biologischem Abbau und Sorptionsprozessen weitestgehend entfernt werden. Die hohe Entfernung in den Kläranlagen wird jedoch durch die ausgesprochen großen Einsatzmengen kompensiert, und so werden Tenside und deren Transformationsprodukte dennoch kontinuierlich in die aquatische Umwelt eingetragen. Die meisten wissenschaftlichen Arbeiten, die sich mit dem Verhalten von Tenside während der Abwasserreinigung befassen, stammen bereits aus den späten 1980er bis frühen 2000er Jahren. Des Weiteren umfassten die bisher publizierten Studien meist nur eine geringe Anzahl an untersuchten Kläranalgen.

Um ein aktuelles und repräsentatives Bild der Konzentrationen von LAS und AES in Kläranlagenabläufen zu erhalten, wurden im Rahmen einer Monitoringkampagne über einen Zeitraum von drei Monaten 7-Tagesmischproben des Ablaufes von 33 Kläranlagen genommen. Zusätzlich wurden an vier der untersuchten Kläranlagen auch der Zulauf beprobt, um die AES- und LAS-Entfernung in konventionellen Kläranlagen abschätzen zu können.

Da für die LAS- und AES-Homologe bzw. -Ethoxymere keine Einzelstandards verfügbar waren, wurde deren Konzentration in zwei Mischstandards experimentell mittels Massenspektrometrie (MS) ermittelt. Dabei konnten in den jeweiligen Mischstandards vier LAS-Homologe (C_{10}–C_{13}) sowie zwei AES-Homologe mit jeweils 10 Ethoxymeren (C_{12} and C_{14}, jeweils mit 0–9 Ethoxygruppen) identifiziert werden. Für diese Substanzen wurden die massenspektrometrischen Messbedingungen optimiert.

Zur Identifikation und Quantifizierung von LAS und AES wurden schließlich zwei analytische Messmethoden, basierend auf der Hochleistungsflüssigkeitschromatographie mit Tandem-Massenspektrometrie-Kopplung (HPLC-MS/MS), entwickelt. Zur Analyse von LAS-Homologen wurde eine C_8-HPLC-Trennsäule ausgewählt, da diese eine ausreichende chromatographische Trennung sowie gute Peakformen ermöglichte. Zur Quantifizierung von AES-Homologen bzw. -Ethoxymeren kam eine Trennsäule mit polar-modifiziertem C_{18}-Material zum Einsatz, da mit dieser gute Peakformen und hohe Sensitivitäten selbst bei kurzen Messzeiten erreicht werden konnte.

Da die Kontamination von Lösungsmitteln und Geräten mit Tensiden ein häufiges Problem in Laboren darstellt, kam ein Rotations-Vakuum-Konzentrator zur Probenvorbereitung zur Anwendung. Diese Aufbereitungstechnik stellte sich als geeigneter gegenüber der Festphasen-extraktion (SPE) heraus, da damit die Bestimmung der Probenkonzentrationen von LAS und AES nicht durch potentiell kontaminierte Lösungsmittel und Laborgeräte beeinträchtigt wurde.

Die Präzision und Richtigkeit der beiden Messmethoden wurde aus der Analyse von jeweils sechs Aliquoten einer dotierten und nativen Trinkwasser- und Kläranlagenablaufprobe abgeleitet. Die Wiederfindungen der untersuchten Homologe bzw. der Ethoxymere von LAS und AES betrugen bei der Extraktion aus Trinkwasser zwischen 91 % und 114 %. Im Falle der Kläranlagenabläufe, wurden Wiederfindungen zwischen 90 % und 120 % erreicht. Die relative Standardabweichung (RSD) bewegte sich für beide Matrices zwischen 3 % und 10 %.

Die Gesamtkonzentrationen von LAS in den untersuchten Kläranlagenabläufen reichten von Konzentrationen unterhalb der Bestimmungsgrenze bis zu einer Konzentration von 47,7 µg/L. Basierend auf den geschätzten mittleren Konzentrationen einzelner Homologe von LAS wurde eine mittlere Gesamtkonzentration von 14,4 µg/L in Kläranlagenabläufen ermittelt. Verglichen mit LAS wurden für AES stets geringere Gesamtkonzentrationen im Ablauf gemessen: Diese reichten von Konzentrationen unterhalb der Bestimmungsgrenze bis 1,9 µg/L. Die mittlere AES-Gesamtkonzentration im Ablauf betrug 0,57 µg/L. Zwischen den Gesamtkonzentrationen von AES und LAS bestand keine Korrelation. In den Zuläufen beprobter Kläranlagen wurden zwischen 2.600 µg/L und 3.500 µg/L LAS gemessen. Damit lag die Entfernung von LAS zwischen 99,2 % und 99,9 %. Die AES-Konzentrationen im Kläranlagenzulauf betrugen zwischen 400 µg/L und 1.000 µg/L, was einer Entfernung von AES von über 99,8 % entspricht. Diese hohen Eleminationsraten sprechen für eine erfolgreiche Implementierung der Europäischen Detergenzien-Verordnung.

Aliquote der untersuchten Kläranlagenabläufe wurden vom Projektpartner mittels Ultrahochleistungsflüssigkeitschromatographie mit Flugzeitmassenspektrometer-Koppelung (UHPLC-QTOF-MS) untersucht. Zum Einsatz kam dabei ein neuartiger Screeningansatz zur Analyse ausgewählter Tenside und deren Transformationsprodukte (TP) in „digital archivierten“ Proben unter Verwendung der von der NORMAN-Gemeinschaft (www.norman-network.net) entwickelten Digital Sample Freezing Platform (DSFP). Das Screening umfasste 1.564 Tenside und deren Metabolite in Ablaufproben und schloss die Target-Analyse der gleichen LAS und AES Homologe bzw. Ethoxymere ein, welche vom Labor des TZW, unter Verwendung identischer Standards, quantifiziert wurden. Dies ermöglichte es, die mit verschiedenen Methoden erzeugten Messergebnisse beider Labore miteinander zu vergleichen. Für LAS zeigte sich eine sehr gute Übereinstimmung der Ergebnisse in Anbetracht der Variabilität der Teilprobe und der Variabilität aufgrund der unterschiedlichen Probenvorbereitungstechniken und Messausstattung. Für AES waren die Abweichungen der Messergebnisse beider Labore größer. In allen Fällen lagen die ermittelten Konzentrationen in derselben Größenordnung.

Bekannte TP von LAS (Sulfophenylalkylcarbonsäuren (SPACs) und Sulfophenylalkyldicarbonsäuren (SPADCs)), die LAS-Nebenprodukte Dialkyltetralinsulfonate (DATSs) und deren TP Sulfotetralinalkylcarbonsäuren (STACs) und Sulfotetralinalkyldicarbonsäuren (STADCs) wurden semi-quantifiziert durch den Vergleich der Signalintensitäten mit der Signalintensität der Muttersubstanz (LAS). Das Suspect-Screening zeigte, dass in vielen Fällen die Konzentration von LAS von der Summe der Konzentrationen der Metaboliten überstiegen wird. Für DATSs lag die maximale Summenkonzentration bei 19 µg/L, für SPACs bei 17 µg/L und für STACs bei 5,3 µg/L.

Zusätzlich zu den Targetsubstanzen LAS und AES und den TP von LAS wurde die Proben auf weitere Tenside hin untersucht. Dabei wurden hohe Konzentrationen von Polyethylenglycolen (PEG) bis zu 7,4 µg/L gemessen. Die Gesamtkonzentration aller quantifizierten Tenside, TPs und Nebenprodukte in einer einzelnen Probe betrug bis zu 82 µg/L. Die Proben wurden auf Rückstände weiterer Tenside hin untersucht. Aufgrund fehlender Referenzstandards konnten diese Substanzen allerdings nicht quantifiziert werden, sondern nur deren

Nachweishäufigkeiten ermittelt werden. Hohe Auftrittshäufigkeiten wurden für NP1Ethoxycarboxylat, Naphthalene-1-sulfonat, Dihexylsulfosuccinat und für Vertreter aus der Gruppe der sekundären Alkylsulfonate gefunden.

1 Introduction

Synthetic surfactants comprise a heterogeneous group of organic compounds that are globally used in large quantities as active ingredients of household and industrial detergents. Moreover, due to their surface-active properties, they find broad application in the production of pharmaceuticals and personal care products, paints and varnishs, foodstuffs, plastics, and pesticides (Fabry 1991). In addition, they have become increasingly important in high technology sectors such as biotechnology and microelectronics in the last decades (Rosen and Kunjappu 2012). Surfactants are amphiphilic compounds with a hydrophilic (polar) head and a hydrophobic (nonpolar) hydrocarbon tail, which makes them soluble in polar and nonpolar liquids. They can be classified according to the ionic charge of the hydrophilic part of the surfactant (nonionic, anionic, cationic, amphoteric) in aqueous solution, with anionic and nonionic classes accounting for the highest production volumes. According to the European Committee of Organic Surfactants and their Intermediates, approximately three million tons of surfactants were manufactured in Western Europe in 2016, about 2.5 times more than 20 years earlier in 1996 (CESIO 2016). Global surfactant production reached 17.6 million tons in 2015 (Credence Research 2017).

Linear alkylbenzene sulfonates (LAS) were introduced in 1964 as the readily biodegradable replacements for branched alkylbenzene sulfonates (ABS). The substitution of ABS by LAS led to the elimination of excessive foaming in sewage treatment plants and receiving waters. Today, LAS are one of the most widely used anionic surfactants in detergents, such as laundry powders and liquids, with up to 25 percent in consumer products and up to 30 percent in products for professional use (UNEP 2005). The total European consumption of LAS was estimated to be about 430 kt in the year 2005 (HERA 2013). The LAS molecule consists of an aromatic ring which is sulfonated at the *para* position, and attached to a linear alkyl chain at any position except the terminal carbons. LAS are commercially available as a mixture of homologs with alkyl chains ranging from C_{10} to C_{13} (Table 1). In currently produced products the C_{11} and C_{12} homologs are dominating, which translates to a weighted average carbon number between 11.7–11.8. The linearity of the alkyl chain is >95% (UNEP 2005). As the benzene sulfonate group may be attached to any internal carbon atom of the alkyl chain, each homolog contains five to seven positional isomers (Traverso-Soto et al. 2015; Ying 2006).

Alkyl ethoxysulfates (AES, also known as alkyl ethoxylated sulfates, alcohol ethoxysulfates or alcohol ethoxylated sulfates) are another important class of anionic surfactants. They are commonly used in various consumer products, such as shampoos, hand dishwashing liquids, and laundry detergents, as well as in industrial cleaning processes, as industrial process aids in emulsion polymerization, and as additives in the plastics and paint production. The total volume of AES used in Europe is estimated to be 276 kt per year (HERA 2004). The chemical structure of AES consists of an aliphatic alkyl chain connected to a varying number of ethoxy (EO) units, terminated by a sulfate group (Popenoe et al. 2002). Consequently, commercially available AES are complex surfactant mixtures containing anionic homologs with alkyl chain lengths ranging from 8 to 18 carbon atoms. Each homolog can exhibit varying degrees of ethoxylation ranging from 0 to 9 EO units (Massey et al. 2010) (Table 1). However, the majority of AES blends manufactured are alkyl chains in the range of C_{12} to C_{15} with 0 to 4 EO units (McAvoy et al. 1998). A high production volume example of AES is sodium lauryl ether sulfate (SLES, sometimes also named sodium laureth sulfate). According to Massey et al. (2010), SLES is the sodium salt of the C_{12} homolog of AES with predominantly three EO units. It should not be confused with sodium dodecyl sulfate (synonymously sodium lauryl sulfate, SLS), which belongs to the group of non-ethoxylated alkyl sulfates (AS). In general, AS can account for up to 50% of a technical AES

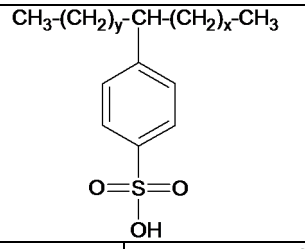
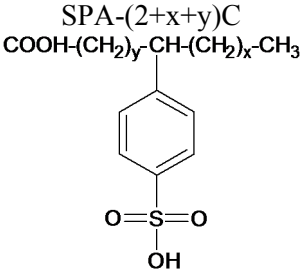
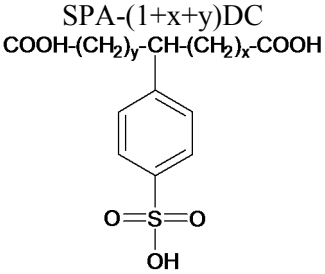
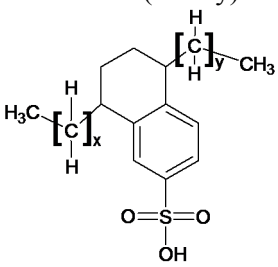
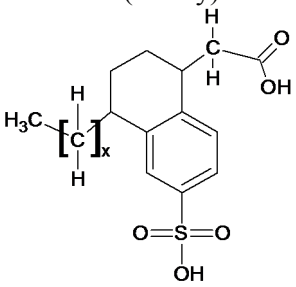
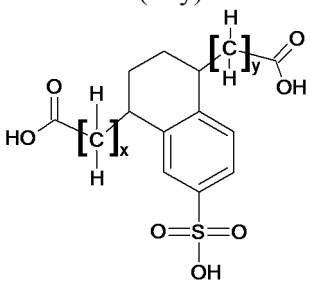
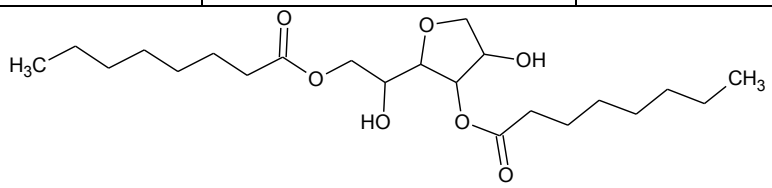
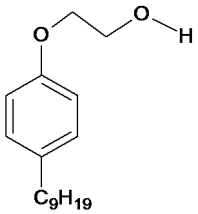
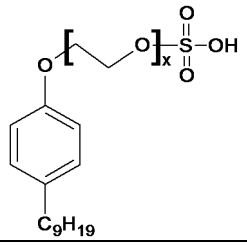
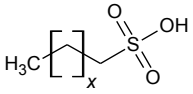
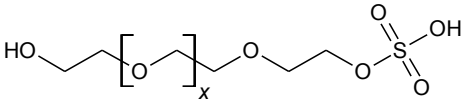
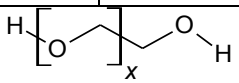
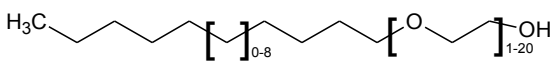
mixture, but are also produced and used separately from AES (Lara-Martín et al. 2008; McAvoy et al. 1998).

Other important representatives are secondary alkane sulfonates (SAS) which are used in the production process for dishwashing and laundry products. Non-ionic surfactants with widespread applications are alcohol ethoxylates (AEO) and nonylphenol ethoxylates (NPEO). The latter are known to be degraded to nonylphenol (NP) and its short-chain ethoxylates. Polyethylenoglycols (PEGs) are widely used in the production of pharmaceuticals, cosmetic products, lubricants, antifreeze mixtures, hydraulic fracturing fluids, and surfactants (Huang et al., 2005; Rogers et al., 2018). Furthermore, the production and application of surfactants imply the simultaneous use of large quantities of chemicals for their synthesis; e. g. commercial LAS mixtures usually contain about 15% of byproducts (Di Corcia et al., 1999b). After application, surfactants and their degradation products enter aquatic environments via discharges of treated or untreated wastewater (Sakai et al. 2017; Traverso-Soto et al. 2015). Studies have shown that modern surfactants are extensively removed by a combination of biodegradation and sorption/settling processes during wastewater treatment (Brunner et al., 1988; Schröder et al., 1999) and generally have low persistence in aquatic and terrestrial ecosystems (Branner et al. 1999; Leon et al. 2004; Scott and Jones 2000). This leads to the release of a complex mixture of numerous surfactants and their transformation products (TPs) into receiving waterbodies. Since the high removal rates are overcompensated by the exceptionally high usage of surfactants and their continuous introduction into the environment, surfactants can be considered as “pseudo-persistent” contaminants. Therefore, they have the same exposure potential as persistent contaminants.

Most studies on the occurrence and behavior of modern surfactants during wastewater treatment were conducted from the late 1980s to the early 2000s. Moreover, in these studies average concentrations were mostly derived from a small number of WWTPs. Therefore, there is need for topical data on the presence of remaining surfactants in WWTP effluents especially against the backdrop of the changing surfactant production in Europe over the last decades. This study aims to evaluate present-day effluent concentrations of two common groups of anionic surfactants, namely LAS and AES, from multiple WWTPs in Germany.

Investigations about the occurrence of emerging substances by target screening alone is not sufficient to cover the multitude of substances occurring in environmental samples. This is due to inherent limitation of the target screening, which focuses on a few pre-selected substances for which reference standards are available. It does not provide information on the occurrence of, e.g., other substances of the same class and their transformation products (TPs). Wide-scope suspect screening using lists of environmentally relevant substances is an effective way to gain a better insight on the occurrence of emerging substances in environmental samples. Hence, samples were additionally analyzed for other substances of the same class and TPs (Table 1) using wide-scope suspect screening in digitally archived chromatograms of sampled effluent wastewater.

Table 1: General structures of linear alkylbenzene sulfonates (LAS), sodium alkyl ethoxysulfates (AES), LAS-related byproducts and TPs as well as other surfactants investigated within this study.

LAS	
LAS TPs	SPA-(2+x+y)C  SPA-(1+x+y)DC 
DATS and its TPs	DATS-(2+x+y)C  STA-(1+x+y)C  STA-(x+y)DC 
AES	
NPEO and NPEO-SO_4	NPEO  NPEO-SO_4 
SAS and GES	SAS- C_{x-2}  GES_{x+1} 
PEGs	
C_{8-18}-AEO_{01-20}	

2 Literature review

2.1 Sampling

The physico-chemical properties of surfactants make representative sampling difficult as they tend to concentrate or adsorb at interfaces. Thus, their distribution may not be homogeneous and rather depends on the degree of physical mixing in the water column. Vertical profiles in a Spanish estuary differed considerably: In one profile, near an underwater wastewater discharge, the highest LAS concentrations were found at 2.5 m depth from the water surface. Not far away from the discharge point, LAS was distributed much more homogeneously. However, in a stagnant zone in the same estuary, concentrations in the uppermost centimeters were up to one order of magnitude higher than in samples from 10 cm depth (León et al. 2002). González-Mazo et al. (1998) also found that the strong accumulation of LAS at the water-atmosphere interface translated into a steep vertical gradient in the LAS concentration in zones close to effluent discharge points. Concentrations found in the top 3-5 mm of water depth were two orders of magnitude higher than those found at a depth of 0.5 m. It was also pointed out that a high variability in daily and weekly surfactant fluxes exists and that spot sampling may not be representative (Traverso-Soto et al. 2015).

Surfactants undergo rather fast degradation processes in the aquatic environments. In order to prevent LAS degradation during storage time prior analysis, Sakai et al. (2017) added 1 mL hydrochloric acid (1 mol/L) to a 2-liter surface water sample and found that biodegradation was "unlikely to happen" if samples were analyzed within one week after sampling. For the preservation of surface water samples from a Mediterranean coastal lagoon, 4% formaldehyde and storage at 4 °C was used (Traverso-Soto et al. 2015). The same procedure was also applied for seawater and wastewater, however the samples were frozen after transport to the laboratory (Lara-Martín et al. 2011). Matthijs et al. (1999) even used 8% formaldehyde by volume for LAS and 0.01 M sodium azide for other surfactants as preservation reagents.

2.2 Reported analytical methods

2.2.1 LAS

In the scientific literature, sample pretreatment by solid phase extraction (SPE) is the method of choice for the purification and pre-concentration of anionic surfactants. Hereby, the sum of dissolved and particle-bound fractions of LAS can be determined. C_{18} is a widely used SPE sorbent for surfactants, including LAS (Clara et al. 2007; Corada-Fernández et al. 2011; Sakai et al. 2017). For a more selective extraction, polymer based SPE (Lara-Martín et al. 2011; McDonough et al. 2016) and anion exchange SPE adsorbents (McAvoy et al. 1998; Ripoll-Seguer et al. 2013) have also been applied.

The general SPE procedure is similar to enrichment procedures known for other micropollutants such as pharmaceuticals, personal care products, and artificial sweeteners. The sample is loaded with a flow rate that allows sufficient interaction between the analytes and the sorbent material. An optional washing step is followed by the drying of the cartridge with nitrogen. Afterwards the analytes are eluted with an organic solvent and the eluate is blown down to dryness to enable a solvent exchange. Due to the rather hydrophobic character of LAS the dry residue is reconstituted with high percentage of organic solvent (usually around 50%) to ensure complete transfer to the HPLC vial and to achieve better chromatographic performance (McAvoy et al. 1998; McDonough et al. 2016). If samples need to be filtered prior to SPE, in order to avoid clogging of the cartridges, LAS can be eluted from the filter cake with methanol and

sonication, and the eluate can be combined with the filtrated water prior to SPE (Sakai et al. 2017).

Predominantly high-performance liquid chromatography (HPLC) coupled with various detection techniques have been used in recent years for the quantification of surfactants in environmental samples. The main advantage of HPLC over gas chromatography (GC) is that a derivatization step is not necessary. For the analysis of LAS, fluorescence (FLD) (Cantarero et al. 2011; McAvoy et al. 1998), ultraviolet (UV) (Mottaleb 1999; Wangkarn et al. 2005), mass spectrometry (MS) (Lara-Martín et al. 2008; Traverso-Soto et al. 2015), time-of-flight mass spectrometry (TOF-MS) (Lara-Martín et al. 2011) and tandem mass spectrometry (MS/MS) (McDonough et al. 2016; Sakai et al. 2017) have been widely used. With the availability of MS/MS, the identification of surfactants has become significantly more reliable and unequivocal, due to the specific monitoring of quasi molecular parent ions and their respective fragment ion, combined with the retention time of the analytes. By this means, an overestimation for LAS as reported for UV detection can be avoided, as MS/MS is considerably more selective (Riu et al. 2000). A popular fragment ion used for the quantification of LAS homologs is characterized by m/z = 183 (Lara-Martín et al. 2008).

As seen in Table 1 the benzene sulfonate moiety of LAS can be attached to different positions of the alkyl chain. As a consequence, several isomers of one LAS homolog exist. Isomers of each LAS homolog have slightly different physico-chemical properties and are separated only to some extent when standard C_{18} columns are used. Consequently, many publications have shown multiple and irregularly shaped chromatographic peaks for each LAS homolog (Castillo et al. 2000a; Lara-Martín et al. 2011; Motteran et al. 2017; Cantarero et al. 2011). This phenomenon was also addressed in a technical note from Japan (GL Sciences Inc. 2012). By applying a less retentive column with weaker hydrophobic interactions (C_8), the compounds eluted as single peaks, which facilitated peak integration. For the quantification of single compounds (isomers and homologs), the availability of pure reference standards or at least an educated estimate of their distribution in a mixture is necessary.

2.2.2 AES

The general statements about the applicability of SPE for the pre-treatment of samples prior LAS analysis also apply to AES. Previous studies have used reversed-phase HPLC coupled to conductivity detection (Morvan et al. 2008), MS (Corada-Fernández et al. 2011; Lara-Martín et al. 2006; McAvoy et al. 1998) and MS/MS (McDonough et al. 2016). Massey et al. (2010) observed the formation of sulfated and desulfated ammonium adducts and used the desulfated ammonium adduct of the C_{12}-homolog with three EO units as a “surrogate marker” to quantify SLES deposition on human skin. No fragment ion was recorded as selected ion recording was used as detection mode. Interestingly, in the same study, highly acidic conditions were reported to hinder the formation of stable precursor ions. When AES were analyzed with HPLC-MS or HPLC-MS/MS in negative ionization mode, the fragment ion with m/z = 97 was used in the published analytical methods (Lara-Martín et al. 2008; McDonough et al. 2016).

2.3 Reported environmental concentrations and fate

2.3.1 Occurrence and fate of LAS

LAS is classified as readily degradable under aerobic conditions according to OECD guidelines. Increased branching of the alkyl chain results in reduction of biodegradability (HERA 2013). The metabolic pathway of LAS biodegradation was studied by several authors and can be described by an initial ω-oxidation of the alkyl chain, followed by subsequent β-oxidations which form

sulfophenyl carboxylic acids (Eichhorn and Knepper 2002; Peressutti et al. 2008). Isomers in which the terminal methyl group is positioned furthest from the sulfophenyl group degrade most easily and isomers having the sulfophenyl moiety at central positions are the most stable ones (Perales et al. 1999). Anaerobic degradation of LAS can occur under some specific environmental conditions, e.g. under methanogenic conditions. However, the anaerobic degradation pathway is of only minor relevance (HERA 2013).

McAvoy et al. (1998) assessed the removal of anionic surfactants during conventional wastewater treatment in the Midwestern United States. The average removal rates of LAS in activated sludge and trickling filter treatment plants were >99% and 82%, respectively. Effluent concentrations ranged from <5 µg/L to 7 µg/L in activated sludge, and from 73 µg/L to 1,500 µg/L in trickling filter treatment plants. Using the average influent concentrations and removal rates, the predicted exposure concentrations for LAS in receiving waters downstream of WWTP outfalls, were less than their corresponding biological predicted no-effect concentrations (PNECs) in more than 98% of the locations under low-flow conditions.

Matthijs et al. (1999) reported an average effluent LAS concentration of 43 µg/L for six municipal WWTPs in the Netherlands (average influent concentration: 5,200 µg/L; >99% removal). The alkyl chain length of LAS in the effluent averaged 11.6 carbon atoms. Their results further indicate that, under normal operating conditions, the removal of the studied surfactants (including LAS and AES) is not affected by WWTP operating characteristics, such as plant size, hydraulic retention time, or sludge retention time.

Riu et al. (2000) determined LAS concentrations in wastewater samples of three Spanish WWTPs, two of them receiving mainly domestic wastewaters whereas the third one treated primarily industrial wastewaters. Additionally, LAS were also analyzed in two samples from coastal waters of the bay of Cadiz, Spain. The concentration levels of total LAS varied from 990 µg/L to 1,300 µg/L in the influents, whereas in the effluents, concentrations from 140 µg/L to 226 µg/L were found. High levels of LAS in coastal wastewaters of the bay of Cadiz were detected (740 to 910 µg/L), indicating that untreated wastewaters were discharged into the bay.

