Chromatographic and Spectroscopic Characterization of Surfactants used for Agrochemical Products

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Für meinen kleinen Sohn

Abstract

Surfactants are key compounds in agrochemical products that ensure properties such as viscosity, dispersity or homogeneity and are assisting the homogenous distribution of the active ingredient(s) over the target crop or promoting its uptake by the plant. There is limited knowledge, however, about the influence of by-products and impurities in a technical surfactant on the properties of the final product. In this context commercial available products of sodium bis(2-ethylhexyl) sulfosuccinate (AOT; anionic) and tristyrylphenol ethoxylates with an average number of 16 ethylene oxide units (TSP-16-ethoxylates; nonionic) - commonly used surfactants in agrochemical products - were investigated for their content of by-products.

The by-products investigated for AOT were its isomeric surface-active mono-esterified sulfosuccinates. An analytical method based on liquid chromatography coupled to time of flight mass spectrometry (LC-ToF-MS) with exact mass measurement was developed to quantify main and by-products in AOT-product. An isomer-selective synthesis for both monoesters was developed for validation of the developed method. Significant differences were observed regarding the content of monoesters among four different suppliers and qualities of AOT-product. A storage stability test performed with a model agrochemical formulation using AOT-product showed decreasing dispersion stability with raised initial content of monoesters. The differences in monoester content were then used for product identification. This could be utilized as additional tool in detection of counterfeit products, as the supplier of the AOT-product in the original agrochemical product is known a priory.

TSP-16-ethoylates were analytically characterized by quantifying all major styrenated ethoxylates against an internal standard using targeted LC-ToF-MS with exact mass measurement in combination with multivariate data analysis. Four suppliers and qualities were analyzed and compared with regard to their content of these styrenated ethoxylates. Significant differences were found between the suppliers based on the content of mono- and tetrastyrylphenol ethoxylates and mono- and distyrylphenol copolymerized propoxylates-ethoxylates, which were successfully utilized for supplier identification.

Analytical characterization and control of surfactants may be a useful tool to avoid unwanted property changes in complex mixtures such as agrochemical products. Moreover, small variations in the composition of surfactants offer further opportunities for identification of counterfeit products.

Future investigations could address the mode of action leading to raised sedimentation in an agrochemical product using AOT with raised content of monoesters. Furthermore, it should be investigated if the method

developed for the characterization of TSP-16-ethoxylatesylates can be adapted to other ethoxylated surfactants and analytes with a comparably broad composition of main and by-products.

Kurzfassung

Tenside sind Schlüsselkomponenten für Pestizide, die für Produkteigenschaften wie Viskosität, Dispersionsstabilität und Homogenität verantwortlich sind und dabei helfen, den Wirkstoff gleichmäßig auf dem Feld zu verteilen und seine Aufnahme in die Pflanze zu erleichtern. Über den Einfluss von Nebenprodukten und Verunreinigungen in technischen Tensiden auf die Eigenschaften des finalen Produkts ist bisher wenig bekannt. In diesem Zusammenhang wurde das Nebenproduktspektrum kommerziell erhältlicher Tenside, Natrium di(2-ethylhexyl) sulfosuccinate (AOT; anionisch) und Tristyrylphenol mit einem mittleren Ethoxylierungsgrad von 16 Ethylenoxideinheiten (TSP-16-ethoxylat), untersucht.

Die im Fall von AOT untersuchten Nebenprodukte waren isomere grenzflächenaktive monoveresterte sulfosuccinate. Für die Analytik dieser Haupt- und Nebenkomponenten in handelsüblichen AOT-Produkt wurde eine analytische Methode mittels Flüssigchromatographie gekoppelt mit einem Flugzeit-Massenspektrometer (LC-ToF-MS), das exakte Massenbestimmung ermöglicht, entwickelt. Für die Validierung der Methode wurde eine isomeren-selektive Synthese der beiden Monoester entwickelt, um die benötigten analytischen Standards für beide Verbindungen zu synthetisieren. Signifikante Unterschiede bezüglich der Monoestergehalte in kommerziellen AOT-Produkten wurden zwischen vier verschiedenen Herstellern und Qualitäten festgestellt. Lagertests mit einer agrochemischen Modellformulierung mit AOT-Produkt als Bestandteil ergaben abnehmende Dispersionsstabilität mit zunehmendem Gehalt an Monoestern in AOT. Darüber hinaus konnten die Unterschiede in Bezug auf die Gehalte an Monoester für die Identifikation der jeweiligen Hersteller genutzt werden. Dies könnte als zusätzliches Merkmal für die Identifikation von Produktfälschungen genutzt werden, da der Hersteller des AOT-Produktes im Originalprodukt von Beginn an bekannt ist.

TSP-16-ethoxylat wurde analytisch charakterisiert, indem alle Styrylphenol ethoxylate gegen einen internen Standard quantifiziert wurde. Hierfür wurde eine analytische Methode basierend auf LC-ToF-MS mit exakter Massenbestimmung in Kombination mit multivarianter Datenanalyse entwickelt und damit TSP-16-ethoxylat von vier verschiedenen Herstellern und Qualitäten untersucht. Dabei wurden signifikante Unterschiede bezüglich des Gehaltes an Mono- und Tetrastyrylphenol ethoylaten sowie an blockcopolymerisiertes Mono- und Distyrylphenol propoxylat-ethxoylat festgestellt, die zur Identifikation der jeweiligen Hersteller genutzt wurden.