In two other WWTPs from Southern Spain, total LAS concentrations of 1,600 µg/L and 1,100 µg/L were measured in the influents and 150 µg/L and 170 µg/L in the effluents, respectively. The LAS distribution found in the wastewater samples was similar to that reported for commercial mixtures with LAS-C_{11} and -C_{12} as prevailing homologs. This was also true for a sample from the Guadalquivir river, where a total LAS concentration of 120 µg/L was measured (Lunar et al. 2006).

Sütterlin (2007) analyzed LAS in 24-h composite samples of one WWTP (600,000 population equivalent) in Germany over the time course of one week. The influent concentrations ranged from 1,500 µg/L to 2,400 µg/L. A considerable decrease in concentration of about 98% was observed which resulted in LAS effluent concentrations between 25 µg/L and 53 µg/L.

An even higher removal of 99.9% was observed in the WWTP of Stony Brook (NY, USA). However, the influent (200 µg/L) and effluent (0.21 µg/L) concentrations were considerably lower as reported in most of the other studies (Lara-Martín et al. 2011).

LAS were also analyzed in untreated and treated sewage of nine municipal WWTPs in Austria. The total influent concentrations of LAS varied between 2,400 µg/L up to 6,700 µg/L, however the compounds were drastically removed during wastewater treatment (as seen by effluent concentrations of 4.2 µg/L to 40 µg/L) resulting in removal rates >99% in all WWTPs. Measured dissolved and sorbed fractions differed considerably in influent and effluent samples:
While about half of LAS in the influents were sorbed onto particles, no concentrations above the

limit of quantification were measured for the bound fraction in the effluents (Clara et al. 2007). Therefore, it is straightforward that sewage sludge has been analyzed for LAS in subsequent studies. Three different extraction techniques were compared and applied to 15 sludge samples from different regions in Spain. The results obtained by the different extraction protocols were similar and revealed LAS concentrations between 0.7 mg/kg and 13.5 mg/kg (Cantarero et al. 2011).

McDonough et al. (2016) carried out a monitoring campaign which collected effluent grab samples from 44 WWTPs across the USA in order to generate statistical distributions of effluent concentrations for various anionic surfactants. Measured LAS concentrations in the effluent ranged from 2.1 µg/L to 105 µg/L with a mean of 15.3 µg/L and an average alkyl chain length of 11.3. The statistical distribution of effluent concentration data was then analyzed in combination with effects data and dilution factors for WWTP mixing zones to evaluate the aquatic safety of the studied surfactants. For all surfactants, including LAS, the toxic units were less than one even under conservative low flow conditions indicating a significant margin of safety for LAS in the aquatic environment. However, a less conservative threshold was used as compared to this study.

Natural-gradient tracer tests were conducted to determine the transport and biodegradation behavior of LAS under in situ conditions in two biogeochemically distinct zones of an aquifer in the state of Massachusetts, USA. No significant loss of LAS mass occurred in the aerobic uncontaminated zone while 20% of the LAS mass injected into the moderately aerobic, sewage-contaminated zone (transition zone) was removed due to biodegradation. The absence of LAS biodegradation in the aerobic zone indicates that aerobic conditions are not the only requisite for the biodegradation of LAS. The removal of LAS mass in the transition zone was accompanied by a decrease in dissolved oxygen concentrations, an increase in the number of free-living bacteria with a concomitant change in bacteria morphology, and the detection of LAS metabolites. Biodegradation preferentially removed the longer alkyl chain homologs and the external isomers (i.e., 2- and 3-phenyl). The authors observed chromatographic separation of the surfactant mixture in both zones, which was attributed to the retardation of the longer alkyl chain homologs during transport. Consequently, sorption and biodegradation enriched the LAS mixture in the more hydrophilic and biologically resistant components (Krueger et al. 1998).

LAS were also found in the catchment of one of the largest freshwater lakes in Asia, Laguna de Bay in the Philippines, which also serves as a drinking water reservoir (Eichhorn et al. 2001). In all streams investigated, LAS were detected in the lower to mid µg/L-range. The concentration levels of LAS were 1.2 µg/L to 73 µg/L in some tributaries of Laguna de Bay and 2.2 µg/L to 102 µg/L in its outlet, the Pasing River, respectively. The authors used the ratio of the easily biodegradable LAS and the more stable ABS to assess the point of time of contamination. However, it was also mentioned that a removal of LAS through sorption and sedimentation must also be taken into account.

LAS concentrations ranging from 14 µg/L to 155 µg/L were measured in the Rio Macacu, which receives discharges of untreated domestic wastewater from several villages located along its river bank (Eichhorn et al. 2002). The authors highlighted the self-purification capacity of the river water, which was demonstrated in the upper course of the river downstream of a town considered as one major discharge point. In other words, a rapid degradation of LAS seems also to occur in surface waters.

In a freshwater water reservoir in Southwest Spain, which is fed by the Guadalete River and receives only primary treated wastewater, LAS concentrations between 10.7 µg/L and 17.4 µg/L were measured (Lara-Martín et al. 2008). With the exception to other surfactants analyzed

within this study the rather polar anionic surfactants were predominantly present in the dissolved form. With regard to LAS, only 13% was attached to particulate matter. Sorption capacity was found to be reduced for homologs with shorter alkyl chain length. As a consequence, the average homolog distribution in water was found to have shifted to shorter chain lengths (C_{10}), whereas C_{11} and C_{12} were the predominant homologs in a commercial LAS standard used for comparison.

LAS were used to assess the anthropogenic impact on pristine karst lakes in Croatia. The total LAS measured in vertical sediment profiles indicated a significant anthropogenic impact in the last decades, most likely from untreated wastewater discharged by hotels and households situated at the lakes´ shorelines. As LAS concentrations in the surface layer of the water column were below 0.1 µg/L, it was assumed that untreated wastewater enters the lake, but subsequently leaks through the bottom sediments and the porous karst rocks underneath (Mikac et al. 2011).

In two river basins in Malaysia, LAS were detected between 1.9 µg/L and 2.4 mg/L in filtered (<0.45 µm) samples. The very high concentrations correlated well with the measured concentrations of ammonia, which supported the assumption that untreated wastewater has been discharged into the surface waters. Based on the LAS concentrations in the dissolved phase and in four different particle size fractions (<0.1 µm; 0.1–1 µm; 1–11 µm; >11 µm), the authors found that the attenuation of LAS in the studied rivers was primarily due to the adsorption of LAS to suspended solids, rather than due to biodegradation, since LAS homologs, particularly in longer alkyl chain lengths, were substantially absorbed to the large size fraction (>11 mm) that settled within a few hours (Sakai et al. 2017).

2.3.2 Occurrence and fate of AES

According to OECD 301 tests, AES are degraded readily and completely under aerobic conditions (Scott and Jones 2000). The degradation of AES starts with one of the following processes: i) ω-/β-oxidation of the alkyl chain; ii) enzymatic cleavage of the sulfate substituent leaving an alcohol ethoxylate; iii) cleavage of an ether bond producing either an alcohol (central cleavage) or an alcohol ethoxylate and an oligo(ethylene glycol) sulfate. Subsequent degradation of the resulting intermediates comprises: i) oxidation of the alcohol to the corresponding fatty acid; ii) degradation of the alcohol ethoxylate via central cleavage or degradation from either end of the molecule, iii) degradation of the oligo(ethylene glycol) sulfate (HERA 2004). The length of the alkyl chain and the number of EO units seemingly do not affect the degree of aerobic biodegradation, however branching of the alkyl chain may slow down the primary biodegradation of AES.

According to a recently published review, only few SLES biodegradation studies have been performed so far (Barra Caracciolo et al. 2017), and further research is necessary to clarify the fate of SLES in real environmental conditions. Biodegradation of SLES was achieved in an enrichment culture with *Citrobacter braakii*. SLES was removed with a high rate of 116 mg of SLES per liter and hour (Dhouib et al. 2003). Even in sea water more than 99% of 1 mg/L of the AES-C_{12} was biologically degraded in 60 hours (Pérez-Carrera et al. 2010).

The degradation of AES also occurs in an anaerobic environment as for the cleavage of the sulfate and ether bonds no molecular oxygen seems to be necessary (Scott and Jones 2000). The Detergents Ingredients Database classifies AES with C_{12} to C_{18} alkyl chains and 1 to 4 EO units as anaerobic biodegradable (European Commission 2016).

In the case of the environmental fate of AES, studies regarding their behavior in WWTPs or receiving waters are more limited than compared to LAS.

The average removal rates for AES (28 analytes: C_{12}–C_{15} with EO0–6) in activated sludge and trickling filter treatment plants in the U.S. were 98% and 83%, respectively. Total AES effluent concentrations ranged from 4 µg/L to 18 µg/L and from 32 µg/L to 164 µg/L for activated sludge and trickling filter treatment, respectively. A modelling approach predicted that only 2% of the anionic surfactants (including AES) exposure concentrations below the WWTP outfalls would be greater than their corresponding biological PNECs under low-flow conditions (McAvoy et al. 1998).

In their study on surfactant concentrations in WWTP effluents in the Netherlands, Matthijs et al. (1999) reported an average total effluent concentration of 6.5 µg/L for AES (36 species: C_{12}–C_{15} with EO0-8) with a removal greater than 99%.

In the previously mentioned study by McDonough et al. (2016) the measured total AES concentrations (24 analytes: C_{12}–C_{16} with EO1–4), in effluents of 44 WWTPs in the US, ranged from 1.2 µg/L to 3.8 µg/L. The predominant AES homologs in the effluent were C_{12}-homologs and the average chain length was 13.5 carbon atoms with an average number of 2.4 EO groups. The derived toxic unit for AES was less than one, indicating a significant margin of safety for AES in the aquatic environment.

In a freshwater reservoir in Spain, total AES concentrations (36 analytes: C_{12}, C_{14}, C_{16} with EO0–11) ranged from <LOQ to 0.1 µg/L in the water, despite of primary treated wastewater having been discharged into the sampling site. Total AES concentration in sediments ranged from 43 µg/kg to 164 µg/kg. Similar to LAS, the long chain homologs of AES show a higher affinity for the particulate phase. Thus, it is not surprising that in the above mentioned study, the homolog distribution of AES in water is quite similar to what is found in technical mixtures (C_{12} dominating), whereas it is shifted towards longer chain lengths when suspended solids or sediments were analyzed (Lara-Martín et al. 2008).

In the middle stretch of the Guadalete River in Spain total AES concentrations ranged from 4 µg/L and 72 µg/L in water samples. Only AES homologs with an even number of carbon atoms (C_{12}, C_{14}, C_{16}) were found. Furthermore, the authors also observed a preferential sorption of the homolog with the longest alkyl chain (C_{16}) on sediments, whereas the aqueous phase contained more polar homologs of short alkyl chains (Corada-Fernández et al. 2011).

3 Materials and methods

3.1 Chemicals

Sodium 4-n-octylbenzenesulfonate (C_8-LAS; 99.9% purity), sodium p-n-decylbenzenesulfonate (C_{10}-LAS; 99.9%), sodium p-n-undecylbenzenesulfonate (C_{11}-LAS; 99.9%), sodium p-n-dodecylbenzenesulfonate (C_{12}-LAS; 99.8%), sodium p-n-tridecylbenzenesulfonate (C_{13}-LAS; 99.5%), and sodium p-n-tetradecylbenzenesulfonate (C_{14}-LAS; 99.1%) were procured from HPC Standards GmbH (Cunnersdorf, Germany). A LAS standard containing a mixture of homologs (CAS-Number.: 68584-22-5; 97%) with an advertised average chain length of 11.4 was purchased from Alfa Aesar (Karlsruhe, Germany).

It should be noted, that every reference standard for individual LAS homologs from HPC contains only one isomer with a terminal benzene sulfate moiety (A. Schulze, HPC Standards GmbH, personal communication), whereas the analytical standard from Alfa Aesar is a mixture with different isomers of every homolog, resulting from the different attachment positions of the benzene sulfonate moiety along the alkyl chain.

AES (CAS-Number.: 9004-82-4; 70.5%) and sodium dodecyl-d_{25} sulfate (SDS-d_{25}; ≥98%) were obtained from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany). Metformin (97%) was procured from Sigma-Aldrich (Steinheim, Germany). Tetraethylene glycol monododecyl ether (CAS Number 5274-68-0; 98%) and PEG-04 (CAS Number 112-27-6; 99%) were purchased from Merck (Chalkidona, Greece). Both laboratories involved in the study used the identical analytical standards for the quantification of LAS and AES.

Solvents and mobile phase additives used by TZW

Methanol (MeOH), acetonitrile (ACN), and formic acid (all LC-MS grade) were purchased from Honeywell (Seelze, Germany). Ultra-pure water (LC-MS grade) was procured from VWR International (Bruchsal, Germany). Glacial acetic acid (100%) was obtained from Merck KGaA (Darmstadt, Germany). Ammonium acetate (≥98%) was provided by Sigma-Aldrich (Steinheim, Germany). Ammonium fluoride (≥98%) was purchased from Carl Roth (Karlsruhe, Germany).

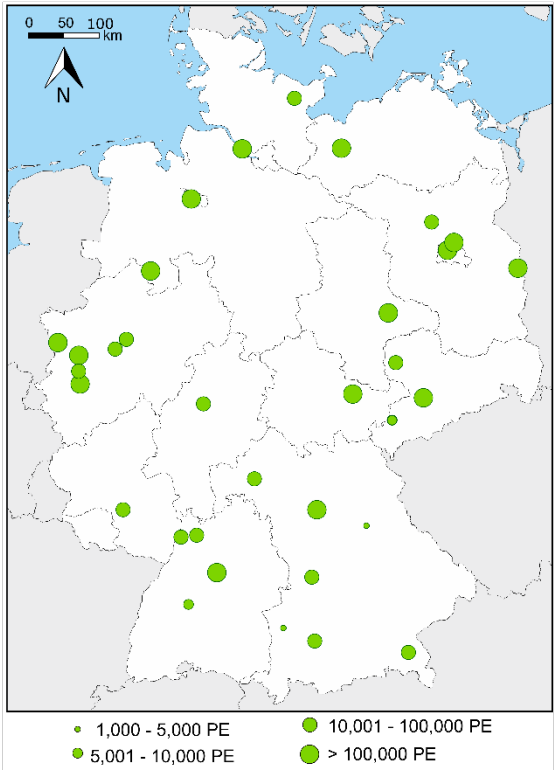
Solvents and mobile phase additives used by the Environmental Institute & the Laboratory of Analytical Chemistry

ACN and MeOH (both LC–MS grade) were purchased from Merck (Darmstadt, Germany) and formic acid (99%) was obtained from Sigma-Aldrich (Buchs, Switzerland). Distilled water was provided by a Milli-Q purification apparatus (Millipore Direct-Q UV, Bedford, MA, USA).

3.2 Sampling strategy

Seven-day composite effluent samples (n=33) were obtained from 33 conventional WWTPs across Germany, which predominantly receive domestic wastewater (Figure 1). The population equivalents (PE) of the sampled WWTPs range from 1,000 PE to 1,300,000 PE. Sampling was conducted from February through April 2018. Samples were taken by automatic samplers, stored in 10 L stainless steel containers, and immediately frozen after sampling. A seven-day composite sample was obtained by combining seven consecutive 24-hour composite samples. After arriving at the laboratory, each seven-day composite sample was thawed at room temperature. Subsequently, the sample was shaken and an aliquot was transferred to a 50 mL polypropylene tube and stored at −18 °C until analysis. 32 WWTPs were each sampled once during dry weather periods, while one WWTP was sampled once during wet weather conditions.

Figure 1: Location of the sampled WWTPs and the population equivalent (PE) of each plant. The map is based on d-maps.com.



(source: TZW, Karlsruhe)

Additionally, four corresponding influents were sampled at four of the 33 monitored WWTPs, using the identical sampling approach as used for the effluents.

3.3 Analytical methods

3.3.1 Target screening (TZW)

High performance liquid chromatography electrospray tandem mass spectrometry (HPLC-MS/MS) analysis using an Agilent 6495B triple-quadrupole mass spectrometer coupled to an Agilent 1290 Infinity II LC system (Agilent Technologies, Waldbronn, Germany) was used for the analysis of LAS. The analysis was carried out in negative-ion electrospray ionization (ESI) mode. Compound specific MS/MS parameters were optimized. The MS/MS settings as well as general interface parameters are summarized in Annex Table 1. Samples were mixed by shaking and an aliquot of 1 mL was transferred to a 10-mL glass vial and spiked with a defined amount of C_8-LAS which served as the internal standard (IS). The aqueous phase was then removed using a RVC 2-33 CDplus rotational vacuum concentrator (Martin Christ, Osterode am Harz, Germany). Subsequently, the sample was reconstituted with 1 mL of ultra-pure water:ACN (50:50, v:v) and transferred to a 2-mL HPLC glass vial. For the analysis of LAS in influent samples, an aliquot of 0.1 mL was extracted and reconstituted with 1 mL of ultra-pure water:ACN (50:50, v:v), leading to a dilution factor of 10. Calibration standards were spiked in empty 10-mL glass vials and processed parallel to environmental samples in order to accommodate for possible contamination in the solvents used for the reconstitution of samples. All glassware used for sample processing was heated to 550 °C overnight prior to use. Chromatographic retention and separation for LAS was achieved using a Kinetex 2.6 µm C_8 100 Å LC column (2.1 mm × 150 mm)

from Phenomenex (Aschaffenburg, Germany) with ultra-pure water containing 0.1 mM ammonium fluoride (A) and MeOH (B) as eluents. The mobile phase gradient was as follows: 0–3.5 min, 40–95% B; 3.5–6 min, 95% B; 6–6.5 min, 95%–40% B. The column was re-equilibrated at 40% of B for 5.5 min between each sample run. The flow rate was 0.27 mL/min and the injection volume was set to 4 µL. The column temperature was set to 30 °C.

For AES, HPLC-MS/MS analysis using an Agilent 1290 Infinity (Agilent Technologies, Waldbronn, Germany) coupled to an API 5500 Q-Trap triple-quadrupole mass spectrometer (Applied Biosystems/MDS Sciex Instruments, Concord, ON, Canada) with an electrospray interface operated in negative ionization mode was used. Optimized compound specific MS/MS settings and interface parameters for the analysis of AES are listed in Annex Table 2. Sample preservation and extraction were identical to LAS, with the exception of a sample volume of 10 mL instead of 1 mL. For the analysis of AES in influent samples, an aliquot of 0.05 mL was extracted and reconstituted with 1 mL of ultra-pure water:ACN (50:50, v:v), leading to a dilution factor of 20. SDS-d_{25}, which corresponds to the fully deuterated C_{12} homolog of AES with zero ethoxy units (AES-C_{12} EO_0), served as the internal standard for all AES homologs/ethoxymers. The separation was carried out on a Luna Omega 1.6 µm Polar C_{18} 100 Å LC column (2.1 mm x 100 mm) from Phenomenex (Aschaffenburg, Germany) with ultra-pure water (25 mM ammonium acetate, pH 3.6 adjusted with glacial acetic acid) (A) and acetonitrile (B) as eluents. The mobile phase gradient was as follows: 0–7 min, 50–98% B; 7–11 min, 98% B; 11–12 min, 50% B. The column was re-equilibrated at 50% of B for 5 min between each sample run. The injection volume was set at 40 µL, the flow rate to 0.22 mL/min and the column compartment was maintained at 30 °C.

3.3.2 Target, suspect and non-target screening (Environmental Institute & Laboratory of Analytical Chemistry)

Ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF) was used for suspect screening of the TPs of LAS and other known surfactants. The UHPLC-QTOF method was also used for cross validation of the screening results with those obtained by HPLC-MS/MS target analysis. The analysis included: (i) two classes of LAS-TPs: SPACs and sulfophenyl alkyl dicarboxylic acids (SPADCs), (ii) the LAS-byproducts DATSs, and (iii) the two classes of DATS-TPs: STACs and sulfo-tetralin alkyl di-carboxylic acids (STADCs). Further analysis comprised (iv) NPEO, (v) nonylphenol ethoxylate sulfate (NPEO-SO_4), (vi) SAS, (vii) glycol ether sulfates (GES), (viii) PEGs, and (ix) AEO (Table 1).

Target screening was accomplished using a UHPLC apparatus (Dionex UltiMate 3000 RSLC, Thermo Fisher Scientific, Dreieich, Germany), coupled to a quadrupole time-of-flight mass spectrometer (QTOF-MS) (Maxis Impact, Bruker Daltonics, Bremen, Germany). The following interface parameters were used: capillary voltage: 2,500 V (positive mode) and 3,500 V (negative mode); end plate offset (500 V); nebulizer (2 bar); drying gas (8 L/min); drying temperature (200 °C).

Samples were cleaned up and pre-concentrated 4,000-fold on an Atlantic HLB-M Disk using HORIZON SPE-DEX 4790 (USA) with 47 mm disk holder according to extraction program presented in Table 2. Extracts were evaporated using a gentle stream of nitrogen and were reconstituted with 500 µL MeOH:water (50:50, v:v) for HPLC-ESI-QTOF-MS analysis. Before instrumental analysis extracts were filtered through RC syringe filters of 4 mm diameter and 0.2 µm pore size (Phenomenex, USA).

Table 2: Conditioning and extraction program used for sample preparation of wastewater samples by HORIZON SPE-DEX 4790.

		Solvent	Soak Time in sec	AirDry Time in sec
Conditioning	PreWet Cycle	Isopropanol	-	5
		Isopropanol	-	5
		Milli-Q water	-	5
		Methanol	-	5
		Ethyl Acetate	-	5
	Rinse Cycle	Methanol	-	5
		Ethyl Acetate	-	5
Extraction	PreWet Cycle	Ethyl Acetate	120	30
		Ethyl Acetate	120	30
		Ethyl Acetate	90	30
		Methanol	120	30
		Methanol	120	30
		Methanol	60	30
		Milli-Q water	120	30
		Milli-Q water	60	30
		Milli-Q water	60	30
	Sample AirDry Cycle	Ethyl Acetate	150	60
		Ethyl Acetate	90	30
		Ethyl Acetate	90	30
		Methanol	150	60
		Methanol	90	30
		Methanol	90	30

The separation was carried out on an Acclaim RSLC C_{18} column (2.1 x 100 mm, 2.2 µm) from Thermo Fisher Scientific (Dreieich, Germany) preceded by a guard column of the same packaging material. In positive ionization mode eluent A consisted of ultra-pure water:MeOH (90:10, v:v) (5 mM ammonium formate with 0.1% formic acid) and eluent B consisted of MeOH (5 mM ammonium formate with 0.1% formic acid). In negative ionization mode eluent A consisted of ultra-pure water:MeOH (90:10, v:v) (5 mM ammonium acetate) and eluent B consisted of MeOH (5 mM ammonium acetate). The mobile phase gradient was as follows: 0–1 min, 1% B; 1–3 min, 1–39% B; 3–14 min, 39–99.9% B; 14–16 min, 99.9% B; 16–16.1 min, 99.9–1% B. The column was re-equilibrated at 1% of B for 4.9 min between each sample run. The flow gradient was as follows: 0.2 mL/min at 0–3 min; 0.4 mL/min at 14 min; 0.48 mL/min at 16–19 min; 0.2 mL/min at 19.1–20 min. The injection volume was set at 5 µL and the column compartment was maintained at 30 °C.

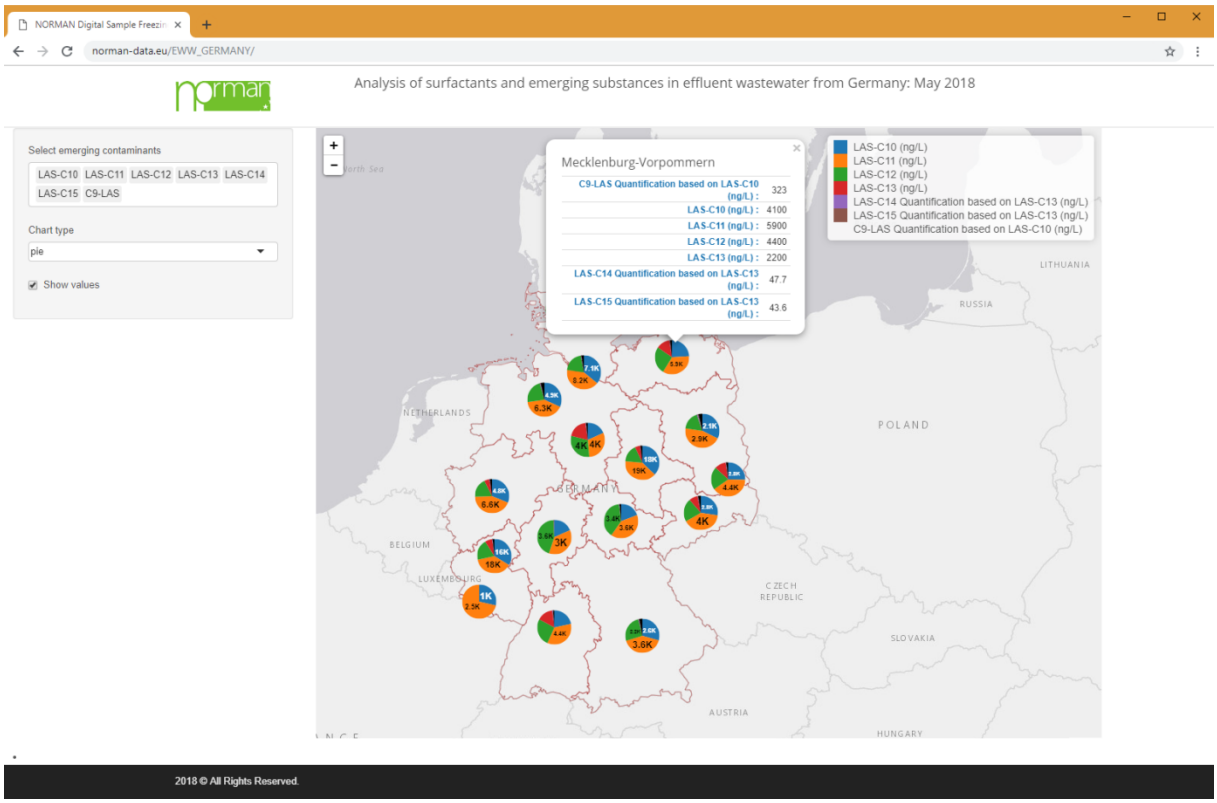
Data processing

HRMS chromatograms were recalibrated using HPC fitting algorithm, which is embedded in DataAnalysis 4.3. (Bruker Daltonics, Bremen, Germany). The manufacturer's calibration method ensures mass accuracy below 2 mDa throughout the chromatographic run for m/z from 50 to 1200 Da. For exporting files in the mzML format, CompassXport 3.0.9.2. (Bruker Daltonics, Bremen, Germany) was used. Chromatograms acquired under data-independent acquisition were separated in low and high collision energy layer chromatograms.