Analytische Charakterisierung und Kontrolle von Tensiden kann hilfreich sein, um unerwünschte Änderungen in den Eigenschaften komplexer Mischungen wie agrochemischen Produkten zu verhindern. Darüber hinaus können kleine Unterschiede in der Zusammensetzung von Tensiden zur Produktidentifizierung im Falle von Produktpiraterie genutzt werden.

Für zukünftige Arbeiten sollte der mechanistische Zusammenhang zwischen zunehmender Sedimenation in der hier verwendeten Modellformulierung mit zunehmendem Monoestergehalt des darin enthaltenen AOT-Produktes untersucht werden. Des Weiteren könnte die Adaptierbarkeit der für die analytische Charakterisierung von TSP-16-ethoxylaten entwickelten Methode auf andere ethoxylierte Tenside sowie auf Analyte mit einem vergleichbar breiten Spektrum an Haupt- und Nebenprodukten geprüft werden.

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List of Abbreviations

-	Not determinable or no information available
%	Percentage
°C	Degree Celsius
µA	Microampere
µg	Microgram
µL	Microliter
µM	Micromole
µm	Micrometer
A	Integrated Peak area
a.i.	Active ingredient
CAN	Acetonitrile
Amu	Atomic mass unit
AOT or Aerosol OT	Sodium bis(2-ethylhexyl) sulfosuccinate
APCI	Atmospheric pressure chemical ionization
APPI	Atmospheric pressure photoionization
Br	Bromine
C	Concentration [mg/L]
C	Carbon
Ca	Calcium
Cl	Chlorine
CMC	Critical micelle concentration
COSY	Correlation spectroscopy
CPP	Critical packing parameter

csv	Comma-separated values
D	Deuterium
DIN	Deutsches Institut für Normung
DMSO	Dimethyl sulfoxide
DSP	Distyrylphenol
e.g.	For example
EC	Emulsion concentrate
EIC	Extracted ion chromatogram
ELSD	Evaporation light scattering detector
EO	Ethylene oxide
ESI	Electrospray ionization
eV	Electronvolt
F	Fluor
FBF	Find-by-formula
FID	Flame ionization detector
F-test	Statistical hypothesis test based on the F-distribution under the null hypothesis
FWHM	Full width at half peak maximum
g	Gram
GC	Gas chromatography
GUM	Guide to the expression of uncertainty in measurement
H	Hydrogen
HCA	Hierarchical clustering
HCl	Hydrochloric acid
HILIC	Hydrophilic interaction liquid chromatography

HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear correlation spectroscopy
HP 921	Hexakis(1H,1H, 3H-fluoropropoxy)phosphazine
HPLC	High performance liquid chromatography
i.e.	Id est
IRMS	Isotope ratio mass spectrometry
k	Coverage factor
K	Potassium
KOH	Potassium hydroxide
L	Liter
LAC	Liquid adsorption chromatography
LC	Liquid chromatography
LD	Linear discriminant
LEAC	Liquid exclusion adsorption chromatography
Li	Lithium
LOC	Limit of capture [mg/L]
LOD	Limit of detection [mg/L]
LOQ	Limit of quantification [mg/L]
M	Molar mass
m	Meter
m/z	Mass to charge ratio [amu]
MALDI	Matrix assisted laser desorption ionization
MeOH	Methanol
MFE	Molecular feature extraction

mg	Milligram
Mg	Magnesium
$MgSO_4$	Magnesium sulfate
Min	Minute
mL	Milliliter
mM	Millimole per liter
Mm	Millimeter
Mmol	Millimole
monoester 2	Sodium 1-carboxy-3-[(2-ethylhexyl)oxy]-3-oxopropane-1-sulfonate
monoester 3	Sodium 3-carboxy-1-[(2-ethylhexyl)oxy]-1-oxopropane-2-sulfonate
MS	Mass spectrometer
MSA	Methanesulfonic acid
MSP	Monostyrylphenol
MTBE	Methyl tert-butyl ether
N	1 mol per liter
N	Nitrogen
Na	Sodium
NaOH	Natrium hydroxide
Neg	Negative
NH_4^+	Ammonium Cation
NIR	Near infrared
NMR	Nuclear magnetic resonance
NP	Normal phase
O	Oxygen

P	Probability
P	Phosphor
p.a.	Per analysis
PCA	Principle component analysis
PEG	Polyethylene glycol
pH	pH-value
Post	Positive
ppm	Part per million
psig	Pound-force per square inch
R	Resolution
R	Correlation coefficient
rpm	Rounds per minute
RFID	Radio frequency identification
RP	Reversed phase
S	Sulfur
SC	Suspension concentrate
SEC	Size exclusion chromatography
SNR	Signal-to-Noise ratio
TeSP	Tetrastyrylphenol
TIC	Total ion chromatogram
TLC	Thin layer chromatography
t_N	Retention time corrected by void volume [min]
ToF	Time-of-Flight
t_R	Retention time [min]

TSP	Tristyrylphenol
TSP-16-ethoxylates	Tristyrylphenol ethoxylates with an average degree of ethoxylation of 16 ethylene oxide units
t-test	Statistical hypothesis test based on the Student's t distribution under the null hypothesis
u	Standard uncertainty of the average values
U	Expanded measurement uncertainty
UV	Ultraviolet
V	Volt
v/v	Volume to volume ratio
Vx0	Relative standard deviation of the method
w	Weight
w/w	Weight to weight ratio

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1. General Introduction

1.1 Surfactants

The term “Surfactant” comprises a large group of molecules having surface-active properties. These molecules are able to adsorb at the interfaces of prior non-miscible media such as water/oil, lowering the surface tension in this process and thus allowing emulsification of both phases in the end. This process is for example responsible for the wetting of a fabric surface and the solubilization of dirt particles in the suds during a washing process [1].