All mzML files and their metadata (instrumental, sample metadata, matrix-specific metadata and retention times of the retention time index (RTI) mixture) were uploaded to a separate section

of the NORMAN Digital Sample Freezing Platform (DSFP) (Alygizakis et al., 2019), which has a built-in integrated standard operating procedure (SOP) to process the mzML files and all metadata for an automated generation of an Excel-based Data Collection Templates (DCTs). DSFP was used to screen the results which were further evaluated and are visualized at a website (www.norman-data.eu/EWW_GERMANY) in order to show the spatial distribution of the analyzed surfactants (Figure 2).

Figure 2: An interactive map for visualization of concentrations of detected surfactants in the studied WWTPs in Germany (www.norman-data.eu/EWW_GERMANY).



(source: Laboratory of Analytical Chemistry, Athens)

Target and suspect screening of common surfactants and their TPs

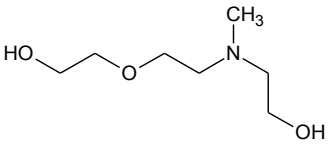
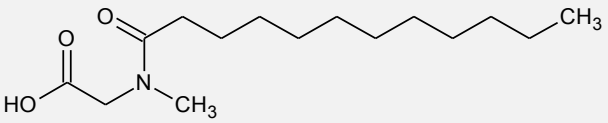
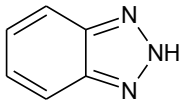
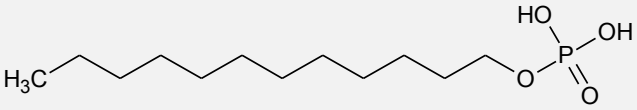
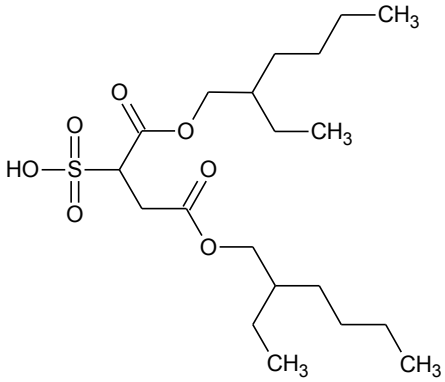
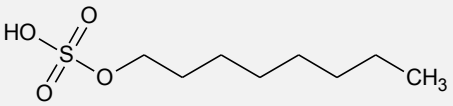
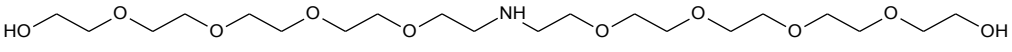
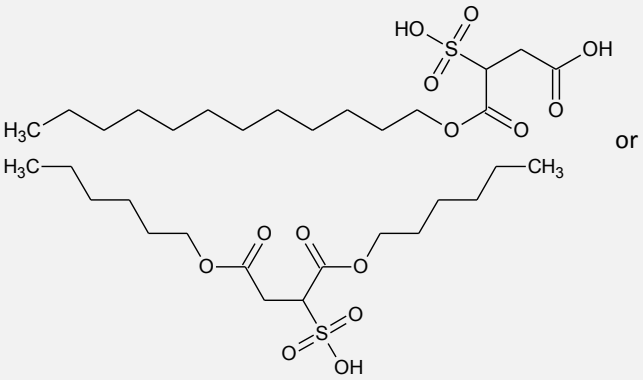
In the first step HRMS was used to confirm the concentrations of AES and LAS obtained by UHPLC-MS/MS. Suspect screening was then applied to search for the presence of TPs of LAS and other known surfactants expected to be present in wastewater treatment plant effluents. All surfactants currently enlisted in NORMAN Suspect List Exchange (more specifically: the lists S7 EAWAGSURF, S8 ATHENSSUS and S23 EIUBASURF) were screened (NORMAN SusDat; NORMAN Suspect List Exchange). These lists have been compiled after a systematic literature review (Alygizakis et al. 2019; Corada-Fernández et al. 2011; Di Corcia et al. 1998; Di Corcia et al. 1999; Field et al. 1994; Gonsior et al. 2011; González et al. 2008; Riediker 2000; Schymanski et al. 2014b) by research groups within the NORMAN network (Dulio and Slobodnik 2009). A summary of chemical structures screened for in the samples is given in Table 1. The lists available in the NORMAN SusDat were extended for screening for PEGs with a higher number of ethoxy groups $(CH_2CH_2O)_x$, since it was found that PEGs with a higher mass are ionized in positive ionization as $[M+NH_4]^{2+}$.

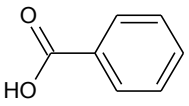
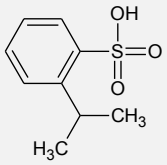
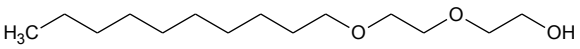
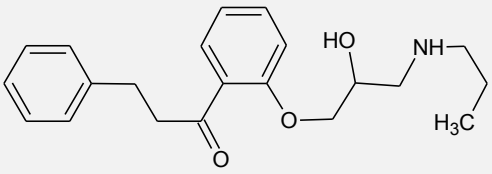
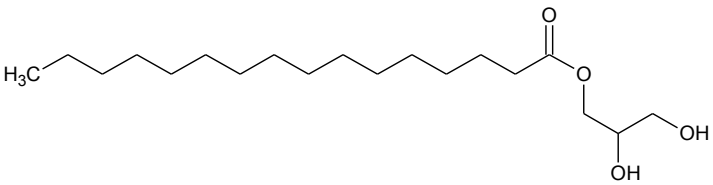
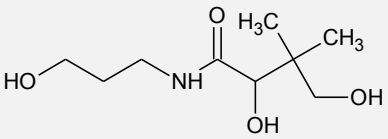
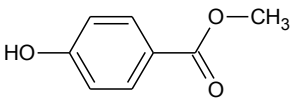
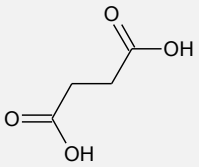
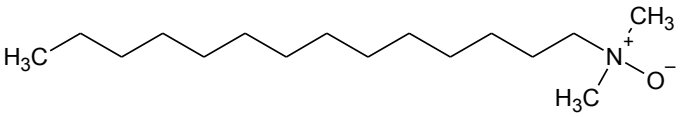
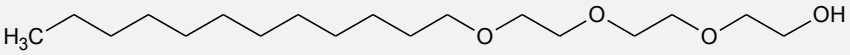
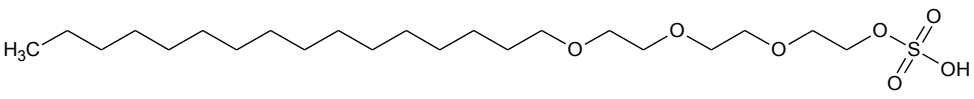
SPACs, SPADCs, DATSs, STACs and STADCs were semi-quantified based on the comparison of their signals to the LAS surfactants. PEGs were semi-quantified based on PEG-04 for which an

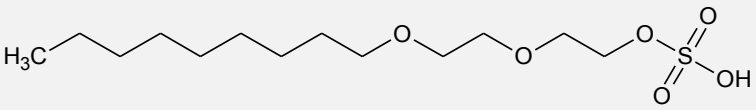
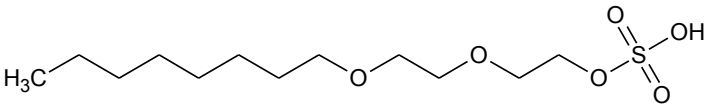
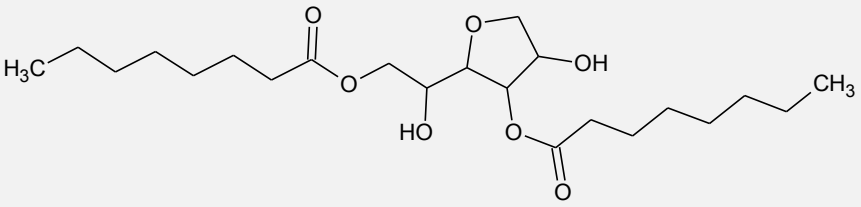
analytical standard was available. AEOs were semi-quantified based on the calibration curve of tetraethylene glycol monododecyl ether. LAS-C_9 was semi-quantified based on the calibration curve of LAS- C_8 and LAS- C_{14}; LAS- C_{15} and LAS-C_{16} were semi-quantified based on the C_{13}-LAS calibration curve.

A new list called S23 EIUBASURF (http://www.norman-network.com/?q=node/236; S23, EIUBASURF) was generated in the context of the current study after assigning structures to Unknown or Variable composition, Complex products or Biological materials (UCVBs). It should be noted that this was a rather time-consuming and challenging procedure and the list will be added to NORMAN SusDat at its next update. The list was generated after assigning structures to the UCVBs included in the Detergents Ingredients Database (DID) version 2016 (European Commission 2016). Generation of the chemical list involved manual assigning of chemical structures to each DID record, automatic retrieval of chemical identifiers and connection to chemical databases, so that the chemical list fits the format requested by the NORMAN SusDat (SMILES, Monoisotopic mass, Molecular Formula, InChI, InChIKey, CAS number, PubChem CID, ChemSpider ID, DTXSID). Examples of chemical structures detected in the wastewater samples are shown in Table 3.

Table 3: Surfactants and additives included in the DID suspect list detected in the WWTP effluent samples and their chemical structures.

Surfactant name	Structure
Amines, tallow, 1+2 EO (R=CH_3)	
Lauroyl sarcosinate	
Benzotriazole	
C_{12}-Alkyl phosphate esters	
di-2-Ethylhexyl sulfosuccinate	
C_8-Alkyl sulfate (linear)	
Amines, tallow, 5+5 EO (R=H)	
Mono-C_{12} Alkyl sulfosuccinate OR di-C_{6-6} Alkyl sulfosuccinate	 or

Surfactant name	Structure
Benzoic acid	
Cumene sulfonate	
C_{10} Alcohol, predominately linear, 2 EO	
Propafenone	
Glycerides, C_{15} mono	
Panthenol	
Methyl-paraben	
Succinic acid	
C_{14}-Alkyl dimethyl amine oxide	
C_{12} Alcohol, predominately linear, 3 EO	
C_{16}-Alkyl 4 ethyl sulfate	

Surfactant name	Structure
C_9-Alkyl 2 ethyl sulfate	
C_8-Alkyl 2 ethyl sulfate	
C_8 Sorbitan diester	

3.4 Methods for interpreting non-detect data

Left-censored observations, sometimes referred to as “non-detects” or “less than” values (e.g. <10 ng/L), are concentrations that are known only to be somewhere between zero and the limit of quantification (LOQ). A commonly used method in environmental chemistry to deal with values below the LOQ is to substitute a fraction of the LOQ for each censored value, or to exclude them from the analysis. However, in recent years research has shown that this produces poor estimates of statistics such as means, correlation coefficients, regression slopes, or hypothesis tests and can obscure trends or other patterns in the data (Helsel 2005, 2006). Better methods for interpreting censored values include regression on order statistics (ROS) and maximum likelihood estimation (MLE). ROS was used in this report, for a better estimate of average concentrations and to draw censored boxplots. The applied techniques are described in (Helsel 2011) and were applied for the LAS and AES target analysis within this study.

4 Results and Discussion

4.1 Method development

4.1.1 Analysis of LAS (TZW)

A first analysis of WWTP effluents, not within the scope of this study, revealed LAS concentrations considerably below the findings reported in the scientific literature. In the beginning, this was attributed to a rapid degradation of LAS in unpreserved environmental water samples. Later, it was found that the acquired LAS standards from HPC were not representative for the LAS used in commercial products. As previously mentioned, the reference standards for individual LAS homologs from HPC contain only one isomer with a terminal benzenesulfate moiety. However, in commercial LAS products the phenyl ring is attached randomly to any position except the terminal one (Lunar et al. 2006). Hence, all subsequent method development and LAS quantification was performed using a LAS mixture standard from Alfa Aesar. Interestingly, the multiple reaction monitoring (MRM) transitions of LAS homologs with terminal benzenesulfate moieties differ from those found in commercial products. For the former, the most intensive fragment ion in negative polarity mode is m/z = 170 (CH_2-C_6H_4-SO_3); for the latter, it is m/z = 183 (CH_2-CH-C_6H_4-SO_3). Hence, the fragment ion with m/z = 183 was used for the quantification of LAS homologs (Annex Table 1).

Since the concentrations of individual LAS homologs in the Alfa Aesar standard were unknown, an experimental determination of individual homolog concentrations using single MS was performed. For this approach the following assumption had to be made: The response of the detector is identical for every homolog, which means that the intensity (I_n) of the mass spectrum measured by single MS for homolog n is directly proportional to its molar concentration (c_n) and B is a constant to all homologs:

$I_n = B \times c_n$ *with* $c_n = \beta_n / M_n$

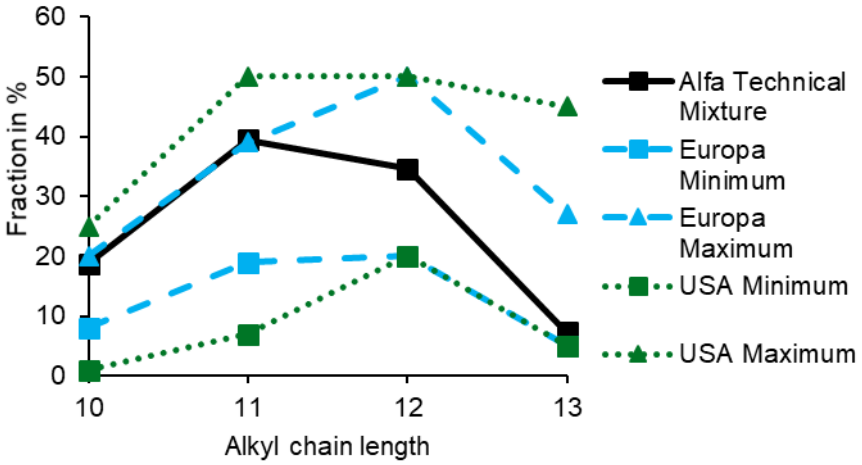
As the total concentration of the LAS standard (β_{tot}) is known, the concentration of an individual homolog in the stock solution can be estimated by using the intensity measured by MS in counts per second, weighted by its molecular weight (MW) in Dalton (Michel et al. 2012). The calculations are based on data from multi-channel acquisition (MCA) scans. The results are displayed in Table 4.

Table 4: Base of calculation for the LAS distribution in the Alfa Aesar standard

LAS homolog	Counts	MW	Counts x MW	Fraction in %
C_{10}-LAS	1.57E+04	297.1	4.65E+06	18.7
C_{11}-LAS	3.14E+04	311.2	9.77E+06	39.4
C_{12}-LAS	2.64E+04	325.2	8.58E+06	34.6
C_{13}-LAS	5.31E+03	339.2	1.80E+06	7.3

As reported in numerous studies (Cantarero et al. 2011; Traverso-Soto et al. 2015), the C_{11}-LAS and C_{12}-LAS also were here the dominant homologs in the standard from Alfa Aesar, with 39% and 35%, respectively. C_{14}-LAS was absent from the standard. The calculated average chain length of the standard based on the experimental determination is 11.4, which is in accordance with the average number provided by the manufacture. The results regarding the distribution of homologs fit well to those reported for LAS used in Europe and the USA (UNEP 2005) (Figure 3).

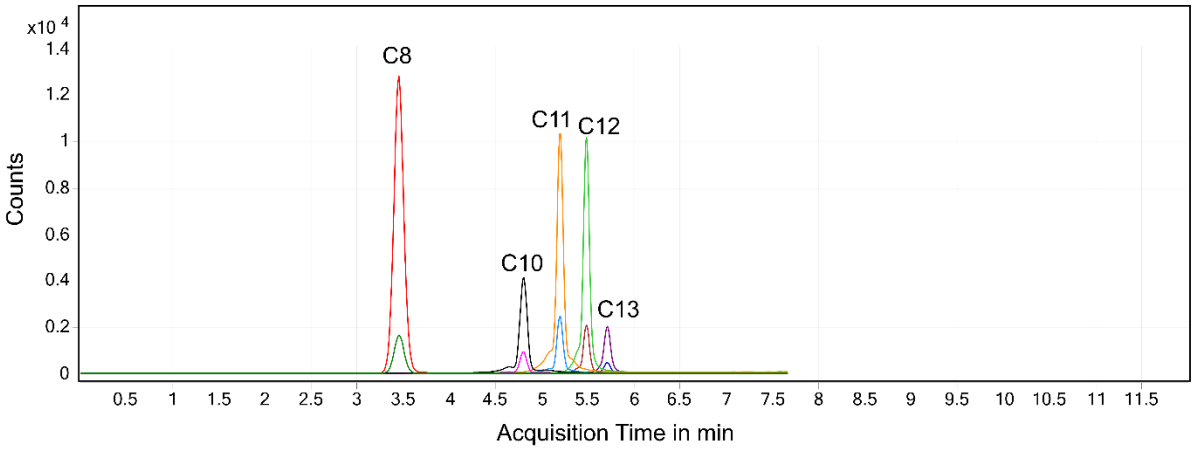
Figure 3: **Distribution of individual LAS homologs (C_{10}-C_{13}) in the Alfa Aesar standard compared to minimum and maximum values reported for Europe and the USA according to UNEP (2005).**



(source: TZW, Karlsruhe)

A variety of reversed-phase LC columns and eluents were tested during the method development process. When using ACN as the organic solvent, retention and peak shapes of LAS homologs were generally poor. In fact, LAS homologs had a similar retention time to that of metformin, which was used to estimate the void time of the analytical method. MeOH was subsequently chosen as the organic mobile phase (eluent B), as it enabled sufficient chromatographic separation as well as good peak shapes. By applying a less retentive C_8 column, isomers of each LAS eluted as single peaks which facilitated peak integration (Figure 4). Ammonium formate (0.1 Mm) in the aqueous mobile phase (eluent A) was found to be an effective mobile phase additive to increase the peak intensities of LAS homologs.

Figure 4: **LC-MS/MS chromatogram of LAS homologs (quantifier & qualifier ions) in water:ACN (50:50). C_8: 100 µg/L; C_{10}: 93.5 µg/L; C_{11}: 197 µg/L; C_{12}: 173 µg/L; C_{13}: 36.5 µg/L. Injection volume: 4 µL.**



(source: TZW, Karlsruhe)

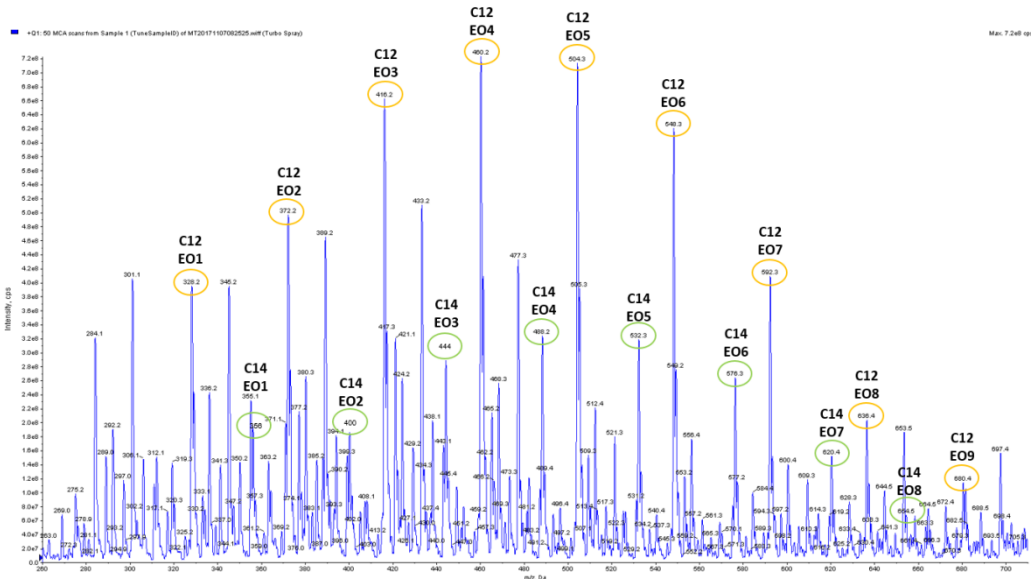
During method development, considerable peaks of LAS in zero volume injections were observed. The contamination originated from the mobile phase(s) and it was found that a low organic content at the beginning of the gradient lead to an accumulation of LAS on the analytical column during the post-run column equilibration, which was subsequently eluted during the

next run. However, by starting with a MeOH content higher than 40%, LAS peaks in zero volume injections could be substantially decreased. Contamination issues with surfactants can be explained by their excessive usage in personal care products as well as in detergents applied in chemical laboratories and have already been reported by several authors (Gray et al. 2011; Knepper et al. 2003; McDonough et al. 2016; Sakai et al. 2017). Due to the issue of LAS contaminated solvents and laboratory equipment, a rotational vacuum concentrator was chosen for the extraction process. Samples (1 mL) and calibration standards were evaporated to dryness and reconstituted again in 1 mL of ultra-pure water:ACN (50:50, v:v). ACN was added to prevent potential adsorption of LAS on glass surfaces. This technique is preferable compared to SPE for two reasons: Firstly, the sample containers can be heated to 550 °C to remove any potential surfactant residues, which is not possible when using the SPE manifold and cartridges. Secondly, all samples including the calibration standards can be reconstituted in the same way, and solvent is only used for the final reconstitution of the analytes. Consequently, the determined LAS concentration of the sample is not altered by any LAS background in the solvents.

4.1.2 Analysis of AES (TZW)

No standards of individual AES homologs/ethoxymers could be purchased. Hence, individual concentrations of AES homologs/ethoxymers in a standard from Santa Cruz Biotechnology were experimentally determined using the same approach as described above for LAS. According to the supplier, the advertised 70.5% of active ingredient refers to the sum of various sodium AES homologs/ethoxymers (S. Kiczka, Santa Cruz Biotechnology, personal communication). Further information on the composition of the standard was not provided. Single MS full mass data showed that AES predominantly forms $[M+NH_4]^+$ (ammonium) adduct ions in positive ionization mode. The spectrum further revealed $[M+NH_4\text{-}SO_3]^+$ (desulfated ammonium) adducts of AES (Figure 5).

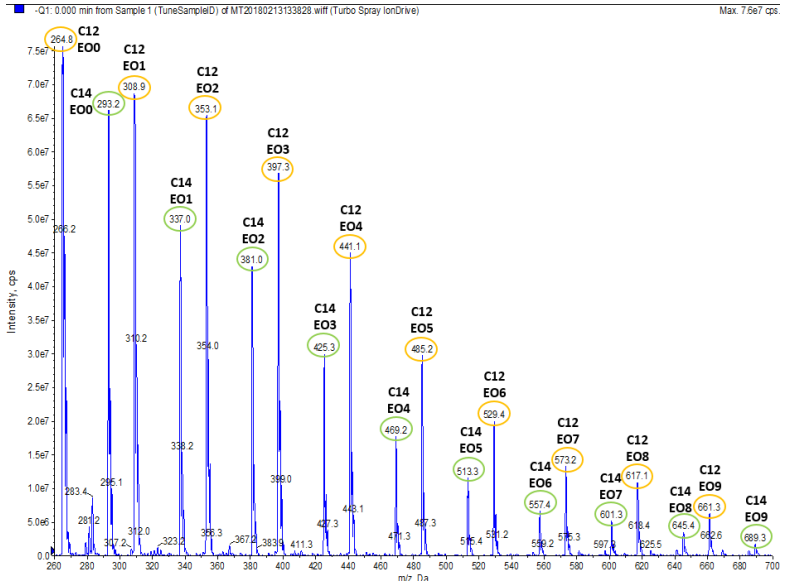
Figure 5: Full mass spectrum obtained by direct injection of the AES standard in positive ionization mode. The highlighted peaks are $[M+NH_4]^+$ species of C_{12}- and C_{14}-homologs with different numbers of EO units.



(source: TZW, Karlsruhe)

In negative ionization mode $[M-H]^-$ ions were formed (Figure 6). The mass spectrum showed C_{12} and C_{14} homologs with EO units from 0 to 9. Other AES homologs (e.g. AES-C_{13}, AES-C_{15}) were not visible in the spectrum.

Figure 6: Full mass spectrum obtained by direct injection of the AES standard in negative ionization mode. The highlighted peaks are $[M-H]^-$ species of C_{12}- and C_{14}-homologs with different numbers of EO units.