There are numerous applications for surfactants, e.g. as cleaning or washing agents or as adjuvant for technical processes and products, respectively, where dispersions and emulsions need to be achieved. One of these technical products surfactants are essential for are agrochemical products. There they have the tasks to stabilize the active ingredient(s) (a.i.) against chemical or physical transformation, ensure homogenous distribution during storage and application and facilitate the uptake of the a.i.(s) into the plant [2;3]. Selected physical-chemical properties of surfactants enabling these applications are described in the following.

1.2 Selected Properties of Surfactants

Surfactants are molecules compromising a hydrophilic head group and a hydrophobic tail as shown exemplarily in Figure 1.

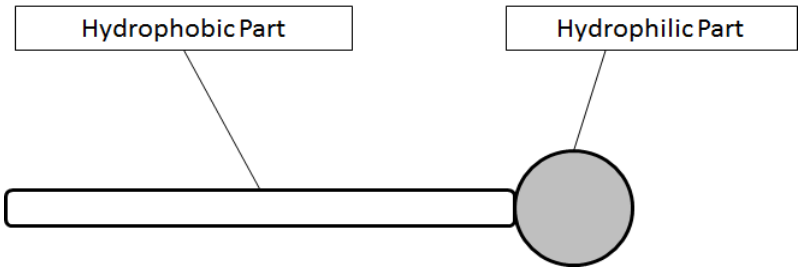


Figure 1: General molecular set-up of a surfactant molecule

In most cases, the hydrophobic group consists of a hydrocarbon chain, whereas the hydrophilic moiety can be categorized with respect to its functionality in four major groups:

- Anionic
- Cationic
- Amphoteric
- Nonionic

The negative charge can be realized via a sulfate group or a phosphate group and the positive charge via an ammonium group. The amphoteric surfactants commonly contain a combination of a quaternary ammonium group carrying a positive charge and a carbonate group containing a negative charge. Nonionic surfactants contain extended polar groups, such as polyethylene glycol chains.

The combination of a hydrophobic and a hydrophilic part in one molecule determines the properties of the surfactants which are able to adsorb at the air/liquid, liquid/liquid or solid/liquid interfaces. Adsorption of a surfactant molecule at interfaces is favored, as its solubility in either of the media is low. After all free space at the interfaces has been occupied, the critical micelle concentration (CMC) is reached. Above this concentration the surfactant molecules start to aggregate in micelles, rods, lamella structures or sponge-phases [4]. Whether a micelle or another kind of aggregate is formed depends on the relation between the effective size of hydrophobic and hydrophilic group in the surfactant molecule. This relation is called critical packing parameter (CPP) and is expressed by the following equation:

$$CPP = \frac{v}{l_c a_0}$$

Equation 1 [5]

v: Hydrocarbon chain volume

a_0: optimal surface area per head group

l_c: critical chain length (correspondences to about the fully extended alkyl chain length)

Depending on the value of the CPP different kinds of aggregates as shown in Figure 2 are formed above the CMC.