(source: TZW, Karlsruhe)

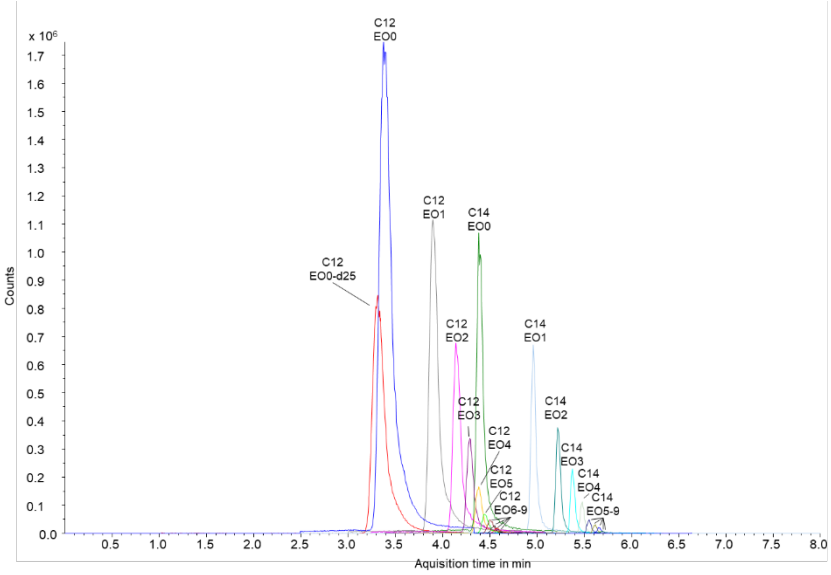
Surprisingly, Figure 5 and Figure 6 show differing ethoxymer distributions for AES: When AES homologs are analyzed as ammonium adduct ions, ethoxymers with 3 to 6 EO units represent the most important fraction of every chain length, while the AES with zero EO units is absent in the mass spectra. In negative ionization mode, the most abundant ethoxymer for C_{12} and C_{14} is the one with zero EO units, and a declining tendency in abundance with an increasing number of EO units is noticeable. These results indicate that AES ethoxymers have a varying affinity to form ammonium adducts. One possible explanation for this could be the formation of crown ethers (Ashton et al. 1995), which involves the complexation of ether oxygens with cations (in this case with ammonium ions). Consequently, the experimental determination of individual concentrations of AES homologs/ethoxymers was based on the mass spectra obtained by direct injection of the AES standard in negative ionization mode. Since other AES homologs were not visible in the mass spectra in negative ionization mode, it was assumed that the obtained standard only contains AES-C_{12} and AES-C_{14} homologs. The results are summarized in Table 5. It can be assumed that the AES standard contains about 72% of AES-C_{12} and 28% of AES-C_{14}. The average number of EO units of AES-C_{12} in the standard is 2.5, which is in accordance with the reported average number of 2.7 for AES for domestic use and 2.4 for the total AES produced (HERA 2004).

Table 5: Base of calculation for the AES distribution in the Santa Cruz Biotechnology standard.

	C_{12}				C_{14}			
Number of EO units	Counts	MW	Counts x MW	Fraction in %	Counts	MW	Counts x MW	Fraction in %
0	2.94E+09	265.1	7.80E+11	14.7	1.15E+09	293.1	3.37E+11	6.3
1	2.17E+09	309.1	6.71E+11	12.6	8.49E+08	337.1	2.86E+11	5.4
2	1.79E+09	353.1	6.32E+11	11.9	6.36E+08	381.2	2.42E+11	4.6
3	1.44E+09	397.2	5.74E+11	10.8	4.93E+08	425.2	2.10E+11	3.9
4	8.43E+08	441.2	3.72E+11	7.0	2.69E+08	469.3	1.26E+11	2.4
5	5.39E+08	485.3	2.62E+11	4.9	1.78E+08	513.3	9.14E+10	1.7
6	3.40E+08	529.3	1.80E+11	3.4	1.24E+08	557.3	6.92E+10	1.3
7	2.45E+08	573.3	1.40E+11	2.6	1.01E+08	601.3	6.06E+10	1.1
8	1.99E+08	617.3	1.23E+11	2.3	7.55E+07	645.3	4.87E+10	0.9
9	1.23E+08	661.3	8.16E+10	1.5	4.24E+07	689.4	2.93E+10	0.6

Two novel stationary phases were tested for the chromatographic separation of AES homologs. The first column was a Luna Omega Polar C_{18} which provides enhanced polar retention compared to standard C_{18} columns due to its polar modified particle surface. The second was a Luna Omega PS C_{18}, which is a mixed-mode stationary column with a surface that contains a positive charge, which aids in the retention of acidic compounds through ionic interactions, while the C_{18} ligand promotes general reversed phase retention. Both columns were tested with different mobile phases and LC gradients. Even though the columns are differently modified C_{18} phases, they provided a nearly identical separation of AES homologs. The best performance in terms of chromatographic resolution, peak shape, intensity, and run time was achieved using the Luna Omega Polar C_{18} column with ultra-pure water (25 mM ammonium acetate, pH 3.6 adjusted with glacial acetic acid) (A) and acetonitrile (B) as eluents. Figure 7 depicts the chromatograms of AES homologs and the internal standard SDS-d_{25} using this optimized method. The method enabled generally good peak shapes, intensities, and short runtime. However, a complete baseline separation of peaks could not be achieved. The fact that AES homologs only differ in the alkyl chain length and/or the number of EO units makes it difficult to separate them using common reversed phase LC columns. An enhanced peak resolution may be achieved with an analytical column that provides retention mechanisms specifically designed for the separation of surfactant homologs. The surface chemistry of the packing material of such columns consists of hydrophobic alkyl chains, tertiary amino groups, and polar amide functional groups, resulting in a multi-mode separation mechanism including reversed-phase, anion-exchange, and dipole-dipole interactions (Liu et al. 2006; Liu et al. 2012). Since MS/MS is capable of distinguishing between co-eluting compounds, the method was not further optimized for better chromatographic resolution. However, scheduled MRM was performed in order to decrease the number of concurrent MRM transitions and therefore to obtain higher sensitivity and more data points per transition.

Figure 7: **LC-MS/MS chromatogram of AES homologs/ethoxymers (quantifier ions) in water:ACN (50:50, v:v) of the standard from Santa Cruz Biotechnology. Concentration: 70 µg/L (sum of C_{12} and C_{14} with EO0–9). Injection volume: 40 µL.**



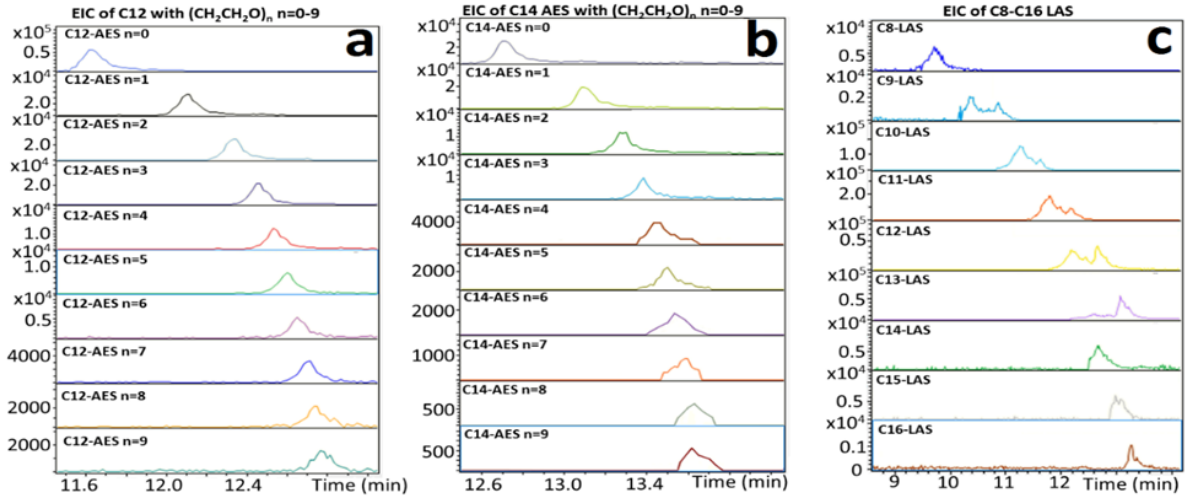
(source: TZW, Karlsruhe)

Due to generally lower AES concentrations in effluent water samples compared to LAS, SPE was initially tested for sample pre-treatment and enrichment. AES were extracted from water samples (50 mL) using 200 mg /6 mL Strata™-X 33 µm SPE cartridges from Phenomenex (Aschaffenburg, Germany). Cartridges were first preconditioned with 10 mL of MeOH, followed by 10 mL of ultrapure water. The samples were then spiked with a defined volume of the IS solution and were then loaded onto the cartridges which were subsequently dried for 60 min using a stream of nitrogen. The analytes were eluted three times with 3 mL of MeOH. The eluate was evaporated to dryness under a gentle flow of nitrogen and reconstituted in 0.5 mL of ultra-pure water:ACN (50:50, v:v). Results indicated high and variable background contamination of AES when SPE was used for sample pre-treatment. McDonough et al. (2016) observed the same problem. In their work, SPE cartridges were soaked overnight with MeOH, dried under vacuum, and additionally rinsed with MeOH to reduce the background of anionic surfactants to acceptable levels. Since this procedure is relatively time and material consuming, sample-pretreatment for AES was carried out in the same way as for LAS using a rotational vacuum concentrator. However, the sample volume was increased to 10 mL, in order to account for the lower concentrations of AES compared to LAS in effluent samples. After evaporating the sample to dryness, the analytes were reconstituted in 1 mL of ultra-pure water:ACN (50:50, v:v).

4.1.3 Analysis of known and unknown surfactants and TPs (Environmental Institute & Laboratory of Analytical Chemistry)

The used HPLC-ESI-QTOF-MS method was previously optimized for detection of several thousands of target substances and proved to be ‘fit for purpose’ of a wide-scope screening, including target, suspect and non-target screening. The method allowed for a good chromatographic separation of all analyzed compounds. In some cases of homolog substances, co-elution occurred (Figure 8). However, the separation was also in these cases feasible based on their exact monoisotopic masses.

Figure 8: Extracted ion chromatogram (EIC) of C_{12}-AES with $(CH_2CH_2O)_n$ where n=0-9 (a), of C_{14}-AES with $(CH_2CH_2O)_n$ where n=0-9 (b) and of C8-16 LAS (c).



(source: Laboratory of Analytical Chemistry, Athens)

4.2 Method validation

4.2.1 Method validation for the analysis of LAS and AES (TZW)

The precision and accuracy of the analytical methods for LAS and AES were determined by extracting six aliquots of a tap and a WWTP effluent sample, respectively. All samples were spiked with a total concentration of 100 µg/L for LAS (Alfa Aesar standard; sum of C_{10}–C_{13}) and 25 µg/L for AES (Santa Cruz standard; sum of C_{12} and C_{14} with EO_{0-9}) prior to extraction. For LAS, relative standard deviations (RSDs) ranged from 3% to 10% for both, tap and effluent water samples. For AES, RSDs ranged from 1% to 6% (tap water) and 3% to 10% (effluent water).

To account for any possible background concentration in the native water samples, two non-spiked tap water and effluent samples were analyzed for LAS and AES, respectively. For LAS, recoveries ranged from 91%–107% and 98%–115% for tap and effluent water samples, respectively. For AES, recoveries ranged from 94%–114% and 90%–120% for tap and effluent water samples, respectively. Both methods showed very good linearity within the calibration range. The LOQ of each homolog/ethoxymer was determined according to DIN 32645. Additional to the statistically derived LOQ, a signal-to-noise ratio of >10 was required for each analyte peak in environmental samples to be considered a detection. All results of the method validation are listed in Table 6 and Table 7 for LAS and AES, respectively.

Table 6: Validation results of the LC-MS/MS method for the analysis of LAS.

Parameter	Matrix	C_{10}	C_{11}	C_{12}	C_{13}
RSD in % (n=6)	tap water	3	5	3	10
RSD in % (n=6)	effluent water	3	5	9	10
Recovery in % (average; n=6)	tap water	103	100	107	91
Recovery in % (average; n=6)	effluent water	98	102	115	104
LOQ in µg/L	tap water	0.96	1.2	2.2	2.2
Linearity (R^2) (5 µg/L - 500 µg/L)	tap water	0.9985	0.9997	0.9996	0.9957

Table 7: Validation results of the LC-MS/MS method for the analysis of AES.

Parameter	Matrix	$C_{12}EO_0$	$C_{12}EO_1$	$C_{12}EO_2$	$C_{12}EO_3$	$C_{12}EO_4$	$C_{12}EO_5$	$C_{12}EO_6$	$C_{12}EO_7$	$C_{12}EO_8$	$C_{12}EO_9$
RSD in % (n=6)	tap water	5	4	4	2	4	5	1	4	4	5
RSD in % (n=6)	effluent water	6	4	4	7	5	4	4	3	10	4
Recovery in % (average; n=6)	tap water	94	101	103	101	101	102	100	105	103	104
Recovery in % (average; n=6)	effluent water	90	101	99	94	94	92	100	107	97	100
LOQ in µg/L	tap water	0.047	0.024	0.013	0.014	0.008	0.005	0.008	0.004	0.006	0.004
Linearity (R^2) (5 µg/L - 175 µg/L)	tap water	0.9989	0.9991	0.9990	0.9995	0.9988	0.9995	0.9981	0.9981	0.9981	0.9988
Parameter	**Matrix**	**$C_{14}EO_0$**	**$C_{14}EO_1$**	**$C_{14}EO_2$**	**$C_{14}EO_3$**	**$C_{14}EO_4$**	**$C_{14}EO_5$**	**$C_{14}EO_6$**	**$C_{14}EO_7$**	**$C_{14}EO_8$**	**$C_{14}EO_9$**
RSD in % (n=6)	tap water	5	4	3	3	4	3	6	4	5	3
RSD in % (n=6)	effluent water	7	7	6	7	6	6	5	5	4	10
Recovery in % (average; n=6)	tap water	98	102	103	102	101	105	104	105	114	108
Recovery in % (average; n=6)	effluent water	102	120	117	114	96	112	106	111	114	113
LOQ in µg/L	tap water	0.043	0.012	0.01	0.008	0.003	0.003	0.004	0.003	0.002	0.001
Linearity (R^2) (5 µg/L - 175 µg/L)	tap water	0.9987	0.9983	0.9990	0.9989	0.9988	0.9992	0.9993	0.9988	0.9991	0.9986

4.2.2 Method validation for the analysis of LAS and AES (Environmental Institute & Laboratory of Analytical Chemistry)

Five-point calibration curves were generated using linear regression analysis. The linearity was qualified by linear correlation coefficient, r^2. The calibration curves obtained for wide concentration ranges were linear with $r^2>0.98$ in all cases. Accuracy of the method was assessed with recovery experiments in wastewater effluent samples. Extraction recoveries for most target analytes showed recovery efficiency between 70% and 110%. To ensure a correct quantification, method precision was determined as %RSD from the recovery experiments. Precision limit <15% RSD was met for all target analytes. Regarding sensitivity, method limit of detection and quantification (LODs and LOQs) were calculated from the recovery experiments at the lowest concentration spiked.

4.3 Environmental concentrations

4.3.1 Target screening of LAS in monitored WWTP effluents (TZW)

Table 8 lists the concentrations of LAS homologs in WWTP effluents. Individual concentrations greater than the LOQ were summed up to calculate the total LAS concentration at each sampling point. Since the data include left-censored values, ROS was used for a better estimate of average concentrations, and to draw censored boxplots. Individual LAS concentrations are in the lower to mid µg/L-range for the monitored WWTP effluents. Individual concentrations of LAS-C_{10} range from <LOQ to 18 µg/L with an estimate average concentration of 4 µg/L. With one exception (WWTP Dortmund-Deusen), individual concentrations of LAS-C_{11} are consistently the highest among all LAS homologs at the same sampling point, with concentrations ranging from <LOQ to 20 µg/L and with an estimated average concentration of 5.4 µg/L. For LAS-C_{12}, individual concentrations range from <LOQ to 11 µg/L and an estimate average concentration of 3.4 µg/L. The lowest individual concentrations were measured for LAS-C_{13}, ranging between <LOQ and 5.2 µg/L with an estimated average of 1.6 µg/L. Total LAS concentrations range from <LOQ to 47.7 µg/L.

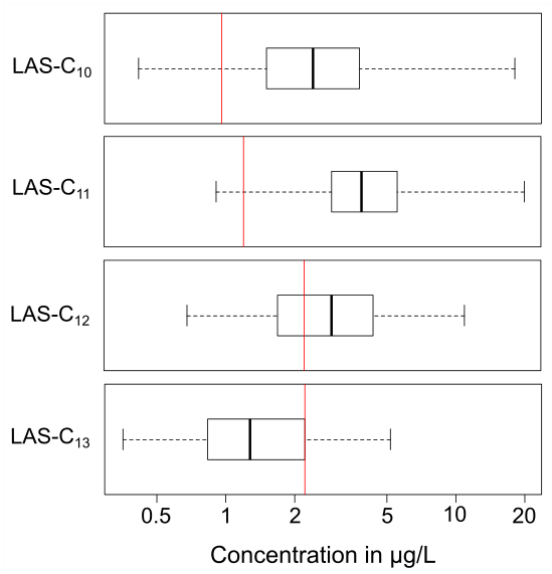
Table 8: Individual and total concentrations of LAS homologs (in ng/L) in monitored WWTP effluents.

LAS homolog LOQ	C_{10} 960	C_{11} 1,200	C_{12} 2,200	C_{13} 2,200	Total (C_{10}–C_{13})	LAS homolog LOQ	C_{10} 960	C_{11} 1,200	C_{12} 2,200	C_{13} 2,200	Total (C_{10}–C_{13})
WWTP 1	16,000	18,000	9,200	3,300	46,500	WWTP 18	1,500	3,000	3,600	<LOQ	8,100
WWTP 2	1,100	2,800	<LOQ	<LOQ	3,900	WWTP 19	2,500	2,900	<BG	<LOQ	5,400
WWTP 3	4,900	6,300	3,500	<LOQ	14,700	WWTP 20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
WWTP 4	3,500	4,400	2,300	<LOQ	10,200	WWTP 21	2,600	3,900	2,500	<LOQ	9,000
WWTP 5	18,000	19,000	8,000	2400	47,400	WWTP 22	1,500	2,500	2,200	<LOQ	6,200
WWTP 6	3,800	4,500	2,300	<LOQ	10,600	WWTP 23	1,800	2,100	<LOQ	<LOQ	3,900
WWTP 7	9,700	7,900	2,900	<LOQ	20,500	WWTP 24	3,200	5,300	4,700	2300	15,500
WWTP 8	1,000	2,500	<LOQ	<LOQ	3,500	WWTP 25	2,400	4,000	4000	2500	12,900
WWTP 9	1,800	3,100	3,300	<LOQ	8,200	WWTP 26	3,100	5,600	4,400	2500	15,600
WWTP 10	2,200	4,600	4,600	<LOQ	11,400	WWTP 27	1,500	2,700	2,200	<LOQ	6,400
WWTP 11	2,300	3,400	<LOQ	<LOQ	5,700	WWTP 28	2,600	3,000	<LOQ	<LOQ	5,600
WWTP 12	1,300	1,800	<LOQ	<LOQ	3,100	WWTP 29	4,100	5,900	4400	2200	16,600
WWTP 13	13,000	20,000	11,000	3,700	47,700	WWTP 30	1,900	5,200	7,400	5,200	19,700
WWTP 14	7,100	8,200	3,900	<LOQ	19,200	WWTP 31	3,500	4,700	3,100	<LOQ	11,300
WWTP 15	6,700	8,300	5,800	2,400	23,200	WWTP 32	1,200	1,300	<LOQ	<LOQ	2,500
WWTP 16	1,700	3,600	3,400	<LOQ	8,700	WWTP 33	<LOQ	3,500	<LOQ	<LOQ	3,500
WWTP 17	2,400	3,600	2,300	<LOQ	8,300	Estim. average	3,966	5,409	3,449	1561	14,385

Based on the estimated average concentrations of individual LAS homologs, the average total LAS concentration in monitored WWTP effluents is 14.4 µg/L. The average LAS chain length of effluent samples is 11.2.

Figure 9 shows censored boxplots of the concentrations of LAS homologs in effluent samples. The vertical red line represents the LOQ of the respective homolog. Percentiles above this line are unaffected by censoring, and accurate comments on the attributes above this line can be made, but not on concentrations below. Percentiles below the line were estimated by using ROS. The highest median concentration was found for LAS-C_{11}. The lowest median concentration was found for the C_{13} homolog, which also had the highest number of observations below the LOQ. The boxplots further show that more than half of the observations for each homolog are within one order of magnitude, indicating that effluent concentrations of monitored WWTPs are similar to each other.

Figure 9: Censored boxplots of the concentrations of LAS homologs in effluents of monitored WWTPs. The vertical red lines depict the limit of quantification for the respective homolog.



(source: TZW, Karlsruhe)

The average LAS chain length of 11.2 is in accordance with the average of 11.3 reported in (McDonough et al. 2016). Since the average chain length in commercial products is between 11.7 and 11.8 (UNEP 2005), this indicates the preferential removal of long alkyl chains during wastewater treatment.

This could be explained by the higher affinity of LAS homologs with long alkyl chains to be adsorbed on suspended solids and sediments (Lara-Martín et al. 2008). However, higher average chain lengths of LAS of 11.6 (Matthijs et al. 1999) and 12.1 (McAvoy et al. 1998) have also been reported in the effluents.

The measured concentrations of LAS homologs in WWTP effluents analyzed in this work are in some cases considerably lower than those reported in other studies. For example, the estimated average total LAS concentration of 14.4 µg/L for WWTP effluents in this study is about ten times lower than the findings for various WWTPs in Spain (Lunar et al. 2006; Riu et al. 2000). However, when comparing effluent concentrations of different WWTPs, it is important to always consider the corresponding influent concentrations/removal rates. For example, it has been found that the removal of LAS in WWTPs equipped with trickling filters is more variable and generally much lower than in WWTPs using the activated sludge process (Holt et al. 2003; McAvoy et al. 1998). In the study presented here, total LAS concentrations of four influent samples (WWTPs: Geldern; Eutin; Landsberg; Stuttgart) ranged between 2,600 µg/L and 3,500 µg/L (Annex Table 3), which translates to very high removal rates between 99.2% and 99.9%. In contrast, average removal rates for LAS in the aforementioned studies by Riu et al. (2000) and Lunar et al. (2006) were only 84.9% and 87.5%, respectively, leading to elevated concentrations of LAS homologs in the effluent. Other authors reported average removal rates and effluent concentrations similar to the values determined in the present study. At nine WWTPs in Austria the average effluent concentration was 13.3 µg/L with an average removal rate of 99.7% (Clara et al. 2007). For six WWTPs in the Netherlands the average effluent concentration was 43 µg/L with an average removal of 99.2% (Matthijs et al. 1999). In a recently published study of effluent concentrations of 44 WWTPs in the U.S. the mean outflow concentration was 15.3 µg/L. However, no influent concentrations or removal rates were reported (McDonough et al. 2016).

The effluent concentration of a specific surfactant is further dependent on the respective inflow concentration, which in turn is foremost controlled by regional differences in the per-capita surfactant use. Such variation is pronounced even within countries of the European Union. For example, Italy is one of the biggest consumers in Europe of linear alkylbenzene, the precursor of LAS, consuming about 23% of the total regional demand in 2012 (MicroMarketMonitor 2018).

Furthermore, it has been demonstrated that the concentration of surfactants can already be substantially reduced in sewers before entering the WWTP. Matthijs et al. (1999) observed up to 68% (average: 50%) of in-sewer removal of LAS, which is expected to be due to a combination of adsorption onto and settling of suspended solids, precipitation as calcium salts, and biodegradation. Their results show that in-sewer removal varies strongly from one location to the other, and is presumably depended on the length of the sewer, travel time, and the degree of microbial activity present in the sewer.

4.3.2 Target screening of AES in monitored WWTP effluents (TZW)

Measured concentrations of AES-C12 and AES-C14 ethoxymers in the 33 WWTP effluents are listed in Table 9 and

Table 10, respectively. For total AES concentrations at each sampling point, individual concentrations of AES-C12 and AES-C14 ethoxymers greater the LOQ were summed up and are included in

Table 10. Again, ROS was used for the estimation of average concentrations.

Individual AES ethoxymer concentrations are in the lower to mid ng/L-range for WWTP effluent samples. Estimated average effluent concentrations for AES-C_{12} ethoxymers range between 12 ng/L (AES-C_{12} EO_9) and 74 ng/L (AES-C_{12} EO_2). AES-C_{12} ethoxymers with zero to three EO units show the highest estimated average effluent concentration, resulting in an average number of 2.65 EO units for the AES-C_{12} homolog in the effluent samples.

Estimated average effluent concentrations for AES-C_{14} ethoxymers range between 4 ng/L (AES-C_{14} EO_9) and 62 ng/L (AES-C_{14} EO_0). Again, AES-C_{14} ethoxymers with zero to three EO units have the highest estimated average effluent concentrations, resulting in an average number of 1.85 EO for the AES-C_{14} homolog in the effluent samples.

Total AES effluent concentrations range from <LOQ to 1.9 µg/L. Based on the estimated average effluent concentrations of individual AES ethoxymers, the average total AES (20 analytes: C_{12} and C_{14} with 0–9 EO) effluent concentration in monitored WWTP effluents is 0.57 µg/L.

Table 9: Concentrations in ng/L of AES-C_{12} ethoxymers in monitored WWTP effluents.

AES ethoxymer LOQ	$C_{12}EO_0$ 47	$C_{12}EO_1$ 24	$C_{12}EO_2$ 13	$C_{12}EO_3$ 14	$C_{12}EO_4$ 8	$C_{12}EO_5$ 5	$C_{12}EO_6$ 8	$C_{12}EO_7$ 4	$C_{12}EO_8$ 6	$C_{12}EO_9$ 4	AES ethoxymer LOQ	$C_{12}EO_0$ 47	$C_{12}EO_1$ 24	$C_{12}EO_2$ 13	$C_{12}EO_3$ 14	$C_{12}EO_4$ 8	$C_{12}EO_5$ 5	$C_{12}EO_6$ 8	$C_{12}EO_7$ 4	$C_{12}EO_8$ 6	$C_{12}EO_9$ 4
WWTP 1	130	62	59	58	27	25	17	16	12	9	WWTP 18	<LOQ	50	60	79	39	28	26	27	24	<LOQ
WWTP 2	360	230	210	170	120	80	60	50	46	27	WWTP 19	<LOQ	31	32	28	18	<LOQ	<LOQ	<LOQ	10	11
WWTP 3	<LOQ	48	66	49	<LOQ	<LOQ	<LOQ	18	<LOQ	<LOQ	WWTP 20	<LOQ	32	35	40	15	21	25	25	23	25
WWTP 4	<LOQ	30	40	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	WWTP 21	<LOQ	41	44	39	21	18	19	16	18	10
WWTP 5	<LOQ	70	61	48	17	<LOQ	14	21	18	14	WWTP 22	<LOQ	<LOQ	18	<LOQ	10	8	10	7	7	4
WWTP 6	130	110	88	73	44	29	23	22	14	<LOQ	WWTP 23	<LOQ	<LOQ	27	27	14	9	12	<LOQ	<LOQ	<LOQ
WWTP 7	<LOQ	32	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	36	<LOQ	<LOQ	WWTP 24	<LOQ	33	40	36	13	<LOQ	11	21	16	<LOQ
WWTP 8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	WWTP 25	52	62	81	57	17	20	13	20	15	<LOQ
WWTP 9	<LOQ	53	38	59	36	28	28	21	24	15	WWTP 26	81	200	330	220	82	62	58	57	54	50
WWTP 10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	WWTP 27	48	180	260	200	91	74	56	56	57	49
WWTP 11	54	49	49	38	21	<LOQ	8	21	<LOQ	<LOQ	WWTP 28	<LOQ	27	29	<LOQ	<LOQ	6	8	6	7	6
WWTP 12	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	WWTP 29	54	69	99	64	31	16	15	28	18	11
WWTP 13	<LOQ	<LOQ	22	26	19	11	10	14	11	9	WWTP 30	<LOQ	26	55	96	77	60	47	38	39	26
WWTP 14	<LOQ	<LOQ	15	<LOQ	<LOQ	<LOQ	<LOQ	8	8	<LOQ	WWTP 31	150	150	160	160	75	55	41	37	35	30
WWTP 15	53	32	39	30	<LOQ	<LOQ	<LOQ	12	10	<LOQ	WWTP 32	<LOQ	31	41	34	13	11	10	17	17	<LOQ
WWTP 16	64	82	73	99	36	31	27	26	30	27	WWTP 33	<LOQ	<LOQ	21	<LOQ	<LOQ	7	<LOQ	<LOQ	<LOQ	<LOQ
WWTP 17	110	320	370	220	97	73	58	51	45	33	Estim. average	48	65	76	63	30	22	19	22	18	12

Table 10: Concentrations in ng/L of AES-C_{14} ethoxymers and total AES (C_{12} and C_{14} with EO_{0-9}) concentration in monitored WWTP effluents.