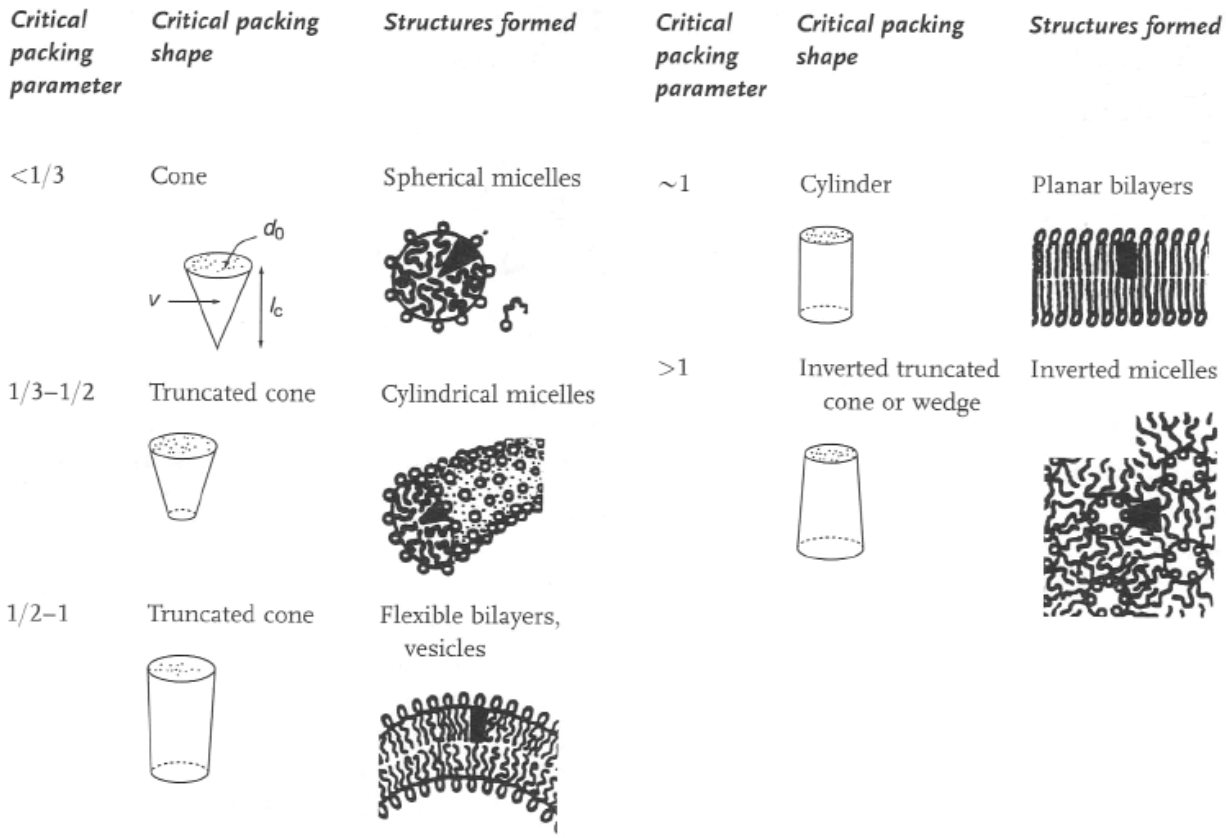


Figure 2: Schematic display of the different forms of surfactant aggregates depending on the value of the CPP [5]

The concentration at the transition between adsorption and aggregation point is called critical-micelle-concentration (CMC) and is depending on the type of surfactant as well as on parameters such as solvent, temperature, salt concentration, etc. [4]. In case of an ionic surfactant containing, for example, a weak acid group its properties and so its CMC are influenced by the pH of the medium. At a pH below its pKa value it is hardly soluble in water, thus having a low CMC and vice versa for a pH value above its pKa value. The counter-ions influence the effective charge of the hydrophilic group as well. Ca^{2+} ions, for example, reduce the charge density of the anionic head group and thus the hydrophilic interaction. Consequently, the solubility of the surfactant molecule will be reduced and so the CMC. The addition of electrolytes has the same effect on the charge density of the ionic hydrophilic group and so on the CMC of anionic surfactants. Moreover, as the charge density is reduced, the repulsion between the hydrophilic groups is reduced as well thus promoting the formation of more complex surfactant aggregates (see also Figure 2).

The properties of anionic surfactants are only to a small degree influenced by temperature in contrast to the properties of nonionic surfactants and so are their CMCs [6]. This is explained by the hydration of its polyethylene oxide chain. A highly oriented sheath of the water molecules is formed, where the water molecules

are aligned towards the polar oxygen atoms of the polyethylene oxide chain. This leads to a higher entropy of the system and thus to lower solubility of the nonionic surfactants, which is about 100 times lower compared to ionic surfactants [7]. With increasing temperature the motion of the water molecules increases and the hydration becomes less favorable. This leads to a lower solubility of the surfactant with a minimum at the cloud point. The name “cloud point” is due to the agglomeration of surfactant molecules as the water phase can no longer solubilize them. The cloud point is depending on the character of the hydrophobic group and length of the polyethylene chain and is characteristic for the respective nonionic surfactant [6].

In Figure 3 the changes in the physico-chemical properties of a surfactant at the CMC are summarized.