AES ethoxymer LOQ	C_{14} EO_0 43	C_{14} EO_1 12	C_{14} EO_2 10	C_{14} EO_3 8	C_{14} EO4 3	C_{14} EO_5 3	C_{14} EO_6 4	C_{14} EO_7 3	C_{14} EO_8 2	C_{14} EO_9 1	C_{12}&C_{14} EO_{0-9}	AES ethoxymer LOQ	C_{14} EO_0 43	C_{14} EO_1 12	C_{14} EO_2 10	C_{14} EO_3 8	C_{14} EO_4 3	C_{14} EO_5 3	C_{14} EO_6 4	C_{14} EO_7 3	C_{14} EO_8 2	C_{14} EO_9 1	C_{12}&C_{14} EO_{0-9}
WWTP 1	83	16	13	18	12	13	10	11	15	9	614	WWTP 18	60	37	33	33	15	18	16	16	19	10	589
WWTP 2	160	84	74	61	39	30	24	19	17	9	1868	WWTP 19	78	47	26	14	<LOQ	<LOQ	<LOQ	8	<LOQ	<LOQ	302
WWTP 3	88	77	45	24	13	7	<LOQ	<LOQ	<LOQ	<LOQ	436	WWTP 20	<LOQ	23	21	15	7	8	<LOQ	4	<LOQ	<LOQ	318
WWTP 4	62	36	22	14	6	<LOQ	6	<LOQ	<LOQ	<LOQ	216	WWTP 21	<LOQ	22	17	12	7	5	4	4	6	<LOQ	303
WWTP 5	82	59	26	18	9	7	7	8	13	10	502	WWTP 22	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	63
WWTP 6	80	44	28	24	11	10	9	7	<LOQ	<LOQ	747	WWTP 23	<LOQ	18	18	11	5	5	<LOQ	3	<LOQ	<LOQ	149
WWTP 7	97	70	41	17	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	293	WWTP 24	86	69	36	19	9	8	7	6	<LOQ	<LOQ	411
WWTP 8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	WWTP 25	60	40	33	22	11	7	7	8	<LOQ	<LOQ	524
WWTP 9	<LOQ	<LOQ	<LOQ	9	8	7	9	6	6	5	352	WWTP 26	76	66	59	26	13	12	14	13	16	9	1498
WWTP 10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	WWTP 27	83	100	87	50	30	26	19	16	17	12	1510
WWTP 11	68	39	27	19	10	6	7	<LOQ	<LOQ	<LOQ	416	WWTP 28	<LOQ	18	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2	109
WWTP 12	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	WWTP 29	84	70	50	28	13	10	10	7	13	<LOQ	691
WWTP 13	<LOQ	<LOQ	<LOQ	8	6	6	4	5	6	3	159	WWTP 30	<LOQ	23	22	29	21	26	25	28	29	19	687
WWTP 14	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3	<LOQ	3	<LOQ	<LOQ	36	WWTP 31	160	110	81	56	27	21	15	12	9	<LOQ	1383
WWTP 15	73	26	21	12	7	4	<LOQ	<LOQ	<LOQ	<LOQ	321	WWTP 32	45	26	15	9	6	4	4	4	<LOQ	<LOQ	286
WWTP 16	48	23	21	15	6	6	<LOQ	7	8	5	635	WWTP 33	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	27
WWTP 17	48	52	37	21	11	11	7	7	4	3	1578	Estim. average	63	39	28	19	10	8	7	7	7	4	566

The estimated average total AES concentration of 0.57 µg/L in WWTP effluents found in this work is lower compared to values reported in other studies. McAvoy et al. (1998) determined average total AES effluent concentrations (28 analytes: C_{12}–C_{15} with $EO_{0–6}$) of 11 µg/L and 73 µg/L for activated sludge (average removal: 98%) and trickling filter treatment (average removal: 83%), respectively. For seven WWTP effluents in the Netherlands, an average effluent concentration of 6.5 µg/L for AES (36 analytes: C_{12}–C_{15} with $EO_{0–8}$) with a removal greater 99% was reported (Matthijs et al. 1999). In the study by McDonough et al. (2016) on 44 WWTPs in the U.S. the average total AES concentration (20 analytes: C_{12}–C_{16} with $EO_{1–4}$) was 1.95 µg/L.

One possible explanation for the overall low average total AES concentration in the present study, is that only homologs with an alkyl chain length of 12 and 14 were considered for the calculation of total AES concentrations. In the effluent of a trickling filter plant in the U.S. sampled by McAvoy et al. (1998) C_{12} and C_{14} homologs only accounted for 57% of the total AES concentration, while C_{13} and C_{15} homologs represented 43%.

The assumption that the standard only consists of C_{12} and C_{14} homologs was made, as no other homologs were visible in the single MS experiment in negative ionization mode. If this assumption is inaccurate, individual AES-C_{12} and AES-C_{14} ethoxymer concentrations in the standard were overestimated, which consequently leads to an underestimation of AES-C_{12} and AES-C_{14} ethoxymer concentrations in environmental samples.

One should also consider that the total concentration of AES may be heavily influenced by the amount of alkyl sulfates in a sample, which are also manufactured and used separately from AES and can be account for up to 50% of a technical AES mixture (Lara-Martín et al. 2008; McAvoy et al. 1998).

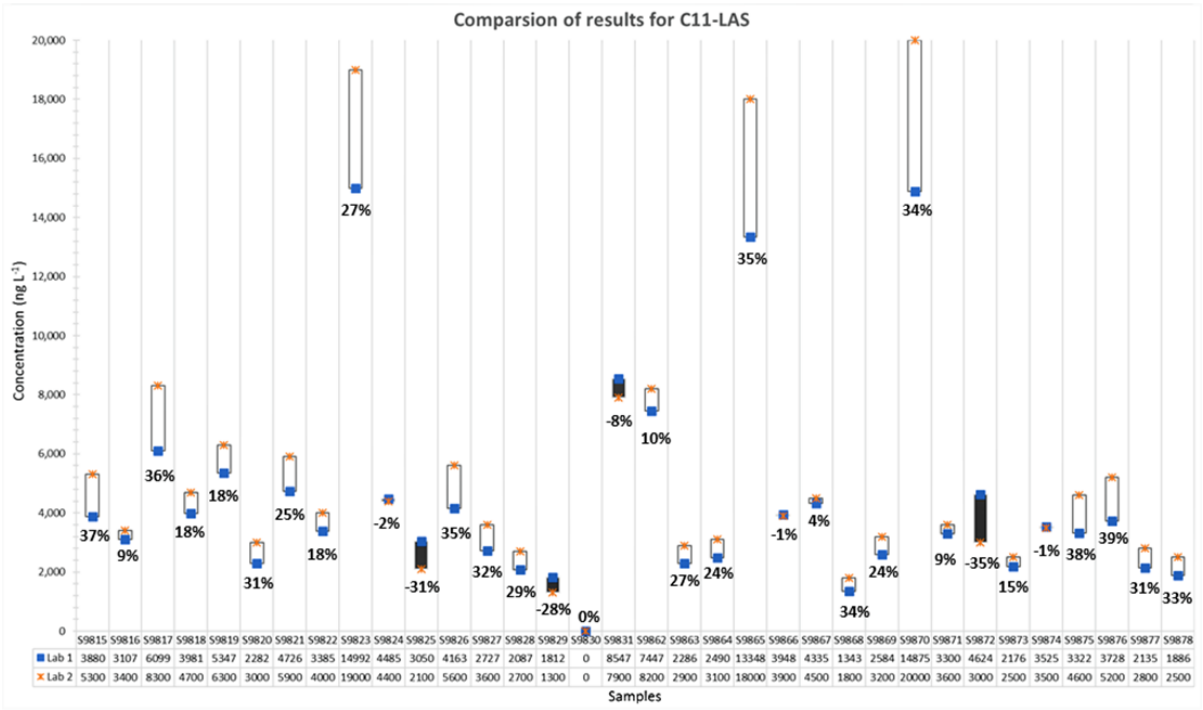
No correlation between total LAS and AES effluent concentrations was found for the WWTPs monitored in the present study. This is in accordance with the findings of McDonough et al. (2016), where no correlation between total LAS and total AES concentrations was observed in 44 WWTP effluents in the U.S. (K. McDonough, personal communication). There are various possible reasons for this lack of a correlation: LAS and AES (and each of their homologs/ethoxymers) are used in different product categories and not necessarily in the same formulations, leading to heterogeneous compositions of surfactants in WWTP influents. Moreover, they are removed at different rates and by different mechanisms (adsorption/biodegradation) in the sewer and during wastewater treatment, resulting in varying total effluent concentrations. However, our results show that the LAS and AES effluent concentrations of the monitored WWTPs are relatively similar to each other.

Total AES concentrations of four influent samples (WWTPs: Geldern; Eutin; Landsberg; Stuttgart) ranged between 400 µg/L and 1,000 µg/L (Annex Table 5). This translates to removal rates >99.8% for AES, which is comparable to the rates found for LAS (>99.2%). Therefore, the data obtained in the present study demonstrates a very high removal of LAS and AES during conventional wastewater treatment. As previously mentioned, LAS were developed as a readily biodegradable substitute for the much more persistent ABS. Hence, our results confirm the feasibility of the “benign by design” concept, which describes the intentional design of alternative chemicals to be more rapidly and completely mineralized during wastewater treatment or in the environment, without losing the desired properties of the compounds they substitute (Kümmerer et al. 2018).

4.3.3 Comparison of the LAS and AES results between the two chemical laboratories

A 'mini collaborative trial' between the two chemical laboratories involved in the study indicated good agreement of the results considering the variability of the subsample, the variability introduced by the different sample preparation techniques applied and the different instrumental facilities. In all cases, concentration levels were at the same order of magnitude. A very good agreement was achieved for the LAS surfactants (deviation below 39% e.g. for LAS-C_{11}; Figure 10). Concentration levels of detected AES surfactants were in the low-ng/L range (close to the LODs of both methods), which could explain higher deviation between the results. The results of both laboratories can be visualized in an interactive map (www.norman-data.eu/EWW_GERMANY).

Figure 10: Comparison of results for LAS-C_{11} between the two laboratories analyzing the same samples.



(source: Laboratory of Analytical Chemistry, Athens)

4.3.4 Suspect screening of other LAS homologs in monitored WWTP effluents

Semi-quantified results for LAS-C_9, LAS-C_{14}, LAS-C_{15} and LAS-C_{16} can be found in Table 11. The decreasing concentrations in all investigated samples were in the order as follows: LAS-C_{10}>LAS-C_{11}>LAS-C_{12}>LAS-C_9>LAS-C_{13}>LAS-C_{14}>LAS-C_{15}. Concentrations of LAS-C_{14}-C_{16} compounds were negligible when comparing with the other LAS compounds. The highest level of LAS-C_9 was determined at 880 ng/L (Table 11).

Table 11: **Semi-quantified LAS compounds. LAS-C_9 was semi-quantified based on the calibration curve of LAS-C_8 and LAS-C_{14}, LAS-C_{15} and LAS-C_{16} were semi-quantified based on LAS-C_{13} calibration curve. Concentrations are in ng/L. N.D.: not detected.**

WWTP	LAS-C_9	LAS-C_{14}	LAS-C_{15}	LAS-C_{16}	WWTP	LAS-C_9	LAS-C_{14}	LAS-C_{15}	LAS-C_{16}
1	770	110	92	N.D.	18	N.D.	33	28	N.D.
2	N.D.	67	58	N.D.	19	420	N.D.	N.D.	N.D.
3	550	72	61	N.D.	20	N.D.	N.D.	N.D.	N.D.
4	300	39	35	N.D.	21	370	85	74	N.D.
5	880	76	60	N.D.	22	200	37	34	N.D.
6	370	55	51	N.D.	23	200	37	30	N.D.
7	640	66	64	N.D.	24	220	41	31	N.D.
8	N.D.	N.D.	N.D.	N.D.	25	210	40	35	N.D.
9	100	35	29	N.D.	26	290	39	36	N.D.
10	160	37	34	N.D.	27	190	43	45	N.D.
11	170	40	35	N.D.	28	280	64	53	N.D.
12	N.D.	N.D.	24	N.D.	29	320	48	44	N.D.
13	730	81	64	N.D.	30	N.D.	28	23	N.D.
14	470	68	57	N.D.	31	180	47	44	N.D.
15	480	130	72	N.D.	32	100	31	30	N.D.
16	130	27	23	N.D.	33	260	52	36	N.D.
17	240	38	33	N.D.					

4.3.5 Suspect screening of DATS and of TPs of LAS and DATS in monitored WWTP effluents

Interesting findings were revealed for TPs of LAS (Table 12 for SPAC and SPADC), for the LAS-byproduct DATS (Table 13) and the TPs of DATS (Table 14 for STAC and STADC). STAC, SPAC and DAT were determined at high concentration levels, whereas STADC and SPADC remained undetected. The highest total concentration was observed for DATS (19 µg/) followed by SPACs (17 µg/L) and STACs (5.3 µg/L). The sum of the concentrations of LAS-related byproducts and TPs surpassed the concentration of LAS in most of the cases. In all cases, both the lower and higher mass homologues remained undetected, while medium mass homologues were detected at high concentration levels. For example, SPA-3C, SPA-4C, SPA-5C and SPA-16C, SPA-17C remained undetected and maximum concentration levels were observed for medium mass homologues (SPA-10C, SPA-11C, SPA-12C and SPA-13C for SPAC, STA-6C and STA-7C for STAC, and DAT-C12 for DATS). The non-detection of high mass homologs (e.g. SPAC with 16 and 17 carbon atoms) is not of surprise given the fact that technical blends barely contain any homologs with more than 13 carbons in the alkyl chain.

DATS was detected at overall higher concentrations (mean: 10 µg/L) than the metabolites STACs (mean: 2,5 µg/L) and SPACs (mean: 5,7 µg/L). Gago-Ferrero et al. (2015) analyzed raw wastewater in Greece using LC-QTOF-MS and also detected DATS in higher number and higher intensity than STACs and STADCs.

Table 12: Occurrence of metabolites of LAS (SPAC and SPADC) in wastewater effluent samples. Semi-quantification was based on calibration curve of LAS-C_{10}. Concentrations are in ng/L. N.D.: not detected.

WWTP	SPA-3C	SPA-4C	SPA-5C	SPA-6C	SPA-7C	SPA-8C	SPA-9C	SPA-10C	SPA-11C	SPA-12C	SPA-13C	SPA-14C	SPA-15C	SPA-16C	SPA-17C	SPA-0-18DC	Total SPAC
1	N.D.	N.D.	N.D.	26	79	240	720	1,700	1,900	2,400	1,400	630	110	N.D.	N.D.	N.D.	9,205
2	N.D.	N.D.	N.D.	N.D.	18	78	190	410	690	770	510	200	55	N.D.	N.D.	N.D.	2,921
3	N.D.	N.D.	N.D.	N.D.	31	96	320	880	1400	2,000	1,400	690	130	N.D.	N.D.	N.D.	6,947
4	N.D.	N.D.	N.D.	11	46	140	470	930	970	1,100	730	390	95	N.D.	N.D.	N.D.	4,882
5	N.D.	N.D.	N.D.	21	58	170	620	1,200	1100	1,500	970	480	97	N.D.	N.D.	N.D.	6,216
6	N.D.	N.D.	N.D.	N.D.	60	240	670	1,100	1,500	2,000	1,300	730	200	N.D.	N.D.	N.D.	7,800
7	N.D.	N.D.	N.D.	20	87	310	1,300	3,300	3,600	4,100	2,000	830	130	N.D.	N.D.	N.D.	15,677
8	N.D.	N.D.	N.D.	N.D.	9.2	36	40	100	200	260	180	110	N.D.	N.D.	N.D.	N.D.	935
9	N.D.	N.D.	N.D.	N.D.	35	120	230	550	790	910	610	320	75	N.D.	N.D.	N.D.	3,640
10	N.D.	N.D.	N.D.	N.D.	26	110	250	750	1,100	1,200	800	380	85	N.D.	N.D.	N.D.	4,701
11	N.D.	N.D.	N.D.	12	39	130	560	900	860	930	570	270	58	N.D.	N.D.	N.D.	4,329
12	N.D.	N.D.	N.D.	N.D.	21	68	160	350	490	600	460	170	55	N.D.	N.D.	N.D.	2,374
13	N.D.	N.D.	N.D.	21	67	210	630	1,500	1,500	1,800	1,100	480	74	N.D.	N.D.	N.D.	7,382
14	N.D.	N.D.	N.D.	33	43	170	510	1,300	1,500	1,900	1,300	600	91	N.D.	N.D.	N.D.	7,447
15	N.D.	N.D.	N.D.	N.D.	64	190	840	2,700	2800	3,600	1,900	960	190	N.D.	N.D.	N.D.	13,244
16	N.D.	N.D.	N.D.	N.D.	N.D.	64	150	390	500	610	460	220	75	N.D.	N.D.	N.D.	2,469
17	N.D.	N.D.	N.D.	N.D.	52	280	740	1,100	1,100	1,100	750	330	86	N.D.	N.D.	N.D.	5,538
18	N.D.	N.D.	N.D.	N.D.	N.D.	46	160	330	510	710	550	250	61	N.D.	N.D.	N.D.	2,617

WWTP	SPA-3C	SPA-4C	SPA-5C	SPA-6C	SPA-7C	SPA-8C	SPA-9C	SPA-10C	SPA-11C	SPA-12C	SPA-13C	SPA-14C	SPA-15C	SPA-16C	SPA-17C	SPA-0-18DC	Total SPAC
19	N.D.	N.D.	N.D.	16	57	270	660	1,300	1,400	1,600	1,300	430	70	N.D.	N.D.	N.D.	7,103
20	N.D.	N.D.	N.D.	N.D.	N.D.	32	700	180	130	94	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1,136
21	N.D.	N.D.	N.D.	N.D.	63	300	1,300	4,300	4,200	4,100	2,000	860	19	N.D.	N.D.	N.D.	17,142
22	N.D.	N.D.	N.D.	N.D.	49	210	540	1,200	1,300	1,300	880	440	110	N.D.	N.D.	N.D.	6,029
23	N.D.	N.D.	N.D.	8.2	32	74	170	500	710	1,000	830	410	80	N.D.	N.D.	N.D.	3,814
24	N.D.	N.D.	N.D.	N.D.	32	120	340	770	980	1,100	750	340	78	N.D.	N.D.	N.D.	4,510
25	N.D.	N.D.	N.D.	6.6	22	76	240	600	810	1,000	730	370	110	N.D.	N.D.	N.D.	3,965
26	N.D.	N.D.	N.D.	N.D.	54	130	320	900	1100	1,400	990	430	110	N.D.	N.D.	N.D.	5,434
27	N.D.	N.D.	N.D.	N.D.	39	130	350	790	1000	1,200	690	300	99	N.D.	N.D.	N.D.	4,598
28	N.D.	N.D.	N.D.	N.D.	31	200	340	980	1300	1,400	920	450	130	N.D.	N.D.	N.D.	5,751
29	N.D.	N.D.	N.D.	N.D.	31	100	280	700	980	1,300	940	460	98	N.D.	N.D.	N.D.	4,889
30	N.D.	N.D.	N.D.	N.D.	18	61	160	410	640	720	410	140	N.D.	N.D.	N.D.	N.D.	2,559
31	N.D.	N.D.	N.D.	18	31	91	390	1,000	1,100	1,300	800	370	73	N.D.	N.D.	N.D.	5,173
32	N.D.	N.D.	N.D.	N.D.	22	69	210	440	620	730	480	260	67	N.D.	N.D.	N.D.	2,898
33	N.D.	N.D.	N.D.	N.D.	32	120	240	500	940	1400	930	460	110	N.D.	N.D.	N.D.	4,732

Table 13: Occurrence of the LAS-byproduct DATS in wastewater effluent samples. Semi-quantification was based on calibration curve of LAS-C_{11}. Concentrations are in ng/L. N.D.: not detected.

WWTP	DATS-C_6	DATS-C_7	DATS-C_8	DATS-C_9	DATS-C_{10}	DATS-C_{11}	DATS-C_{12}	DATS-C_{13}	DATS-C_{14}	DATS-C_{15}	DATS-C_{16}	DATS-C_{17}	DATS-C_{18}	DATS-C_{19}	DATS-C_{20}	DATS-C_{21}	Total DATS
1	N.D.	N.D.	N.D.	240	650	2,700	7,500	5,100	2,100	600	61	N.D.	N.D.	N.D.	N.D.	N.D.	18,951
2	N.D.	N.D.	N.D.	160	350	1,300	3,000	2,200	970	370	71	N.D.	N.D.	N.D.	N.D.	N.D.	8,421
3	N.D.	83	63	190	530	1,800	5,700	3,900	1,500	420	52	N.D.	N.D.	N.D.	N.D.	N.D.	14,238
4	N.D.	N.D.	N.D.	140	360	1,400	4,000	2,800	1,000	280	33	N.D.	N.D.	N.D.	N.D.	N.D.	10,013
5	N.D.	N.D.	N.D.	190	500	2,100	6,000	4,200	1,600	420	42	N.D.	N.D.	N.D.	N.D.	N.D.	15,052
6	N.D.	27	N.D.	220	570	2,200	5,600	3,500	1,300	360	46	N.D.	N.D.	N.D.	N.D.	N.D.	13,823
7	N.D.	N.D.	N.D.	180	450	1,900	5,700	4,000	1,500	420	42	N.D.	N.D.	N.D.	N.D.	N.D.	14,192
8	N.D.	N.D.	N.D.	110	220	800	1,800	1,200	440	140	23	N.D.	N.D.	N.D.	N.D.	N.D.	4,733
9	N.D.	N.D.	N.D.	180	360	1,300	3,200	2,000	740	210	31	N.D.	N.D.	N.D.	N.D.	N.D.	8,021
10	N.D.	N.D.	N.D.	230	470	1,600	3,800	2,500	920	260	32	N.D.	N.D.	N.D.	N.D.	N.D.	9,812
11	N.D.	N.D.	12	130	300	1,200	3,300	2,500	910	270	35	N.D.	N.D.	N.D.	N.D.	N.D.	8,657
12	N.D.	N.D.	N.D.	97	210	670	1,700	1,200	450	120	17	N.D.	N.D.	N.D.	N.D.	N.D.	4,464
13	N.D.	N.D.	N.D.	170	480	1,900	5,100	3,900	1,600	430	39	N.D.	N.D.	N.D.	N.D.	N.D.	13,619
14	N.D.	N.D.	N.D.	210	530	2,000	5,400	3,600	1,400	370	43	N.D.	N.D.	N.D.	N.D.	N.D.	13,553
15	N.D.	N.D.	N.D.	200	450	1,700	4,700	2,900	1,200	350	48	N.D.	N.D.	N.D.	N.D.	N.D.	11,548
16	N.D.	N.D.	N.D.	190	400	1,400	3,500	2,300	720	180	28	N.D.	N.D.	N.D.	N.D.	N.D.	8,718
17	N.D.	N.D.	N.D.	150	360	1,400	3,500	2,400	850	240	26	N.D.	N.D.	N.D.	N.D.	N.D.	8,926
18	N.D.	N.D.	N.D.	120	220	720	2,000	1,400	520	150	22	N.D.	N.D.	N.D.	N.D.	N.D.	5,152

WWTP	DATS-C_6	DATS-C_7	DATS-C_8	DATS-C_9	DATS-C_{10}	DATS-C_{11}	DATS-C_{12}	DATS-C_{13}	DATS-C_{14}	DATS-C_{15}	DATS-C_{16}	DATS-C_{17}	DATS-C_{18}	DATS-C_{19}	DATS-C_{20}	DATS-C_{21}	Total DATS
19	N.D.	N.D.	N.D.	310	730	2,500	5,300	2,900	840	160	21	N.D.	N.D.	N.D.	N.D.	N.D.	12,761
20	N.D.	N.D.	N.D.	41	52	160	310	200	59	21	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	843
21	N.D.	N.D.	N.D.	200	410	1,300	3,500	2,300	890	290	44	N.D.	N.D.	N.D.	N.D.	N.D.	8,934
22	N.D.	N.D.	N.D.	250	460	1,300	3,300	2,000	790	240	34	N.D.	N.D.	N.D.	N.D.	N.D.	8,374
23	N.D.	N.D.	N.D.	260	560	2,200	5,500	3,300	1,100	260	35	N.D.	N.D.	N.D.	N.D.	N.D.	13,215
24	N.D.	N.D.	N.D.	240	510	1,700	4,800	3,100	1,000	280	36	N.D.	N.D.	N.D.	N.D.	N.D.	11,666
25	N.D.	N.D.	N.D.	170	370	1,200	3,100	2,300	870	250	30	N.D.	N.D.	N.D.	N.D.	N.D.	8,290
26	N.D.	N.D.	N.D.	220	560	2,100	4,800	3,100	980	240	30	N.D.	N.D.	N.D.	N.D.	N.D.	12,030
27	N.D.	N.D.	N.D.	220	320	1,100	2,700	1,900	690	210	38	N.D.	N.D.	N.D.	N.D.	N.D.	7,178
28	N.D.	N.D.	N.D.	270	530	1,900	5,100	3,700	1,600	500	74	N.D.	N.D.	N.D.	N.D.	N.D.	13,674
29	N.D.	N.D.	N.D.	270	650	2,300	5,600	3,500	1,200	340	42	N.D.	N.D.	N.D.	N.D.	N.D.	13,902
30	N.D.	N.D.	N.D.	170	290	950	3,200	2,100	750	200	33	N.D.	N.D.	N.D.	N.D.	N.D.	7,693
31	N.D.	N.D.	N.D.	95	230	870	2,400	1,900	810	250	28	N.D.	N.D.	N.D.	N.D.	N.D.	6,583
32	N.D.	N.D.	N.D.	150	320	1,200	2,600	1,800	620	180	22	N.D.	N.D.	N.D.	N.D.	N.D.	6,892
33	N.D.	N.D.	N.D.	280	550	2,000	5,300	3,400	1,300	370	50	N.D.	N.D.	N.D.	N.D.	N.D.	13,250

Table 14: Occurrence of metabolites of DATS (STAC and STADC) in wastewater samples. Semi-quantification was based on calibration curve of LAS-C_{10}. Concentrations are in ng/L. N.D.: not detected.

WWTP	STA-2C	STA-3C	STA-4C	STA-5C	STA-6C	STA-7C	STA-8C	STA-9C	STA-10C	STA-11C	STA-12C	STA-13C	STA-0-15DC	Total STAC
1	63	N.D.	200	720	810	740	360	270	N.D.	N.D.	N.D.	N.D.	N.D.	3,163
2	N.D.	N.D.	130	320	560	550	280	170	N.D.	N.D.	N.D.	N.D.	N.D.	2,010
3	8.6	N.D.	78	290	460	560	360	300	N.D.	N.D.	N.D.	N.D.	N.D.	2,057
4	N.D.	N.D.	140	430	560	560	350	300	N.D.	N.D.	N.D.	N.D.	N.D.	2,340
5	N.D.	N.D.	140	47	530	520	350	300	N.D.	N.D.	N.D.	N.D.	N.D.	1,887
6	N.D.	22	190	670	910	870	460	360	86	N.D.	N.D.	N.D.	N.D.	3,568
7	35	39	280	740	980	1200	720	470	N.D.	N.D.	N.D.	N.D.	N.D.	4,464
8	7.1	N.D.	N.D.	99	200	220	120	91	N.D.	N.D.	N.D.	N.D.	N.D.	737
9	N.D.	N.D.	140	330	550	490	360	220	N.D.	N.D.	N.D.	N.D.	N.D.	2,090
10	N.D.	N.D.	140	420	730	670	330	290	N.D.	N.D.	N.D.	N.D.	N.D.	2,580
11	N.D.	N.D.	150	480	520	480	270	220	N.D.	N.D.	N.D.	N.D.	N.D.	2,120
12	N.D.	N.D.	65	230	370	440	230	150	N.D.	N.D.	N.D.	N.D.	N.D.	1,485
13	16	28	140	420	480	510	350	240	N.D.	N.D.	N.D.	N.D.	N.D.	2,184
14	N.D.	N.D.	120	410	450	550	440	350	N.D.	N.D.	N.D.	N.D.	N.D.	2,320
15	N.D.	55	170	690	860	910	480	390	N.D.	N.D.	N.D.	N.D.	N.D.	3,555
16	N.D.	N.D.	110	280	480	460	260	210	74	N.D.	N.D.	N.D.	N.D.	1,874
17	N.D.	N.D.	240	680	680	480	240	150	N.D.	N.D.	N.D.	N.D.	N.D.	2,470
18	N.D.	9.6	36	160	300	380	270	180	N.D.	N.D.	N.D.	N.D.	N.D.	1,336
19	N.D.	N.D.	230	660	1,100	820	410	250	100	N.D.	N.D.	N.D.	N.D.	3,570

WWTP	STA-2C	STA-3C	STA-4C	STA-5C	STA-6C	STA-7C	STA-8C	STA-9C	STA-10C	STA-11C	STA-12C	STA-13C	STA-0-15DC	Total STAC
20	N.D.	N.D.	67	210	240	140	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	657
21	N.D.	33	250	950	1,300	1,500	820	480	N.D.	N.D.	N.D.	N.D.	N.D.	5,333
22	N.D.	N.D.	250	650	1,000	830	450	240	N.D.	N.D.	N.D.	N.D.	N.D.	3,420
23	N.D.	N.D.	93	300	510	520	320	240	N.D.	N.D.	N.D.	N.D.	N.D.	1,983
24	N.D.	61	140	390	660	660	430	260	80	N.D.	N.D.	N.D.	N.D.	2,681
25	N.D.	N.D.	69	270	490	530	340	230	N.D.	N.D.	N.D.	N.D.	N.D.	1,929
26	N.D.	N.D.	160	470	760	770	450	340	73	N.D.	N.D.	N.D.	N.D.	3,023
27	N.D.	N.D.	150	400	680	820	440	280	73	N.D.	N.D.	N.D.	N.D.	2,843
28	N.D.	N.D.	170	540	890	880	500	340	110	N.D.	N.D.	N.D.	N.D.	3,430
29	N.D.	N.D.	110	350	650	750	470	370	88	N.D.	N.D.	N.D.	N.D.	2,788
30	N.D.	N.D.	130	250	510	510	280	150	N.D.	N.D.	N.D.	N.D.	N.D.	1,830
31	N.D.	N.D.	87	330	490	490	250	180	N.D.	N.D.	N.D.	N.D.	N.D.	1,827
32	N.D.	N.D.	85	260	490	460	270	190	N.D.	N.D.	N.D.	N.D.	N.D.	1,755
33	N.D.	N.D.	110	370	630	700	400	260	N.D.	N.D.	N.D.	N.D.	N.D.	2,470

4.3.6 Suspect screening of AEO surfactants with known fragmentation

Samples were also screened for a total of 290 homologs of AEOs (C_8-C_{18}). Concentration levels of AEOs were semi-quantified based on the calibration curve of tetraethylene glycol monododecyl ether. This substance class also showed remarkable occurrence in the effluent wastewater samples up to 650 ng/L (Annex Table 6). Homologs with medium ethoxy group content generally showed higher frequencies of appearance and concentrations.

4.3.7 Suspect screening of PEG surfactants with known fragmentation

41 PEG compounds with repeating ethoxy groups were screened in positive ionization mode. It is known that PEGs result in other adducts instead of [M+H]+ under electrospray ionization (Gago-Ferrero et al. 2015; Schymanski et al. 2014b). In this study, we discovered that PEG homologs with high molecular mass are ionized as $[M+NH4]^{2+}$ adducts, which resulted in the positive detection of the longest homolog series in effluent wastewater samples so far reported in the literature. Concentration levels of PEGs were semi-quantified based on the calibration curve of PEG-04. Generally, PEGs are efficiently removed during biological wastewater treatment. However, they can also be generated during wastewater treatment when precursor molecules are biologically degraded. For example, low molecular PEGs have been described as the main metabolites of the nonionic surfactants AEOs (Sparham et al. 2008; Traverso-Soto et al. 2013) and nonylphenol ethoxylates (NPOEs) (Castillo et al. 2000b). In an aerobic biodegradation test under OECD 301 test conditions, PEGs biodegraded more slowly than the parent AEOs and were removed by hydrolysis, thus leading to shorter PEG oligomers, and by hydrolysis, thus forming carboxylated PEGs (Marcomini et al. 2000).

Maximum estimated concentration levels occurred in most of the cases for PEG-08 and PEG-09, followed by PEG-10 and PEG-07 (Table 15, Table 16, Table 17). However, estimated concentrations for PEG-4 (maximum concentration: 780 ng/L) are considerably lower than reported in the work of (Castillo et al. 2000b). In their study, 6,400 ng/L and 13,000 ng/L of PEG-4 were detected in two effluent samples of a conventional wastewater treatment plant in Spain.

Table 15: Occurrence of polyethylene glycols (PEG) PEG-04 to PEG-21 in wastewater effluent samples. Semi-quantification was based on calibration curve of PEG-04. Concentrations are in ng/L. N.D.: not detected.

WWTP	PEG-04	PEG-05	PEG-06	PEG-07	PEG-08	PEG-09	PEG-10	PEG-11	PEG-12	PEG-13	PEG-14	PEG-15	PEG-16	PEG-17	PEG-18	PEG-19	PEG-20	PEG-21
1	230	34	160	370	300	220	220	160	92	64	42	28	43	35	51	56	48	37
2	360	N.D.	27	47	95	170	160	74	34	34	21	45	63	65	64	64	57	41
3	89	32	130	400	1,100	1,100	690	270	130	95	65	30	54	41	36	28	23	17
4	200	13	61	53	130	180	150	77	38	35	24	40	83	76	82	92	78	60
5	200	15	44	150	440	600	490	220	100	81	55	61	100	110	100	85	73	51
6	100	23	140	360	380	320	320	200	120	76	45	37	58	56	67	84	77	66
7	200	16	100	240	490	610	480	280	190	180	150	160	200	160	130	110	67	46
8	250	16	78	200	240	290	330	220	130	94	54	120	160	190	210	230	200	160
9	150	13	56	120	130	120	150	140	93	78	44	39	57	59	63	80	80	77
10	600	5.9	63	220	490	630	520	270	140	100	73	91	120	140	150	150	120	93
11	90	20	100	210	200	260	230	110	54	51	34	27	55	50	57	63	67	50
12	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
13	780	44	240	680	770	730	690	360	190	130	89	91	140	170	170	190	170	130
14	190	20	100	250	230	150	170	180	140	130	84	49	49	32	38	52	56	61
15	130	22	97	250	380	430	320	150	87	85	66	58	95	77	77	77	72	53
16	64	9.7	36	120	380	440	300	130	73	58	41	53	84	69	65	42	35	35
17	180	89	610	970	670	450	370	190	78	56	35	35	62	58	77	70	71	57
18	22	N.D.	45	110	250	340	170	82	61	59	37	16	38	19	31	21	21	8.6
19	39	44	280	920	840	540	440	400	260	180	85	92	85	63	72	89	87	90
20	130	34	71	140	250	240	220	120	53	39	20	55	78	70	68	70	55	33
21	160	29	130	390	410	370	380	250	150	100	70	53	78	86	93	110	99	89
22	61	14	97	160	150	170	170	90	42	32	16	24	33	30	38	46	40	30
23	74	14	33	68	290	440	380	210	100	84	60	82	120	110	120	110	90	64
24	340	31	110	210	300	350	270	130	64	56	37	25	51	37	53	42	37	24
25	130	9.6	31	60	200	280	240	120	63	51	34	44	73	67	78	63	60	41

26	170	14	50	150	310	340	220	80	33	32	22	14	23	13	27	29	17	7.1
27	93	22	97	310	610	660	450	210	110	92	65	68	100	90	110	87	73	50
28	280	13	72	160	240	250	180	93	53	48	29	24	34	43	53	60	49	41
29	300	12	29	23	160	300	240	120	58	51	35	43	71	67	54	62	49	31
30	410	8.2	54	150	300	390	350	200	110	69	48	67	70	70	110	100	110	64
31	150	40	190	430	590	550	280	100	67	63	45	51	44	73	38	46	27	33
32	170	14	27	96	370	570	440	240	120	81	54	87	130	130	130	120	100	72
33	420	14	95	440	840	880	780	550	300	190	120	170	220	230	270	260	210	170

Table 16: Occurrence of polyethylene glycols (PEG) PEG-22 to PEG-39 in wastewater effluent samples. Semi-quantification was based on calibration curve of PEG-04. Concentrations are in ng/L. N.D.: not detected.

WWTP	PEG-22	PEG-23	PEG-24	PEG-25	PEG-26	PEG-27	PEG-28	PEG-29	PEG-30	PEG-31	PEG-32	PEG-33	PEG-34	PEG-35	PEG-36	PEG-37	PEG-38	PEG-39
1	42	35	29	21	23	19	21	20	20	17	18	20	15	16	13	12	8.4	5
2	32	33	29	30	24	23	24	27	23	22	22	19	17	16	12	10	8.1	4.7
3	11	9.5	9.1	10	9.9	10	10	12	9.1	7.8	6.6	5.9	3.8	1.9	2.3	1.9	2	1.2
4	40	35	26	27	26	27	24	28	22	22	20	18	16	12	11	9	7	3.5
5	42	42	39	34	38	35	36	37	34	32	32	29	27	23	20	18	14	6.8
6	52	49	43	36	29	28	24	22	21	21	18	18	16	16	12	13	11	6
7	32	34	32	32	31	29	29	31	22	22	20	18	14	13	11	9.7	7.5	3.5
8	120	110	93	78	64	57	51	51	41	39	34	28	25	22	18	15	12	5.9
9	72	73	67	63	51	46	38	42	32	34	28	31	25	24	20	18	13	7.9
10	63	63	60	52	43	41	38	40	33	34	30	28	23	23	18	16	12	6.3
11	38	40	35	32	26	23	23	25	22	22	17	18	14	16	12	9.8	7	3.6
12	N.D.	1.6	N.D.	1.9	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
13	86	86	87	81	68	68	63	67	57	57	51	53	48	46	41	37	32	18
14	59	67	63	62	51	48	41	43	30	35	31	33	27	28	23	22	19	10
15	43	46	46	50	48	51	52	58	48	46	43	43	36	31	29	23	19	10
16	30	33	32	29	29	22	25	24	21	20	19	19	18	14	13	14	10	7
17	45	41	40	38	30	31	27	31	24	27	22	21	18	19	14	16	11	6.3

18	9.3	17	12	15	15	15	17	15	13	12	11	9.8	8.1	8.5	5.9	5.9	5.4	2.4
19	82	80	69	64	50	43	36	36	26	25	21	21	18	12	12	11	7.6	3.9
20	21	21	19	19	15	13	13	15	12	13	6.3	11	9.2	6.8	6.3	4.8	3	1.8
21	77	72	62	53	41	37	33	36	28	26	26	26	22	22	18	15	13	6.5
22	26	23	17	15	15	12	13	13	13	11	11	9.8	9.3	7.5	5.9	5.1	4.1	2.3
23	50	40	39	43	36	34	37	36	32	34	32	27	26	24	20	17	14	7.4
24	18	17	15	15	15	15	15	18	16	14	15	16	12	11	9.6	9.8	7.5	3.1
25	22	26	24	24	23	22	24	27	21	23	22	22	20	18	16	14	12	6.3
26	9.9	8	8.9	8.8	12	13	16	17	16	16	18	19	18	17	15	14	11	4.4
27	44	41	40	39	34	37	33	40	31	29	26	26	20	21	17	16	13	6.5
28	32	30	25	23	17	14	16	N.D.	12	7.1	12	8.4	9.7	8.7	6.9	5.3	4.9	2.8
29	15	23	22	20	21	19	21	23	18	15	17	17	14	11	10	9.6	7.5	3.7
30	49	40	34	36	30	27	29	30	26	24	23	18	16	17	13	11	10	4.9
31	23	19	26	27	25	11	21	26	16	14	11	12	7.7	3.5	5.5	4.5	2.8	1.7
32	49	45	39	39	35	31	32	33	27	28	23	20	16	16	12	9.1	8.1	4.6
33	130	120	100	92	83	77	75	80	59	62	55	52	45	42	33	30	25	13

Table 17: Occurrence of polyethylene glycols (PEG) PEG-40 to PEG-44 in wastewater effluent samples. Semi-quantification was based on calibration curve of PEG-04. Concentrations are in ng/L. N.D.: not detected.

WWTP	PEG-40	PEG-41	PEG-42	PEG-43	PEG-44	Total PEGs
1	3.9	3.1	2	1.5	0.9	2,556
2	3.8	3.2	2.1	1.4	1.2	1,809
3	1	N.D.	N.D.	N.D.	N.D.	4,455
4	2.7	2.4	1.5	N.D.	N.D.	1,852
5	5.2	4.3	3	1.6	1.2	3,529
6	4.7	3.8	2.5	1.3	1.1	2,977
7	2.8	2.3	1.8	0.9	N.D.	4,208
8	4.1	3.3	2.6	1.3	0.9	4,048
9	5.7	4.3	3.2	1.7	1.3	2,250

10	4.6	3.9	3	1.7	1.3	4,614
11	3.2	2.2	1.7	0.8	0.8	2,120
12	N.D.	N.D.	N.D.	N.D.	N.D.	3.5
13	14	12	10	5.6	4.6	6,856
14	8.2	6.5	5	2.2	1.6	2,697
15	7.1	5.6	4.4	2	1.6	3,269
16	4.8	4.1	3.1	1.7	1.3	2,429
17	5.7	4.6	4.2	2	1.6	4,607
18	2	1.5	0.9	0.4	N.D.	1,533
19	2.6	1.9	1.4	0.5	0.5	5,230
20	1.5	N.D.	N.D.	N.D.	N.D.	1,958
21	5	3.5	2.8	1.2	1	3,674
22	1.8	1.5	N.D.	N.D.	0.4	1,460
23	5.8	4.5	3.4	1.8	0.9	3,014
24	2.3	1.5	1.2	N.D.	N.D.	2,414
25	4.6	4.1	2.8	1.5	1.1	2,025
26	3.7	3.3	2.3	0.9	N.D.	1,803
27	5.2	3.7	2.9	1	0.9	3,824
28	1.9	1.8	N.D.	N.D.	N.D.	1,961
29	3	2.4	1.7	0.8	0.7	2,000
30	3.6	3.2	2.4	1.3	1	3,130
31	1.4	N.D.	N.D.	N.D.	N.D.	3,075
32	3.1	2.1	1.7	0.8	0.7	3,426
33	10	8.2	5.1	2.9	2.1	7,360

4.3.8 Suspect screening of other surfactants with known fragmentation

A number of other surfactants have previously been reported in the literature but could not be semi-quantified here due to lack of standards with similar structure (Table 18). However, their fragmentation pattern was known and thus they were identified at the level of 'possible structure by library spectrum match' (Level 2A; (Schymanski et al. 2014a)). High frequency of appearances (FoA) were observed for SAS- C_{12} and SAS-C_{14}, which were detected in all wastewater samples, C11-SAS was detected with a FoA of 91%, while C13-SAS was detected with a FoA of 73%. SAS-C_{10} and SAS-C_{16} were detected in only two wastewater effluent samples, while the rest of SAS surfactants remained undetected. The highest signal was observed for SAS-C_{12}.

Other surfactants with widespread occurrence were NP1ethoxycarboxylate, naphthalene-1-sulfonate and dihexyl sulfosuccinate (DHSS), which were detected with very high FoA (100% for NP1ethoxycarboxylate and naphthalene-1-sulfonate, 97% for DHSS). On the contrary, the Water Framework Directive (WFD) priority substance 4-nonylphenol was scarcely detected (FoA 12%). However, its homolog compound NPEO1 was detected in almost all wastewater samples (FoA of 97%). NPEO3 was detected only in a few samples (FoA of 18%), while NPEO4 was found in more than half of the samples (FoA of 58%). The rest of NPEO compounds (NPEO5–NPEO17) remained undetected.

4-Octylphenol was frequently (FoA of 85%) detected in the wastewater effluent samples. Its homolog substances OP1 ethoxy carboxylate and OP2 ethoxy carboxylate were also detected in 73% and 18% of the samples, respectively. GES surfactants were detected less frequently. A maximum FoA of 55% was observed for GES9, followed by GES11 (FoA of 39 %) and GES10 (FoA of 36 %).

Table 18: Occurrence profile of surfactants included in the two suspect surfactant lists (EAWAGSURF, ATHENSSUS DID), which were screened for their presence in the 33 wastewater effluents samples using DSFP (0: non-detect; 1: positive detection).

	blank	WWTP 1	WWTP 2	WWTP 3	WWTP 4	WWTP 5	WWTP 6	WWTP 7	WWTP 8	WWTP 9	WWTP 10	WWTP 11	WWTP 12	WWTP 13	WWTP 14	WWTP 15	WWTP 16	WWTP 17	WWTP 18	WWTP 19	WWTP 20	WWTP 22	WWTP 22	WWTP 23	WWTP 24	WWTP 25	WWTP 26	WWTP 27	WWTP 28	WWTP 29	WWTP 30	WWTP 31	WWTP 32	WWTP 33
SAS-C_{12}	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SAS-C_{14}	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
NP1 ethoxy carboxylate	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Naphthalene-1-sulfonate	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
NPEO1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
Dihexyl sulfosuccinate (DHSS)	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
SAS-C_{11}	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	0	1
4-octylphenol (OP)	0	0	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1
SAS-C_{13}	0	1	1	0	1	0	0	0	1	1	0	1	1	0	1	0	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
OP1 ethoxy carboxylate	0	1	1	0	1	1	0	0	1	1	1	0	1	0	1	0	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1
NPEO3	0	0	1	1	1	1	1	1	0	1	1	1	0	0	0	1	0	1	0	1	0	0	0	1	1	1	1	0	1	0	1	1	0
GES9	0	1	1	0	1	1	0	0	0	0	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	1	1	0	0	0	1	1	1
GES11	0	1	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	1	0	0	0	0	1	0	1
GES10	0	1	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0	0	1	0	1	0	0	0	0	1	1	1
GES7	0	1	0	0	0	0	1	0	0	0	1	1	0	1	0	0	0	1	0	1	0	1	0	0	0	0	1	1	0	0	0	0	1	0
GES8	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	1	0	0	0	0	1	1	0	0	0	1	1	1
GES12	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1
NPEO2	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0
OP2 ethoxy carboxylate	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0
Benzenesulfonate	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
GES13	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1
4-nonylphenol (NP)	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
SAS-C_{10}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
SAS-C_{15}	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
GES14	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0
NPEO1-SO_4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
NPEO2-SO_4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
NPEO3-SO_4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
NPEO4-SO_4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
NPEO5-SO_4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
NPEO6-SO_4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
GES15	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

4.3.9 Suspect screening of surfactants from the DID list using in silico predicted fragmentation

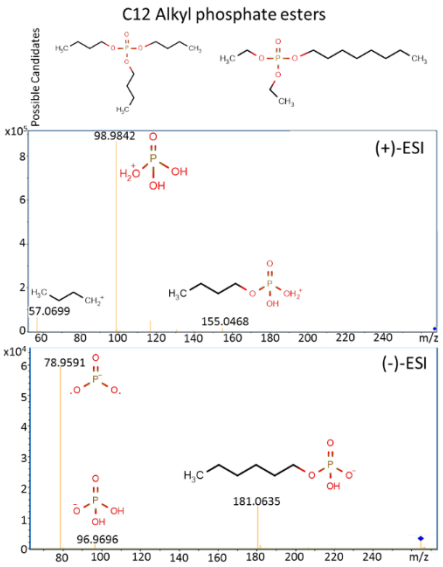
In silico predicted fragmentation patterns (Allen et al. 2015) were generated for the candidate suspect compounds of the DID list (European Commission 2016). The list and the samples were uploaded to DSFP and 33 wastewater effluent samples were screened for these substances. Substances with a match of more than three in silico predicted fragments were prioritized and then further investigated. The investigation involved the acquisition of HRMS/MS spectra and structural explanation of the spectra (procedure termed as 'annotation'). Candidates that could adequately explain the HRMS/MS spectra were summarized in Table 19. The number in a respective cell indicates the number of fragments explained. Structures of tentatively identified compounds can be found in Table 3. Table 20 shows the normalized intensities of signals of the identified compounds in the samples. All these compounds were investigated in-depth, following the NTS identification workflow (Gago-Ferrero et al. 2015), and were tentatively identified (Level 3, (Schymanski et al. 2014a). The presence of 1H-benzotriazole, propafenone and benzoic acid in the samples could be successfully confirmed with authentic standards (Level 1). Benzoic acid and benzotriazole were detected in all samples, while propafenone was detected with FoA 48%. Other compounds with widespread occurrence were mono-C_{12} alkyl sulfosuccinate and lauroyl sarcosinate (FoA 100%), di-2-ethylhexyl sulfosuccinate and C_8-alkyl sulfate (linear) (FoA 91%), "amines, tallow, 1+2 EO (R=CH_3)" and "amines, tallow, 5+5 EO (R=H)" (88%), cumene sulfonate (FoA 85%), panthenol (FoA 67%) and methylparaben (55%). Compounds detected in less than half of the samples were C_{10} alcohol, predominately linear, 2 EO (FoA 48%), succinic acid (36%) and glycerides, C_{15} mono (27%). Finally, sulfate related surfactants were detected only scarcely (C_{16}-alkyl 4 ethyl sulfate with FoA 18%, C_9-alkyl 2 ethyl sulfate with FoA 15% and C_8-alkyl 2 ethyl sulfate with FoA 6%).