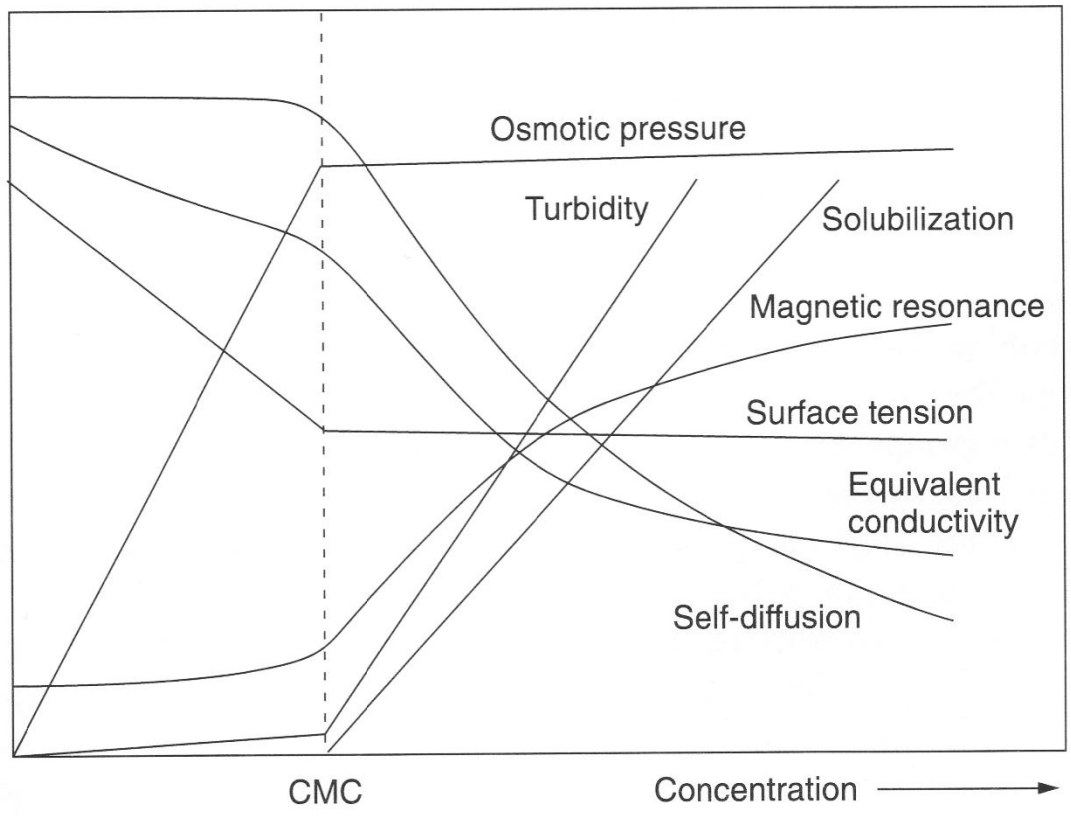


Figure 3: Illustration of changes in the properties of a surfactant at the CMC [8].

As described the CMC depends on the choice of surfactant and the physical-chemical conditions in the respective media. A low CMC is viewed as beneficial as less amount of surfactant is needed until all available interface areas in a system are covered and aggregates are formed. Nevertheless, the surfactant has to be still soluble in the respective medium. The aggregates of surfactants formed above the CMC are depending on the actual CCP value of the surfactant as shown before in Figure 2.

These aggregates are available as a repository for the surfactant molecules in many different applications. If new active surface area is created, for example during emulsification of an agrochemical product in water for preparation of a spray liquid, aggregates are readily disintegrated and adsorb at the newly created interface between oil droplets and the aqueous continuous phase. The surfactant molecules form a barrier at the interface of the oil droplets which hinders aggregation and coalescence of the oil droplets thus stabilizing the emulsion. This barrier is realized through electrostatic repulsion and/or steric hindrance. This is schematically displayed for electrostatic repulsion in Figure 4 (a) and for steric hindrance in Figure 4 (b)

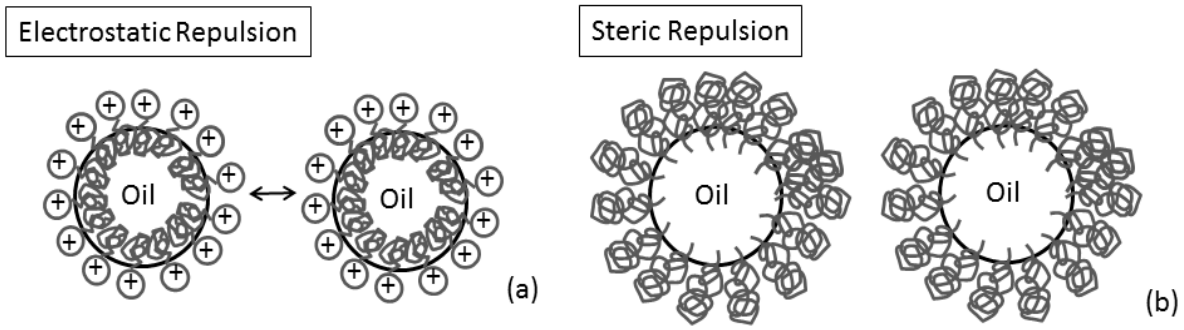


Figure 4: Barrier function of surfactant layers at the interfaces of oil droplets in water through electrostatic repulsion (a) and/or steric hindrance (b)

The effectiveness of the barrier is depending on the speed (kinetic) in which it is formed and on the thermodynamic equilibrium between the interface and the continuous phase. In complex mixtures of different surfactants, for example in agrochemical formulations, the equilibrium is influenced by all surface active compounds. As a consequence, formation and persistence of the interfacial barriers can only be determined via storage or application tests where coalescence of emulsions or particle aggregation and sedimentation in suspensions are observed over time. Based on the results, the composition of the formulation may be adjusted to improve the efficacy of the surfactant system with respect to stability of the formulation during storage and / or the stability of the spray broth during application.

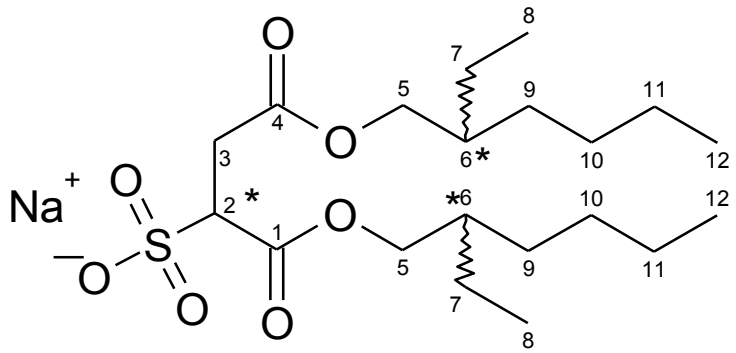
In the focus of this work were two commercially available surfactants, sodium bis(2-ethylhexyl) sulfosuccinate (anionic) and tristyrylphenol (TSP) ethoxylates with an average number of 16 ethylene oxide units (TSP-16-ethoxylates; nonionic). As described, the properties of complex mixtures of surfactants, such as in agrochemical products, are depending on many factors, which makes it very difficult to predict and influence processes like coalescence of emulsions or sedimentation in suspensions. This is in particular the case, if technical products rather than pure surfactants are used that vary in their content of by-products. In the following the composition

and properties for both target surfactants are described with the focus on potential by-products in the technical products originating for the production process.

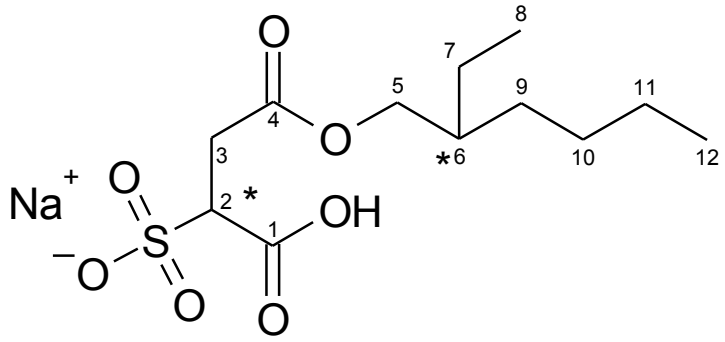
1.2.1 Anionic Surfactant: Sodium bis(2-ethylhexyl) Sulfosuccinate (Aerosol OT or AOT)

Anionic surfactants are the most commonly used type of surfactants in industrial applications. Typically they consist of a linear alkyl chain with 12 – 16 carbon atoms [9]. The negative charge is introduced via carboxylate, sulfate, sulfonate or phosphate groups, usually with sodium as counter ion.

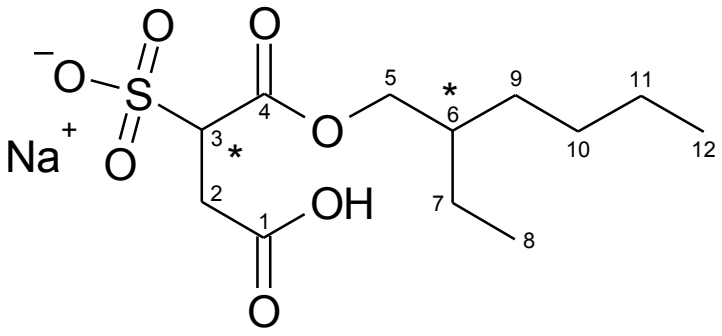
The investigated surfactant was sodium bis(2-ethylhexyl) sulfosuccinate (also called Aerosol OT or AOT) a commonly used anionic surfactant in agrochemical formulations (see Figure 5, 1) [10-12]. In commercial AOT product, pure AOT is purchased dissolved in light aromatic naphtha solvent (Trade name: Solvesso 100).



Structure of sodium bis(2-ethylhexyl) sulfosuccinate (1) (a)



Structure of sodium 1-carboxy-3-[(2-ethylhexyl)oxy]-3-oxopropane-1-sulfonate (2) (b)



Structure of sodium 3-carboxy-1-[(2-ethylhexyl)oxy]-1-oxopropane-2-sulfonate (3) (c)

Figure 5: Structural formulae of AOT (a) and monoesters 2 (b) and 3 (c) including their centers of chirality indicated by [*]

Through previous work it is known that commercially available AOT product is not pure but contains two monoester sulfosuccinate isomers as by-products [13]. Synthesis of sodium bis(2-ethylhexyl) sulfosuccinate is schematically displayed in Figure 6.