Table 19: **Occurrence profile of surfactants and additives included in the DID suspect surfactants list which were detected in the studied wastewater effluent samples. The number in a respective cell indicates the number of fragments detected in the mass spectrum of each substance. The higher the number of detected fragments, the higher is the confidence in identification of the substance.**

	blank	WWTP 1	WWTP 2	WWTP 3	WWTP 4	WWTP 5	WWTP 6	WWTP 7	WWTP 8	WWTP 9	WWTP 10	WWTP 11	WWTP 12	WWTP 13	WWTP 14	WWTP 15	WWTP 16	WWTP 17	WWTP 18	WWTP 19	WWTP 20	WWTP 21	WWTP 22	WWTP 23	WWTP 24	WWTP 25	WWTP 26	WWTP 27	WWTP 28	WWTP 29	WWTP 30	WWTP 31	WWTP 32	WWTP 33
Amines, tallow, 1+2 EO (R=CH_3)	0	6	6	6	6	6	6	6	6	6	6	6	0	6	6	6	0	6	6	6	0	6	6	6	6	6	0	6	6	6	6	6	6	6
Lauroyl Sarcosinate	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Benzotriazole	0	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
C_{12} Alkyl phosphate esters	0	5	5	5	5	5	5	5	5	5	0	5	0	0	5	5	5	5	5	0	5	5	0	5	5	5	0	5	0	5	0	5	5	5
Di-2-ethylhexyl sulfosuccinate	0	4	4	4	4	4	4	4	4	4	4	0	4	4	4	4	4	4	4	4	0	0	4	4	4	4	4	4	4	4	4	4	4	4
C8-Alkyl Sulfate	0	4	0	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0	0	4	4	4	4
Amines, tallow, 5+5 EO (R=H)	0	4	4	4	4	4	4	4	0	4	4	4	0	4	4	4	4	4	0	4	4	4	4	4	4	4	0	0	4	4	4	4	4	4
Mono-C_{12} Alkyl sulfosuccinate	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Benzoic acid	0	0	3	3	3	3	3	3	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Cumene sulfonate	0	3	3	3	3	3	0	3	0	3	0	3	0	3	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	3	3	3
C_{10} Alcohol, predom. linear, 2 EO	0	5	5	5	0	0	0	0	0	0	0	0	0	0	5	5	0	5	5	0	0	0	5	5	5	5	0	5	5	0	0	5	5	0
Propafenone	0	5	0	0	5	5	0	0	0	5	5	5	5	5	5	0	0	0	5	0	5	0	0	0	0	5	0	0	5	5	0	0	5	5
Glycerides, C_{15} mono	0	8	0	0	0	0	8	0	8	8	0	0	0	8	8	0	0	8	0	8	0	8	0	0	0	0	0	0	8	0	0	0	0	0
Panthenol	0	0	0	0	3	0	3	0	0	3	3	3	0	3	3	3	3	3	3	0	3	3	3	3	3	3	3	3	0	3	0	3	3	0
Methylparaben	0	3	0	3	3	0	0	0	0	0	0	0	0	0	3	0	3	3	3	0	0	3	3	3	3	3	3	0	3	3	0	3	3	0
Succinic acid	0	0	0	0	0	0	3	0	0	3	3	3	0	0	3	3	3	0	0	0	0	0	0	3	3	0	0	0	0	3	0	0	3	0
C_{14}-Alkyl dimethyl amine oxide	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	7	0	0	7	0
C_{12} Alcohol, predomi. linear, 3 EO	0	5	0	0	0	0	0	0	0	0	0	0	0	0	5	5	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0
C_{16}-Alkyl 4 ethyl sulfate	0	0	0	3	0	0	0	3	0	3	0	0	0	0	3	0	0	0	0	0	0	0	3	0	0	0	3	0	0	0	0	0	0	0
C_9-Alkyl 2 ethyl sulfate	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	0	0	0	0	3	0	0	0	0	3	0	0
C_8-Alkyl 2 ethyl sulfate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	3	0	0	0	0	0	0	0	0
C_8 Sorbitan diester	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diethylene glycol monoethyl ether	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0

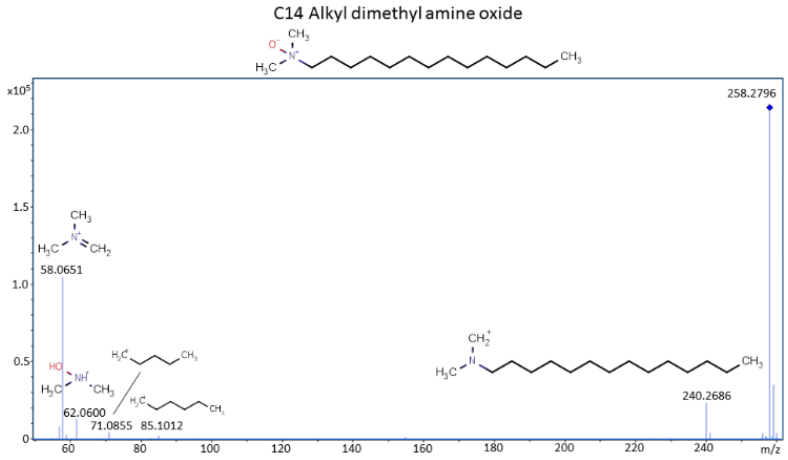
Two examples of such in-depth investigations to tentatively identify compounds are shown in Figure 11 (C_{12} alkyl phosphate ester) and Figure 12 (C_{14} alkyl dimethyl amine oxide). C_{12} alkyl phosphate ester was detected in both ionization modes and gave fragments of diagnostic evidence (e.g. 78.9591 and 96.9690 for negative and 98.9842 for positive ionization, respectively). C_{14} alkyl dimethyl amine oxide structure did not result in fragments of diagnostic evidence because of the structure of the compound. However, the obtained spectrum was clearly explainable and all fragments could be annotated with respective structural fragments. Table 19 gives the number of fragments explained for each substance and sample. All these compounds were investigated in-depth, following the NTS identification workflow (Gago-Ferrero et al. 2015), and were tentatively identified (Level 3, (Schymanski et al. 2014a)). Among the exceptions were benzotriazole, propafenone and benzoic acid, whose presence in samples was successfully confirmed with authentic standards (Level 1). Benzoic acid and benzotriazole were detected in all samples (FoA of 100%), while propafenone was detected with a FoA of 48%. Compounds with widespread occurrence were mono-C_{12} alkyl sulfosuccinate and lauroyl sarcosinate (FoA 100%), di-2-ethylhexyl sulfosuccinate and C_8-alkyl sulfate (linear) (FoA of 91%), "amines, tallow, 1+2 EO (R=CH_3)" and "amines, tallow, 5+5 EO (R=H)" (88%), cumene sulfonate (FoA of 85%), C_{12} alkyl phosphate esters (FoA of 7 %), panthenol (FoA of 67%) and methylparaben (55%). Compounds detected in less than half of the samples were C_{10} alcohol, predominately linear, 2 EO (48%), succinic acid (36%) and glycerides, C_{15} mono (27%). Finally, sulfates were detected only scarcely (C_{16}-alkyl 4 ethyl sulfate with a FoA of 18%, C_9-alkyl 2 ethyl sulfate with a FoA of 15% and C_8-alkyl 2 ethyl sulfate with a FoA of 6%).

Figure 11: Tentative identification of C_{12} alkyl phosphate esters (level 3; ramification possible). Annotated fragment structures for positive and negative electrospray ionization (ESI) indicate that compound contains PO_4 group and a carbon chain.



(source: Laboratory of Analytical Chemistry, Athens)

Figure 12: HRMS/MS spectra of tentatively identified C_{14} alkyl dimethyl amine oxide (level 3; ramification possible) and annotated fragments.



(source: Laboratory of Analytical Chemistry, Athens)

Table 20: Surfactants and additives included in the DID suspect list detected in the WWTP effluent samples and their peak intensities. N.D.: not detected.

WWTP	Amines, tallow, 1+2 EO (R=CH_3)	Lauroyl Sarcosinate	Benzotriazole	C_{12} Alkyl phosphate esters	Di-2-ethylhexyl sulfo-succinate	C_8-Alkyl Sulfate (linear)	Amines, tallow, 5+5 EO (R=H)	Mono-C_{12} Alkyl sulfo-succinate	Benzoic acid	Cumene sulfonate	C_{10} Alcohol, predom. linear, 2 EO
WWTP 1	65,000	7,800	43,000	290,000	150,000	16,000	94,000	58,000	N.D.	120,000	61,000
WWTP 2	19,000	1,400	470,000	64,000	25,000	N.D.	20,000	10,000	31,000	4,500	22,000
WWTP 3	28,000	68,000	520,000	190,000	49,000	31,000	200,000	61,000	35,000	5,700	150,000
WWTP 4	37,000	210,000	930,000	140,000	46,000	6,800	5,900	41,000	45,000	15,000	N.D.
WWTP 5	23,000	13,000	290,000	61,000	20,000	8,500	41,000	42,000	69,000	77,000	N.D.
WWTP 6	50,000	82,000	590,000	74,000	11,000	7,000	33,000	26,000	35,000	N.D.	N.D.
WWTP 7	37,000	710,000	800,000	55,000	75,000	44,000	39,000	37,000	33,000	32,000	N.D.
WWTP 9	31,000	210,000	590,000	63,000	8,900	2,500	75,000	20,000	28,000	5,000	N.D.
WWTP 10	22,000	53,000	780,000	N.D.	11,000	2,800	98,000	100,000	29,000	N.D.	N.D.
WWTP 11	44,000	230,000	480,000	81,000	N.D.	49,000	34,000	20,000	58,000	25,000	N.D.
WWTP 12	N.D.	17,000	370,000	N.D.	5,400	1,900	N.D.	15,000	35,000	N.D.	N.D.
WWTP 13	20,000	130,000	570,000	N.D.	90,000	88,000	74,000	31,000	42,000	12,000	N.D.
WWTP 14	26,000	38,000	380,000	100,000	18,000	12,000	51,000	80,000	42,000	N.D.	40,000
WWTP 15	34,000	13,000	350,000	49,000	31,000	46,000	150,000	56,000	41,000	25,000	37,000
WWTP 16	N.D.	260,000	1,000,000	520,000	64,000	4,600	12,000	75,000	32,000	3,100	N.D.
WWTP 17	20,000	95,000	880,000	960,000	35,000	5,200	54,000	14,000	52,000	45,000	42,000
WWTP 18	33,000	34,000	75,000	150,000	66,000	11,000	N.D.	21,000	26,000	4,500	190,000

WWTP	Amines, tallow, 1+2 EO (R=CH_3)	Lauroyl Sarcosinate	Benzotriazole	C_{12} Alkyl phosphate esters	Di-2-ethylhexyl sulfo-succinate	C_8-Alkyl Sulfate (linear)	Amines, tallow, 5+5 EO (R=H)	Mono-C_{12} Alkyl sulfo-succinate	Benzoic acid	Cumene sulfonate	C_{10} Alcohol, predom. linear, 2 EO
WWTP 19	51,000	1,400	160,000	N.D.	N.D.	3,100	9,200	15,000	25,000	17,000	N.D.
WWTP 20	N.D.	9,700	200,000	100,000	N.D.	990	1,500	2,000	15,000	6,000	N.D.
WWTP 21	55,000	96,000	960,000	60,000	37,000	36,000	160,000	31,000	50,000	31,000	N.D.
WWTP 22	25,000	6,300	570,000	N.D.	24,000	11,000	46,000	12,000	26,000	9,000	34,000
WWTP 23	7,300	270,000	290,000	55,000	29,000	110,000	28,000	40,000	36,000	11,000	81,000
WWTP 24	76,000	360,000	230,000	91,000	67,000	11,000	28,000	25,000	65,000	15,000	59,000
WWTP 25	19,000	33,000	650,000	94,000	46,000	3,600	24,000	23,000	37,000	11,000	43,000
WWTP 26	N.D.	12,000	380,000	N.D.	200,000	80,000	N.D.	46,000	49,000	15,000	N.D.
WWTP 27	24,000	39,000	450,000	160,000	16,000	6,300	N.D.	22,000	28,000	12,000	25,000
WWTP 28	26,000	120,000	900,000	N.D.	81,000	N.D.	41,000	44,000	46,000	8,800	28,000
WWTP 29	43,000	110,000	450,000	78,000	38,000	N.D.	50,000	56,000	110,000	8,300	N.D.
WWTP 30	25,000	130,000	370,000	N.D.	16,000	4,100	35,000	13,000	26,000	N.D.	N.D.
WWTP 31	21,000	860,000	960,000	36,000	73,000	200,000	140,000	18,000	71,000	37,000	22,000
WWTP 32	30,000	6,600	480,000	73,000	63,000	5,600	40,000	9,400	42,000	3,800	24,000
WWTP 33	24,000	11,000	410,000	90,000	29,000	4,200	6,500	19,000	24,000	4,600	N.D.

WWTP	Propafenone	Glycerides, C_{15} mono	Panthenol	Methylparaben	Succinic acid	C_{14} Alkyl dimethyl amine oxide	C_{12} Alcohol, predominately linear, 3 EO	C_{16}-Alkyl 4 ethyl sulfate	C_9-Alkyl 2 ethyl sulfate	C_8-Alkyl 2 ethyl sulfate	C_8 Sorbitan diester
WWTP 1	12,000	340,000	N.D.	5,800	N.D.	N.D.	21,000	N.D.	N.D.	N.D.	N.D.
WWTP 2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 3	N.D.	N.D.	N.D.	4,900	N.D.	N.D.	N.D.	1,300	N.D.	N.D.	N.D.
WWTP 4	240,000	N.D.	460	5,200	N.D.	N.D.	N.D.	N.D.	1,200	N.D.	N.D.
WWTP 5	18,000	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 6	N.D.	130,000	390	N.D.	910	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 7	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1,900	N.D.	N.D.	N.D.
WWTP 9	25,000	250,000	560	N.D.	2,900	N.D.	N.D.	1,500	N.D.	N.D.	N.D.
WWTP 10	56,000	N.D.	1100	N.D.	1,400	170,000	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 11	26,000	N.D.	1500	N.D.	1,500	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 12	830	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 13	29,000	100,000	710	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 14	21,000	320,000	670	5,500	1,000	N.D.	16,000	1,400	N.D.	N.D.	N.D.
WWTP 15	N.D.	N.D.	1100	N.D.	1,800	N.D.	11,000	N.D.	N.D.	N.D.	N.D.
WWTP 16	N.D.	N.D.	580	3,500	1,600	N.D.	N.D.	N.D.	N.D.	N.D.	3,600
WWTP 17	N.D.	92,000	15000	11,000	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 18	11,000	N.D.	610	2,500	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	93,000
WWTP 19	N.D.	250,000	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2,400	N.D.	N.D.

WWTP	Propafenone	Glycerides, C_{15} mono	Panthenol	Methylparaben	Succinic acid	C_{14} Alkyl dimethyl amine oxide	C_{12} Alcohol, predominately linear, 3 EO	C_{16}-Alkyl 4 ethyl sulfate	C_9-Alkyl 2 ethyl sulfate	C_8-Alkyl 2 ethyl sulfate	C_8 Sorbitan diester
WWTP 20	6,000	N.D.	280	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 21	N.D.	240,000	720	6,000	N.D.	N.D.	140,000	N.D.	1,800	3,000	N.D.
WWTP 22	N.D.	N.D.	440	4,400	N.D.	95,000	N.D.	1,300	N.D.	N.D.	N.D.
WWTP 23	N.D.	N.D.	670	3,400	1,400	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 24	N.D.	N.D.	460	4,700	1,400	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 25	13,000	N.D.	360	4,000	N.D.	N.D.	N.D.	N.D.	N.D.	2,400	N.D.
WWTP 26	N.D.	N.D.	360	4,800	N.D.	N.D.	N.D.	1,000	2,600	N.D.	N.D.
WWTP 27	N.D.	N.D.	560	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 28	25,000	120,000	N.D.	6,600	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 29	30,000	N.D.	670	4,200	830	61,000	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 30	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 31	N.D.	N.D.	1,300	7,800	N.D.	N.D.	N.D.	N.D.	730	N.D.	N.D.
WWTP 32	13,000	N.D.	580	3,200	6,300	42,000	N.D.	N.D.	N.D.	N.D.	N.D.
WWTP 33	15,000	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

5 Conclusions

Two analytical methods have been developed for the identification and quantification of four LAS homologs (C_{10}–C_{13}) and two AES homologs with each 10 ethoxymers (C_{12} and C_{14} with 0–9 ethoxy units) in WWTP effluent samples. It was found that a rotational vacuum concentrator is a suitable sample pretreatment technique for the analysis of surfactants, as the measured concentration of an environmental sample is not altered by any potential background contamination from solvents or laboratory equipment.

The analytical performance of the methods was evaluated and validated in tap and effluent waters, obtaining fast runtimes, good peaks shapes, as well as adequate trueness and precision. Recoveries of analytes ranged from 91% to 114% for tap water samples and from 90% to 120% for effluent water sample. Relative standard deviations (RSDs) ranged from 3% to 10% for both, tap and effluent water samples.

Total LAS concentrations in the monitored WWTP effluents ranged from below the limit of quantification (LOQ) to 47.7 µg/L. Based on the estimated average effluent concentrations of individual LAS homologs, the average total LAS effluent concentration in monitored WWTP effluents was 14.4 µg/L. Therefore, the obtained results are similar to concentrations reported in former studies conducted in Europe and the U.SA.

Total AES effluent concentrations were lower compared to LAS and ranged from <LOQ to 1.9 µg/L, with an average total AES effluent concentration of 0.57 µg/L. The estimated average total AES concentration found in this work is lower compared to values reported by other authors.

No correlation between total LAS and AES effluent concentrations was found for the WWTPs monitored in the present study, indicating regional variations in the surfactant use and/or differences in the removal mechanisms of surfactants in the sewer and the WWTP. However, our results indicate similar LAS and AES effluent concentrations for various WWTPs in Germany. Very high removal rates were found for LAS (>99.2%) and for AES (>99.8%). Therefore, these results confirm that both surfactants are extensively removed during conventional wastewater treatment.

A screening of 1,564 surfactants and their metabolites in the effluent samples was performed by HPLC-ESI-QTOF-MS analysis by the project partner. Target screening of LAS and AES was performed for the same set of homologs/ethoxymers using the identical analytical standards provided by the other laboratory (TZW). The screening has shown that in many cases the sum of concentration of all LAS-related byproducts and TPs surpassed the concentration of LAS themselves; all surfactants together accounted for concentrations of up to 94 µg/L in a single sample; high total concentrations of LAS up to 47.7 µg/L and PEGs up to 7.4 µg/L were determined in the samples and DATs, SPACs and STACs reached concentration levels of 19 µg/L, 17 µg/L and 5.3 µg/L, respectively. An interactive map for visualization of concentrations of detected surfactants in the studied WWTPs is at www.norman-data.eu/EWW_GERMANY.

A ‘mini collaborative trial’ between the two laboratories involved in the study indicated good agreement of the results considering the variability of the subsample, the variability introduced by the different sample preparation techniques applied and the different instrumental facilities. In all cases, concentration levels were at the same order of magnitude.

In non-target screening all surfactants currently enlisted in NORMAN Suspect List Exchange (SusDat; S7 EAWAGSURF, S8 ATHENSSUS and S23 EIUBASURF) were searched for, i.e. additional to the target substances and TPs of LAS, occurrence of other surfactants was investigated. In this

study, it has been discovered that PEG homologs with high molecular mass are ionized as $[M+NH4]^{2+}$ adducts, which resulted in the positive detection of the longest homolog series so far reported in the literature (41 molecules) in wastewater effluent samples. Concentration levels of PEGs were semi-quantified based on the calibration curve of PEG-04 and the cumulative concentration level of all PEGs together reached up to 7.4 µg/L. Maximum concentration levels occurred in most of the cases for PEG-08 and PEG-09, followed by PEG-10 and PEG-07.

Several other surfactants were screened for without a possibility to estimate their concentrations due to unavailability of structurally similar reference standard chemicals. High frequency of appearance (FoA) was observed for secondary alkane sulfonate (SAS) surfactants; SAS-C_{12} and SAS-C_{14} were detected in all wastewater samples, SAS-C_{11} was detected with FoA 91%, SAS- C_{13} was detected with FoA 73%. The highest intensities of signals were observed for C_{12}-SAS. Other surfactants with widespread occurrence were NP1ethoxycarboxylate, naphthalene-1-sulfonate and dihexyl sulfosuccinate (DHSS), which were detected with very high FoA (100% for NP1ethoxycarboxylate and naphthalene-1-sulfonate, 97% for DHSS).

An interactive map for the visualization of concentrations of the quantified surfactants in the studied WWTPs is available at www.norman-data.eu/EWW_GERMANY. Based on the "Detergents Ingredients Database" (EC, 2016) a Suspect List of all surfactants and their metabolites was created including their exact masses and chemical structures (SMILES, INCHIKEYs). It is available at www.norman-network.com/?q=node/236 as a list S23 coded "EIUBASURF". All UHPLC-ESI-QTOF-MS raw data were uploaded into NORMAN Digital Sample Freezing Platform (www.norman-data.eu) and are available for future retrospective efforts.

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7 Annex

Annex Table 1: **MS/MS conditions for LAS homologs in ESI− mode. General interface parameters: Gas Temp.: 200 °C; Gas Flow: 14 L/min; Nebulizer: 45 psi; Sheath Gas Temp.: 350 °C; Sheath Gas Flow: 11 L/min; Capillary: −3000 V; Nozzle Voltage: −500 V; High Pressure RF: −90 V: Low Pressure RF: −40 V.**

Compound	Precursor Ion in Da	Product Ion in Da	Collision Energy in eV	Cell Accelerator Voltage in V	Dwell time in ms
C_8	269.1	182.9	41	4	25
C_8	269.1	170.0	33	4	25
C_{10}	297.2	183.0	45	4	25
C_{10}	297.2	119.1	61	4	25
C_{11}	311.2	182.9	45	4	25
C_{11}	311.2	119.1	69	4	25
C_{12}	325.2	183.1	41	4	25
C_{12}	325.2	119.1	69	4	25
C_{13}	339.2	182.9	45	4	25
C_{13}	339.2	119.0	69	4	25

Annex Table 2: **MS/MS conditions for AES homologs in ESI− mode. General interface parameters: Curtain Gas: 40 psi; Collision Gas: 7 psi; Ionspray Voltage: −4500 V; Temperature: 400 °C; Ion Source Gas 1: 40 psi; Ion Source Gas 2: 70 psi. Target scan time: 0.4 s.**

Compound	Precursor Ion in Da	Product Ion in Da	Declustering Potential in V	Entrance Potential in V	Collision energy in V	Collision Cell Exit Potential in V
C_{12} EO_0-d_{25}	290.2	97.9	-115	-10	-38	-11
C_{12} EO_0-d_{25}	290.2	79.9	-115	-10	-96	-9
C_{12} EO_0	264.98	96.8	-125	-10	-34	-7
C_{12} EO_0	264.98	79.8	-125	-10	-92	-9
C_{12} EO_1	309.02	96.8	-25	-10	-36	-9
C_{12} EO_1	309.02	80.0	-25	-10	-106	-11
C_{12} EO_2	353.02	96.8	-55	-10	-40	-7
C_{12} EO_2	353.02	79.9	-55	-10	-118	-9
C_{12} EO_3	397.03	97.0	-35	-10	-46	-9
C_{12} EO_3	397.03	79.9	-35	-10	-128	-7
C_{12} EO_4	441.07	96.9	-165	-10	-50	-9
C_{12} EO_4	441.07	79.9	-165	-10	-124	-11

Compound	Precursor Ion in Da	Product Ion in Da	Declustering Potential in V	Entrance Potential in V	Collision energy in V	Collision Cell Exit Potential in V
C_{12} EO_5	485.09	80.0	-55	-10	-130	-11
C_{12} EO_5	485.09	96.4	-55	-10	-130	-15
C_{12} EO_6	529.13	97.0	-60	-10	-106	-15
C_{12} EO_6	529.13	79.9	-60	-10	-130	-11
C_{12} EO_7	573.14	96.8	-40	-10	-100	-11
C_{12} EO_7	573.14	79.9	-40	-10	-130	-13
C_{12} EO_8	617.16	96.8	-90	-10	-126	-15
C_{12} EO_8	617.16	80.0	-90	-10	-128	-7
C_{12} EO_9	661.21	96.8	-100	-10	-130	-13
C_{12} EO_9	661.21	80.0	-100	-10	-126	-9
C_{14} EO_0	293.06	96.9	-120	-10	-36	-13
C_{14} EO_0	293.06	79.9	-120	-10	-102	-11
C_{14} EO_1	337.06	96.8	-90	-10	-40	-13
C_{14} EO_1	337.06	80.0	-90	-10	-114	-7
C_{14} EO_2	381.07	96.9	-85	-10	-44	-7
C_{14} EO_2	381.07	80.0	-85	-10	-130	-11
C_{14} EO_3	425.05	96.9	-70	-10	-46	-7
C_{14} EO_3	425.05	80.1	-70	-10	-124	-5
C_{14} EO_4	469.09	96.9	-125	-10	-48	-9
C_{14} EO_4	469.09	80.0	-125	-10	-126	-11
C_{14} EO_5	513.13	96.9	-55	-10	-116	-9
C_{14} EO_5	513.13	80.0	-55	-10	-130	-9
C_{14} EO_6	557.15	97.0	-150	-10	-130	-11
C_{14} EO_6	557.15	79.8	-150	-10	-130	-7
C_{14} EO_7	601.19	97.0	-40	-10	-130	-11
C_{14} EO_7	601.19	79.9	-40	-10	-130	-13
C_{14} EO_8	645.1	96.9	-30	-10	-128	-9
C_{14} EO_8	645.1	80.0	-30	-10	-130	-11
C_{14} EO_9	689.2	96.9	-55	-10	-130	-13
C_{14} EO_9	689.2	80.0	-55	-10	-130	-9

Annex Table 3: Individual and total concentrations of LAS homologs (in µg/L) in monitored WWTP influents.

LAS homolog	C_{10}	C_{11}	C_{12}	C_{13}	Total (C_{10}-C_{13})
Eutin	630	1200	950	670	3450
Geldern	630	1100	820	820	3370
Landsberg	540	1100	960	720	3320
Stuttgart	520	950	720	440	2630

Annex Table 4: Concentrations in µg/L of AES-C_{12} ethoxymers in monitored WWTP influents.

AES ethoxymer	C_{12} EO0	C_{12} EO1	C_{12} EO2	C_{12} EO3	C_{12} EO4	C_{12} EO5	C_{12} EO6	C_{12} EO7	C_{12} EO8	C_{12} EO9
Eutin	8.7	23	31	59	16	21	30	46	61	45
Geldern	42	54	64	110	48	48	49	56	64	44
Landsberg	10	91	100	140	76	73	66	72	77	55
Stuttgart	16	32	21	36	11	17	24	41	58	44

Annex Table 5: Concentrations in µg/L of AES-C_{14} ethoxymers and total AES (C_{12} and C_{14} with EO0-9) concentration in monitored WWTP influents.

AES ethoxymer	C_{14} EO0	C_{14} EO1	C_{14} EO2	C_{14} EO3	C_{14} EO4	C_{14} EO5	C_{14} EO6	C_{14} EO7	C_{14} EO8	C_{14} EO9	C_{12}&C_{14} EO0-9
Eutin	8.1	43	37	18	6.4	6.3	11	17	20	15	523
Geldern	17	42	35	21	10	8.8	12	16	17	12	770
Landsberg	11	60	53	36	18	15	21	26	27	18	1045
Stuttgart	8.8	23	15	6.3	2.1	1.7	3.5	8.4	14	12	395

Annex Table 6: **Occurrence of AEOs in wastewater samples. The table illustrates the compound name, the molecular formula, the frequency of appearance (FoA), and the concentration range of AEOs in ng/L (only detects are considered). Identification level of detected chemicals is level 3 (ramification isomers possible), and the screened adduct was $[M+NH_4]^+$ in all cases.**

Compound Name	Formula	FoA (%)	C_{range} (ng/L)
C08 Alcohol, predominately linear, 01 EO	C10H22O2	0.0	N.D.
C08 Alcohol, predominately linear, 02 EO	C12H26O3	0.0	N.D.
C08 Alcohol, predominately linear, 03 EO	C14H30O4	24.2	1-31
C08 Alcohol, predominately linear, 04 EO	C16H34O5	45.5	1-48
C08 Alcohol, predominately linear, 05 EO	C18H38O6	3.0	48
C08 Alcohol, predominately linear, 06 EO	C20H42O7	54.5	1-74
C08 Alcohol, predominately linear, 07 EO	C22H46O8	12.1	1-446
C08 Alcohol, predominately linear, 08 EO	C24H50O9	93.9	13-650
C08 Alcohol, predominately linear, 09 EO	C26H54O10	90.9	2-82
C08 Alcohol, predominately linear, 10 EO	C28H58O11	9.1	4-51
C08 Alcohol, predominately linear, 11 EO	C30H62O12	45.5	2-138
C08 Alcohol, predominately linear, 12 EO	C32H66O13	6.1	1-4
C08 Alcohol, predominately linear, 13 EO	C34H70O14	9.1	4-14
C08 Alcohol, predominately linear, 14 EO	C36H74O15	0.0	N.D.
C08 Alcohol, predominately linear, 15 EO	C38H78O16	6.1	4-6
C08 Alcohol, predominately linear, 16 EO	C40H82O17	3.0	4
C08 Alcohol, predominately linear, 17 EO	C42H86O18	0.0	N.D.
C08 Alcohol, predominately linear, 18 EO	C44H90O19	3.0	2
C08 Alcohol, predominately linear, 19 EO	C46H94O20	3.0	1
C08 Alcohol, predominately linear, 20 EO	C48H98O21	0.0	N.D.
C09 Alcohol, predominately linear, 01 EO	C11H24O2	0.0	N.D.
C09 Alcohol, predominately linear, 02 EO	C13H28O3	3.0	2
C09 Alcohol, predominately linear, 03 EO	C15H32O4	30.3	1-3
C09 Alcohol, predominately linear, 04 EO	C17H36O5	39.4	1-2
C09 Alcohol, predominately linear, 05 EO	C19H40O6	87.9	2-278

Compound Name	Formula	FoA (%)	C_{range} (ng/L)
C09 Alcohol, predominately linear, 06 EO	C21H44O7	36.4	1-4
C09 Alcohol, predominately linear, 07 EO	C23H48O8	21.2	1-4
C09 Alcohol, predominately linear, 08 EO	C25H52O9	30.3	1-4
C09 Alcohol, predominately linear, 09 EO	C27H56O10	90.9	4-368
C09 Alcohol, predominately linear, 10 EO	C29H60O11	90.9	1-59
C09 Alcohol, predominately linear, 11 EO	C31H64O12	12.1	3-36
C09 Alcohol, predominately linear, 12 EO	C33H68O13	30.3	4-165
C09 Alcohol, predominately linear, 13 EO	C35H72O14	6.1	2-19
C09 Alcohol, predominately linear, 14 EO	C37H76O15	3.0	1-1
C09 Alcohol, predominately linear, 15 EO	C39H80O16	3.0	5
C09 Alcohol, predominately linear, 16 EO	C41H84O17	6.1	3
C09 Alcohol, predominately linear, 17 EO	C43H88O18	3.0	1
C09 Alcohol, predominately linear, 18 EO	C45H92O19	6.1	1-5
C09 Alcohol, predominately linear, 19 EO	C47H96O20	3.0	1
C09 Alcohol, predominately linear, 20 EO	C49H100O21	0.0	N.D.
C10 Alcohol, predominately linear, 01 EO	C12H26O2	0.0	N.D.
C10 Alcohol, predominately linear, 02 EO	C14H30O3	0.0	N.D.
C10 Alcohol, predominately linear, 03 EO	C16H34O4	93.9	5-142
C10 Alcohol, predominately linear, 04 EO	C18H38O5	90.9	3-64
C10 Alcohol, predominately linear, 05 EO	C20H42O6	90.9	3-74
C10 Alcohol, predominately linear, 06 EO	C22H46O7	78.8	2-54
C10 Alcohol, predominately linear, 07 EO	C24H50O8	87.9	2-36
C10 Alcohol, predominately linear, 08 EO	C26H54O9	6.1	2-69
C10 Alcohol, predominately linear, 09 EO	C28H58O10	57.6	1-25
C10 Alcohol, predominately linear, 10 EO	C30H62O11	93.9	4-345
C10 Alcohol, predominately linear, 11 EO	C32H66O12	78.8	2-74
C10 Alcohol, predominately linear, 12 EO	C34H70O13	36.4	3-44
C10 Alcohol, predominately linear, 13 EO	C36H74O14	15.2	2-146

Compound Name	Formula	FoA (%)	C_{range} (ng/L)
C10 Alcohol, predominately linear, 14 EO	C38H78O15	21.2	2-5
C10 Alcohol, predominately linear, 15 EO	C40H82O16	12.1	2-5
C10 Alcohol, predominately linear, 16 EO	C42H86O17	15.2	1-3
C10 Alcohol, predominately linear, 17 EO	C44H90O18	3.0	4
C10 Alcohol, predominately linear, 18 EO	C46H94O19	0.0	N.D.
C10 Alcohol, predominately linear, 19 EO	C48H98O20	0.0	N.D.
C10 Alcohol, predominately linear, 20 EO	C50H102O21	0.0	N.D.
C11 Alcohol, predominately linear, 01 EO	C13H28O2	0.0	N.D.
C11 Alcohol, predominately linear, 02 EO	C15H32O3	6.1	1
C11 Alcohol, predominately linear, 03 EO	C17H36O4	90.9	2-60
C11 Alcohol, predominately linear, 04 EO	C19H40O5	72.7	3-13
C11 Alcohol, predominately linear, 05 EO	C21H44O6	21.2	1-6
C11 Alcohol, predominately linear, 06 EO	C23H48O7	78.8	2-15
C11 Alcohol, predominately linear, 07 EO	C25H52O8	24.2	2-59
C11 Alcohol, predominately linear, 08 EO	C27H56O9	57.6	2-12
C11 Alcohol, predominately linear, 09 EO	C29H60O10	15.2	2-6
C11 Alcohol, predominately linear, 10 EO	C31H64O11	9.1	5
C11 Alcohol, predominately linear, 11 EO	C33H68O12	93.9	4-411
C11 Alcohol, predominately linear, 12 EO	C35H72O13	78.8	2-82
C11 Alcohol, predominately linear, 13 EO	C37H76O14	6.1	5-32
C11 Alcohol, predominately linear, 14 EO	C39H80O15	12.1	2-86
C11 Alcohol, predominately linear, 15 EO	C41H84O16	3.0	1
C11 Alcohol, predominately linear, 16 EO	C43H88O17	6.1	3-11
C11 Alcohol, predominately linear, 17 EO	C45H92O18	3.0	2
C11 Alcohol, predominately linear, 18 EO	C47H96O19	0.0	N.D.
C11 Alcohol, predominately linear, 19 EO	C49H100O20	0.0	N.D.
C11 Alcohol, predominately linear, 20 EO	C51H104O21	0.0	N.D.
C12 Alcohol, predominately linear, 01 EO	C14H30O2	87.9	1-5

Compound Name	Formula	FoA (%)	C_{range} (ng/L)
C12 Alcohol, predominately linear, 02 EO	C16H34O3	36.4	2-6
C12 Alcohol, predominately linear, 03 EO	C18H38O4	81.8	1-38
C12 Alcohol, predominately linear, 04 EO	C20H42O5	42.4	3-22
C12 Alcohol, predominately linear, 05 EO	C22H46O6	18.2	1-8
C12 Alcohol, predominately linear, 06 EO	C24H50O7	33.3	3-10
C12 Alcohol, predominately linear, 07 EO	C26H54O8	51.5	3-11
C12 Alcohol, predominately linear, 08 EO	C28H58O9	18.2	2-55
C12 Alcohol, predominately linear, 09 EO	C30H62O10	24.2	2-24
C12 Alcohol, predominately linear, 10 EO	C32H66O11	3.0	114
C12 Alcohol, predominately linear, 11 EO	C34H70O12	3.0	245
C12 Alcohol, predominately linear, 12 EO	C36H74O13	93.9	4-380
C12 Alcohol, predominately linear, 13 EO	C38H78O14	87.9	1-73
C12 Alcohol, predominately linear, 14 EO	C40H82O15	18.2	2-53
C12 Alcohol, predominately linear, 15 EO	C42H86O16	6.1	6-35
C12 Alcohol, predominately linear, 16 EO	C44H90O17	3.0	2
C12 Alcohol, predominately linear, 17 EO	C46H94O18	3.0	1
C12 Alcohol, predominately linear, 18 EO	C48H98O19	0.0	N.D.
C12 Alcohol, predominately linear, 19 EO	C50H102O20	0.0	N.D.
C12 Alcohol, predominately linear, 20 EO	C52H106O21	0.0	N.D.
C12 Alcohol, predominately linear, 21 EO	C54H110O22	0.0	N.D.
C12 Alcohol, predominately linear, 22 EO	C56H114O23	0.0	N.D.
C12 Alcohol, predominately linear, 23 EO	C58H118O24	0.0	N.D.
C12 Alcohol, predominately linear, 24 EO	C60H122O25	0.0	N.D.
C12 Alcohol, predominately linear, 25 EO	C62H126O26	0.0	N.D.
C12 Alcohol, predominately linear, 26 EO	C64H130O27	0.0	N.D.
C12 Alcohol, predominately linear, 27 EO	C66H134O28	0.0	N.D.
C12 Alcohol, predominately linear, 28 EO	C68H138O29	0.0	N.D.
C12 Alcohol, predominately linear, 29 EO	C70H142O30	0.0	N.D.

Compound Name	Formula	FoA (%)	C_{range} (ng/L)
C12 Alcohol, predominately linear, 30 EO	C72H146O31	0.0	N.D.
C13 Alcohol, predominately linear, 01 EO	C15H32O2	0.0	N.D.
C13 Alcohol, predominately linear, 02 EO	C17H36O3	24.2	1-236
C13 Alcohol, predominately linear, 03 EO	C19H40O4	90.9	15-347
C13 Alcohol, predominately linear, 04 EO	C21H44O5	87.9	5-153
C13 Alcohol, predominately linear, 05 EO	C23H48O6	90.9	3-120
C13 Alcohol, predominately linear, 06 EO	C25H52O7	48.5	3-129
C13 Alcohol, predominately linear, 07 EO	C27H56O8	30.3	2-6
C13 Alcohol, predominately linear, 08 EO	C29H60O9	9.1	2-6
C13 Alcohol, predominately linear, 09 EO	C31H64O10	57.6	2-71
C13 Alcohol, predominately linear, 10 EO	C33H68O11	6.1	2-3
C13 Alcohol, predominately linear, 11 EO	C35H72O12	30.3	3-37
C13 Alcohol, predominately linear, 12 EO	C37H76O13	6.1	11-36
C13 Alcohol, predominately linear, 13 EO	C39H80O14	90.9	3-320
C13 Alcohol, predominately linear, 14 EO	C41H84O15	48.5	1-47
C13 Alcohol, predominately linear, 15 EO	C43H88O16	9.1	1-30
C13 Alcohol, predominately linear, 16 EO	C45H92O17	3.0	11
C13 Alcohol, predominately linear, 17 EO	C47H96O18	3.0	2
C13 Alcohol, predominately linear, 18 EO	C49H100O19	0.0	N.D.
C13 Alcohol, predominately linear, 19 EO	C51H104O20	0.0	N.D.
C13 Alcohol, predominately linear, 20 EO	C53H108O21	0.0	N.D.
C13 Alcohol, predominately linear, 21 EO	C55H112O22	0.0	N.D.
C13 Alcohol, predominately linear, 22 EO	C57H116O23	0.0	N.D.
C13 Alcohol, predominately linear, 23 EO	C59H120O24	0.0	N.D.
C13 Alcohol, predominately linear, 24 EO	C61H124O25	0.0	N.D.
C13 Alcohol, predominately linear, 25 EO	C63H128O26	0.0	N.D.
C13 Alcohol, predominately linear, 26 EO	C65H132O27	0.0	N.D.
C13 Alcohol, predominately linear, 27 EO	C67H136O28	0.0	N.D.

Compound Name	Formula	FoA (%)	C_{range} (ng/L)
C13 Alcohol, predominately linear, 28 EO	C69H140O29	0.0	N.D.
C13 Alcohol, predominately linear, 29 EO	C71H144O30	0.0	N.D.
C13 Alcohol, predominately linear, 30 EO	C73H148O31	0.0	N.D.
C14 Alcohol, predominately linear, 01 EO	C16H34O2	60.6	1-3
C14 Alcohol, predominately linear, 02 EO	C18H38O3	36.4	2-6
C14 Alcohol, predominately linear, 03 EO	C20H42O4	87.9	5-19
C14 Alcohol, predominately linear, 04 EO	C22H46O5	87.9	2-20
C14 Alcohol, predominately linear, 05 EO	C24H50O6	81.8	3-53
C14 Alcohol, predominately linear, 06 EO	C26H54O7	72.7	3-10
C14 Alcohol, predominately linear, 07 EO	C28H58O8	51.5	3-46
C14 Alcohol, predominately linear, 08 EO	C30H62O9	45.5	2-4
C14 Alcohol, predominately linear, 09 EO	C32H66O10	39.4	1-4
C14 Alcohol, predominately linear, 10 EO	C34H70O11	78.8	1-113
C14 Alcohol, predominately linear, 11 EO	C36H74O12	21.2	2-18
C14 Alcohol, predominately linear, 12 EO	C38H78O13	24.2	2-17
C14 Alcohol, predominately linear, 13 EO	C40H82O14	3.0	184
C14 Alcohol, predominately linear, 14 EO	C42H86O15	90.9	2-219
C14 Alcohol, predominately linear, 15 EO	C44H90O16	54.5	1-34
C14 Alcohol, predominately linear, 16 EO	C46H94O17	15.2	2-16
C14 Alcohol, predominately linear, 17 EO	C48H98O18	9.1	1-2
C14 Alcohol, predominately linear, 18 EO	C50H102O19	0.0	N.D.
C14 Alcohol, predominately linear, 19 EO	C52H106O20	0.0	N.D.
C14 Alcohol, predominately linear, 20 EO	C54H110O21	0.0	N.D.
C14 Alcohol, predominately linear, 21 EO	C56H114O22	0.0	N.D.
C14 Alcohol, predominately linear, 22 EO	C58H118O23	0.0	N.D.
C14 Alcohol, predominately linear, 23 EO	C60H122O24	0.0	N.D.
C14 Alcohol, predominately linear, 24 EO	C62H126O25	0.0	N.D.
C14 Alcohol, predominately linear, 25 EO	C64H130O26	0.0	N.D.

Compound Name	Formula	FoA (%)	C_{range} (ng/L)
C14 Alcohol, predominately linear, 26 EO	C66H134O27	0.0	N.D.
C14 Alcohol, predominately linear, 27 EO	C68H138O28	0.0	N.D.
C14 Alcohol, predominately linear, 28 EO	C70H142O29	0.0	N.D.
C14 Alcohol, predominately linear, 29 EO	C72H146O30	0.0	N.D.
C14 Alcohol, predominately linear, 30 EO	C74H150O31	0.0	N.D.
C15 Alcohol, predominately linear, 01 EO	C17H36O2	0.0	N.D.
C15 Alcohol, predominately linear, 02 EO	C19H40O3	30.3	2-4
C15 Alcohol, predominately linear, 03 EO	C21H44O4	24.2	3-11
C15 Alcohol, predominately linear, 04 EO	C23H48O5	18.2	2-4
C15 Alcohol, predominately linear, 05 EO	C25H52O6	69.7	2-6
C15 Alcohol, predominately linear, 06 EO	C27H56O7	63.6	2-4
C15 Alcohol, predominately linear, 07 EO	C29H60O8	42.4	1-6
C15 Alcohol, predominately linear, 08 EO	C31H64O9	3.0	2
C15 Alcohol, predominately linear, 09 EO	C33H68O10	27.3	1-35
C15 Alcohol, predominately linear, 10 EO	C35H72O11	39.4	2-32
C15 Alcohol, predominately linear, 11 EO	C37H76O12	84.8	1-138
C15 Alcohol, predominately linear, 12 EO	C39H80O13	30.3	1-20
C15 Alcohol, predominately linear, 13 EO	C41H84O14	39.4	1-144
C15 Alcohol, predominately linear, 14 EO	C43H88O15	12.1	2-16
C15 Alcohol, predominately linear, 15 EO	C45H92O16	87.9	1-113
C15 Alcohol, predominately linear, 16 EO	C47H96O17	36.4	1-5
C15 Alcohol, predominately linear, 17 EO	C49H100O18	6.1	1-8
C15 Alcohol, predominately linear, 18 EO	C51H104O19	0.0	N.D.
C15 Alcohol, predominately linear, 19 EO	C53H108O20	0.0	N.D.
C15 Alcohol, predominately linear, 20 EO	C55H112O21	0.0	N.D.
C15 Alcohol, predominately linear, 21 EO	C57H116O22	0.0	N.D.
C15 Alcohol, predominately linear, 22 EO	C59H120O23	0.0	N.D.
C15 Alcohol, predominately linear, 23 EO	C61H124O24	0.0	N.D.

Compound Name	Formula	FoA (%)	C_{range} (ng/L)
C15 Alcohol, predominately linear, 24 EO	C63H128O25	0.0	N.D.
C15 Alcohol, predominately linear, 25 EO	C65H132O26	0.0	N.D.
C15 Alcohol, predominately linear, 26 EO	C67H136O27	0.0	N.D.
C15 Alcohol, predominately linear, 27 EO	C69H140O28	0.0	N.D.
C15 Alcohol, predominately linear, 28 EO	C71H144O29	0.0	N.D.
C15 Alcohol, predominately linear, 29 EO	C73H148O30	0.0	N.D.
C15 Alcohol, predominately linear, 30 EO	C75H152O31	0.0	N.D.
C16 Alcohol, predominately linear, 01 EO	C18H38O2	0.0	N.D.
C16 Alcohol, predominately linear, 02 EO	C20H42O3	81.8	1-3
C16 Alcohol, predominately linear, 03 EO	C22H46O4	24.2	3-10
C16 Alcohol, predominately linear, 04 EO	C24H50O5	93.9	3-17
C16 Alcohol, predominately linear, 05 EO	C26H54O6	93.9	4-19
C16 Alcohol, predominately linear, 06 EO	C28H58O7	18.2	6-28
C16 Alcohol, predominately linear, 07 EO	C30H62O8	81.8	2-17
C16 Alcohol, predominately linear, 08 EO	C32H66O9	78.8	2-11
C16 Alcohol, predominately linear, 09 EO	C34H70O10	12.1	3-11
C16 Alcohol, predominately linear, 10 EO	C36H74O11	57.6	3-6
C16 Alcohol, predominately linear, 11 EO	C38H78O12	75.8	2-6
C16 Alcohol, predominately linear, 12 EO	C40H82O13	84.8	4-135
C16 Alcohol, predominately linear, 13 EO	C42H86O14	18.2	3-11
C16 Alcohol, predominately linear, 14 EO	C44H90O15	42.4	3-14
C16 Alcohol, predominately linear, 15 EO	C46H94O16	57.6	3-14
C16 Alcohol, predominately linear, 16 EO	C48H98O17	75.8	3-49
C16 Alcohol, predominately linear, 17 EO	C50H102O18	0.0	N.D.
C16 Alcohol, predominately linear, 18 EO	C52H106O19	0.0	N.D.
C16 Alcohol, predominately linear, 19 EO	C54H110O20	0.0	N.D.
C16 Alcohol, predominately linear, 20 EO	C56H114O21	0.0	N.D.
C16 Alcohol, predominately linear, 21 EO	C58H118O22	0.0	N.D.

Compound Name	Formula	FoA (%)	C_{range} (ng/L)
C16 Alcohol, predominately linear, 22 EO	C60H122O23	0.0	N.D.
C16 Alcohol, predominately linear, 23 EO	C62H126O24	0.0	N.D.
C16 Alcohol, predominately linear, 24 EO	C64H130O25	0.0	N.D.
C16 Alcohol, predominately linear, 25 EO	C66H134O26	0.0	N.D.
C16 Alcohol, predominately linear, 26 EO	C68H138O27	0.0	N.D.
C16 Alcohol, predominately linear, 27 EO	C70H142O28	0.0	N.D.
C16 Alcohol, predominately linear, 28 EO	C72H146O29	0.0	N.D.
C16 Alcohol, predominately linear, 29 EO	C74H150O30	0.0	N.D.
C16 Alcohol, predominately linear, 30 EO	C76H154O31	0.0	N.D.
C17 Alcohol, predominately linear, 01 EO	C19H40O2	0.0	N.D.
C17 Alcohol, predominately linear, 02 EO	C21H44O3	0.0	N.D.
C17 Alcohol, predominately linear, 03 EO	C23H48O4	3.0	1
C17 Alcohol, predominately linear, 04 EO	C25H52O5	15.2	1-5
C17 Alcohol, predominately linear, 05 EO	C27H56O6	27.3	1-3
C17 Alcohol, predominately linear, 06 EO	C29H60O7	15.2	1-3
C17 Alcohol, predominately linear, 07 EO	C31H64O8	24.2	1-6
C17 Alcohol, predominately linear, 08 EO	C33H68O9	18.2	2-6
C17 Alcohol, predominately linear, 09 EO	C35H72O10	18.2	2-14
C17 Alcohol, predominately linear, 10 EO	C37H76O11	12.1	2-25
C17 Alcohol, predominately linear, 11 EO	C39H80O12	36.4	1-30
C17 Alcohol, predominately linear, 12 EO	C41H84O13	42.4	1-32
C17 Alcohol, predominately linear, 13 EO	C43H88O14	87.9	4-101
C17 Alcohol, predominately linear, 14 EO	C45H92O15	3.0	3-3
C17 Alcohol, predominately linear, 15 EO	C47H96O16	21.2	1-93
C17 Alcohol, predominately linear, 16 EO	C49H100O17	3.0	65
C17 Alcohol, predominately linear, 17 EO	C51H104O18	0.0	N.D.
C17 Alcohol, predominately linear, 18 EO	C53H108O19	0.0	N.D.
C17 Alcohol, predominately linear, 19 EO	C55H112O20	0.0	N.D.

Compound Name	Formula	FoA (%)	C_{range} (ng/L)
C17 Alcohol, predominately linear, 20 EO	C57H116O21	0.0	N.D.
C17 Alcohol, predominately linear, 21 EO	C59H120O22	0.0	N.D.
C17 Alcohol, predominately linear, 22 EO	C61H124O23	0.0	N.D.
C17 Alcohol, predominately linear, 23 EO	C63H128O24	0.0	N.D.
C17 Alcohol, predominately linear, 24 EO	C65H132O25	0.0	N.D.
C17 Alcohol, predominately linear, 25 EO	C67H136O26	0.0	N.D.
C17 Alcohol, predominately linear, 26 EO	C69H140O27	0.0	N.D.
C17 Alcohol, predominately linear, 27 EO	C71H144O28	0.0	N.D.
C17 Alcohol, predominately linear, 28 EO	C73H148O29	0.0	N.D.
C17 Alcohol, predominately linear, 29 EO	C75H152O30	0.0	N.D.
C17 Alcohol, predominately linear, 30 EO	C77H156O31	0.0	N.D.
C18 Alcohol, predominately linear, 01 EO	C20H42O2	0.0	N.D.
C18 Alcohol, predominately linear, 02 EO	C22H46O3	0.0	N.D.
C18 Alcohol, predominately linear, 03 EO	C24H50O4	3.0	3
C18 Alcohol, predominately linear, 04 EO	C26H54O5	3.0	1
C18 Alcohol, predominately linear, 05 EO	C28H58O6	12.1	1-3
C18 Alcohol, predominately linear, 06 EO	C30H62O7	0.0	N.D.
C18 Alcohol, predominately linear, 07 EO	C32H66O8	3.0	4
C18 Alcohol, predominately linear, 08 EO	C34H70O9	9.1	1-4
C18 Alcohol, predominately linear, 09 EO	C36H74O10	9.1	1-11
C18 Alcohol, predominately linear, 10 EO	C38H78O11	0.0	N.D.
C18 Alcohol, predominately linear, 11 EO	C40H82O12	6.1	1-24
C18 Alcohol, predominately linear, 12 EO	C42H86O13	6.1	1-2
C18 Alcohol, predominately linear, 13 EO	C44H90O14	9.1	1-21
C18 Alcohol, predominately linear, 14 EO	C46H94O15	87.9	2-66
C18 Alcohol, predominately linear, 15 EO	C48H98O16	0.0	N.D.
C18 Alcohol, predominately linear, 16 EO	C50H102O17	12.1	1-74
C18 Alcohol, predominately linear, 17 EO	C52H106O18	0.0	N.D.

Compound Name	Formula	FoA (%)	C_{range} (ng/L)
C18 Alcohol, predominately linear, 18 EO	C54H110O19	0.0	N.D.
C18 Alcohol, predominately linear, 19 EO	C56H114O20	0.0	N.D.
C18 Alcohol, predominately linear, 20 EO	C58H118O21	0.0	N.D.
C18 Alcohol, predominately linear, 21 EO	C60H122O22	0.0	N.D.
C18 Alcohol, predominately linear, 22 EO	C62H126O23	0.0	N.D.
C18 Alcohol, predominately linear, 23 EO	C64H130O24	0.0	N.D.
C18 Alcohol, predominately linear, 24 EO	C66H134O25	0.0	N.D.
C18 Alcohol, predominately linear, 25 EO	C68H138O26	0.0	N.D.
C18 Alcohol, predominately linear, 26 EO	C70H142O27	0.0	N.D.
C18 Alcohol, predominately linear, 27 EO	C72H146O28	0.0	N.D.
C18 Alcohol, predominately linear, 28 EO	C74H150O29	0.0	N.D.
C18 Alcohol, predominately linear, 29 EO	C76H154O30	0.0	N.D.
C18 Alcohol, predominately linear, 30 EO	C78H158O31	0.0	N.D.