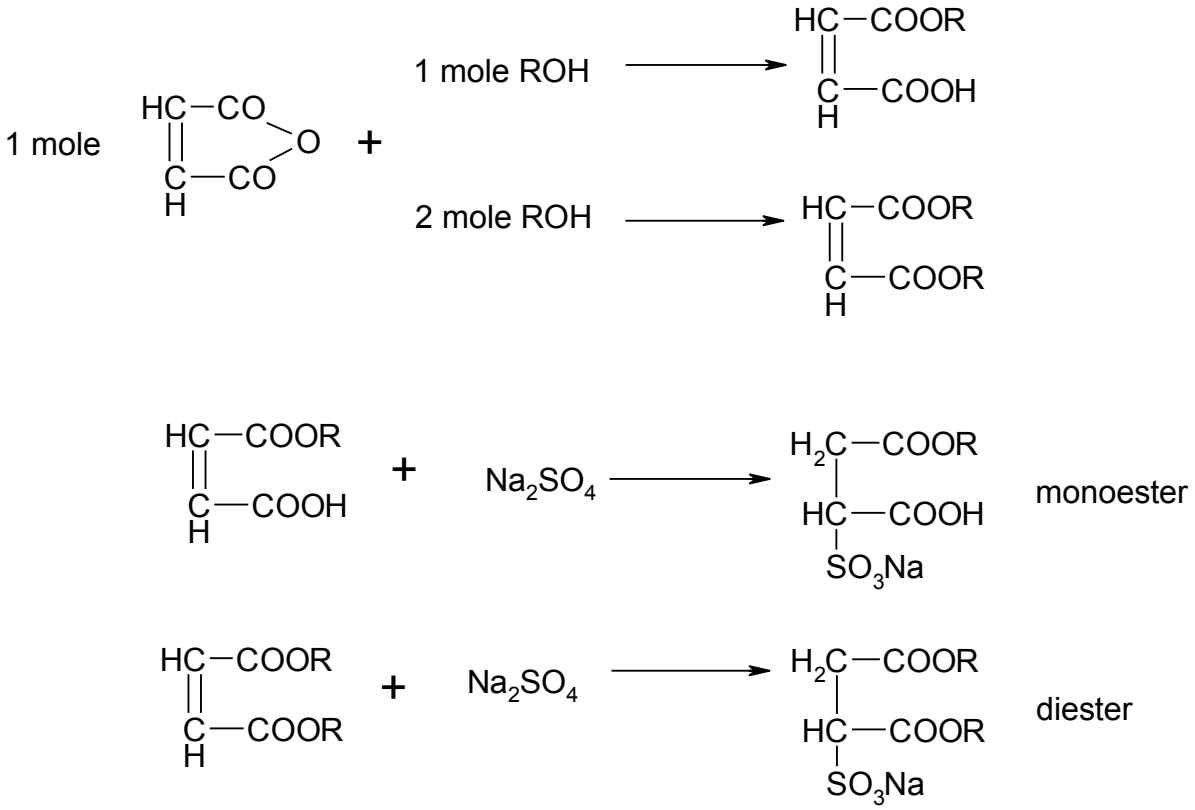


Figure 6: Reactions in the synthesis of sulfosuccinic surfactants [14]

These monoesters are surface active and have been used in the past as wetting agents [15].

1.2.2 Nonionic Surfactant: Tristyrylphenol Ethoxylates

Nonionic surfactants commonly have a polyethylene oxide chain as hydrophilic group bound via either hydroxyl or carboxyl groups or primary or secondary amines to various hydrocarbons. For the synthesis of nonionic surfactants the hydroxyl group is functionalized with ethylene oxide either by base or acid catalysis. In the following Figure 7 a base catalyzed polymerization is shown.

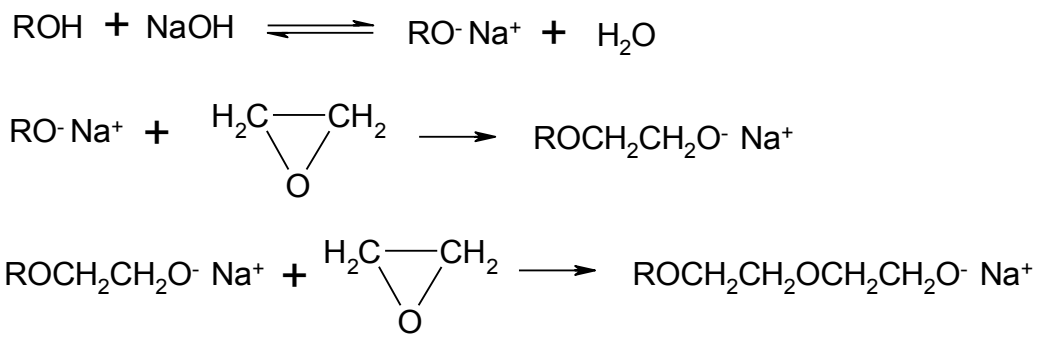


Figure 7: Scheme of synthesis of nonionic surfactants [16]

As a result of the polymerization reaction a mixture of homologues with different degrees of ethoxylation is obtained. In addition to the reaction conditions, the distribution of products depends on the acidity of the hydroxyl group. For less acidic hydroxyl groups such as of alcohols or sugars unreacted starting material remains and has to be removed afterwards. Besides, polyethylene glycol is formed in all cases, which may be necessary or unwanted depending on the application [17].

For phenol derivatives, such as the tristyrylphenol (TSP), the acidity of the hydroxyl group (pKa (TSP) = 11.0) is higher than the acidity of the hydroxyl group of the already reacted phenol ethoxylates. As a consequence, the addition of polyethylene oxide to phenol is thermodynamically favored over the addition to already reacted phenol ethoxylate so that no residual phenol remains after polymerization [17-19]. The starting material, however, may have different qualities, comprising a variable number of styrenes bound to the phenol group. Therefore, a complex mixture of different molecules is expected as shown in Figure 8.

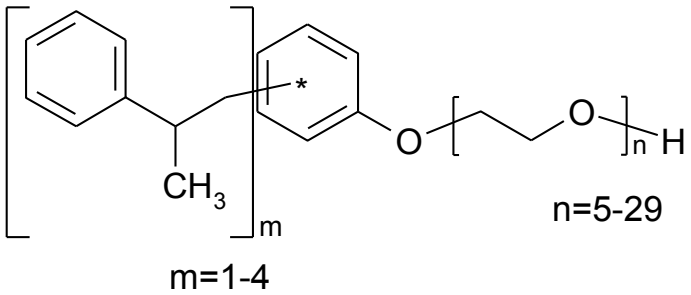


Figure 8: Structure of commercially available tristyrylphenol (m=3) with an average number of ethylene oxide of n = 16.

1.3 Agrochemical Formulations

Agrochemical formulations are mixtures of one or more active ingredient(s) (a.i.) and inerts such as surfactants, solvents, defoamer, stabilizer and partially sticker among others, which are added to provide stable and well applicable products. Depending on the physico-chemical properties of the a.i. different forms of formulations can be developed. Typical ones are emulsion concentrates (EC) and suspension concentrates (SC) [20]. EC are chosen for an a.i. which has a high solubility in organic solvent and a good stability against chemical transformation (e.g., by hydrolysis, oxidation, etc.). For an a.i. with poor solubility in water and organic solvents or when stability against chemical transformation is limited, a SC is a better choice. For this purpose the a.i. has to be milled (micronized) to achieve particle sizes in the micrometer scale in order to ensure uniform distribution of the a.i. in the final product. As the a.i. is not dissolved, chemical reactivity and chemical transformation is reduced. Nevertheless the micronized a.i. particles have to be stabilized against agglomeration or sedimentation in the formulation. Agglomeration can be prevented by using surfactants which adsorb to the interface of particle and media and thus build up a barrier against agglomeration of the a.i. particles. For this purpose usually large polymeric surfactants are used, which are also kinetically hindered in their adsorption-desorption processes due to their structure. This further stabilizes the barrier and moreover hampers crystal growth of the particles [3]. Whereas for larger particles the surfactant is adsorbing at the particle surface smaller particles may be solubilized within formed micelles, because the hydrophobic hydrocarbon chains act as a liquid in which the a.i. is solved [4;21]. The viscosity of the formulation can be adjusted against sedimentation via thickeners or gelling agents, slowing the sedimentation processes to an acceptable degree. In addition to the function to preserve the a.i. and the agrochemical product against degradation or unwanted changes in its rheological properties surfactants have the task to enable homogenous distribution of the a.i. in the spraying liquid. For this task wetting agents are used that spontaneously adsorb to the interface of the a.i. particles during the mixing process thus making them dispersible in the aqueous continuous phase of the spraying liquid. Moreover, some surfactants have the ability to facilitate the uptake of the a.i. by the plants. Thereby they are assisted by solvents tailored to dissolve the a.i. and lead to swelling the waxy layer of the leaf surface thus allowing the migration of the a.i. from the leaf surface into the cuticle and then into the plant [3].

All in all, the formulation has the task to preserve the a.i. until its use, to ensure maximum homogeneity of a.i. in the final application and to enhance its performance, e.g., by promoting its uptake by the plant. As described this is achieved with various surface active agents specialized for their specific task. The selection of a.i.(s) and inerts has to consider all these requirements, and in addition their potential interaction in the formulation. Some of the effects such as solubility may be anticipated, others may only be elucidated during storage test, such as chemical

stability of the a.i.(s) or long term processes such as sedimentation in a SC or coalescence of emulsion droplets in EC [22;23]. Nevertheless, “to date, such a choice is made by trial and error procedure ” [24] and setting-up an agrochemical formulation requires experience paired with theoretical knowledge of colloid chemistry [18].

1.4 Analysis of Surfactants

The various tasks surfactants are prepared for require defined production quality and their control. In order to control and monitor the composition and content of the actual surfactant, analytical methods are necessary.

For ethoxylated surfactants there is no analytical standard available for each single component. In consequence, quantitative methods rather focus on determining a sum parameter than the quantitative content of the single components. One possibility to determine the total content of a nonionic surfactant is using modified Dragendorff reagent to precipitate the ethoxylated surfactant with electrochemical quantification of the precipitate. The use of this method has been described for example for the determination of the total amount of nonionic surfactants in waste water, however not for agrochemical formulations [25-27].

Another approach is the identification and determination of the single ethoxylated entities. For separation of the single components several techniques are available. One of the earlier ones is thin layer chromatography, which separates the ethoxylated surfactant either according to the hydrophobic hydrocarbon group using a reversed phase stationary phase or according to the degree of ethoxylation using a normal phase. For detection staining derivates with ultraviolet(UV)-active groups have to be used [28;29].

For nonionic surfactants with lower degree of ethoxylation separation via gas chromatography (GC) and detection either via flame ionization detector (FID) or mass spectrometry (MS) is possible. As the FID is considered a universal detector because its response depends mainly on the number of carbon atoms in the analyte the quantitative distribution of the single ethoxylates can be estimated without the use of an analytical standard [30-32]. The detection via MS provides structural information for the respective component, which enables structure elucidation. The signal response, however, is very dependent on the components structure [33].

Using liquid chromatography (LC) nonionic surfactants with a higher degree of ethoxylation can be analyzed which are not accessible to the analysis via GC. Separation according to the degree of ethoxylation can be achieved via normal phase-liquid chromatography (NP-LC) [34] or via hydrophilic interaction liquid chromatography (HILIC) [35]. Both have highly polar stationary phases, which interact with the hydrophilic polyethylene chain. In case of HILIC the mode of separation is partition chromatography between an immobilized ionic aqueous stationary phase and an organic mobile phase, such as acetonitrile. The aqueous

phase is formed by water molecules which adsorb to a hydrophilic stationary phase formed for example by amphoteric surfactants bound to porous silica. To sustain the aqueous phase the mobile phase consists of a organic/aqueous mixture with a ratio of at least 9:1 (v/v). Typically, the aqueous phases have a salt concentration of 5-20 mM, in order to avoid peak tailing [36]. Separation according to the hydrocarbon chain is achieved via reversed phase liquid chromatography (RP-LC) [37-40]. Another possibility to chromatographically separate according to the degree of ethoxylation and/or the hydrophobic group is liquid adsorption chromatography (LAC). The separation here is determined by the number of repeating structural units adsorbing to the stationary phase. In combination with size exclusion chromatography (SEC) liquid exclusion adsorption chromatography (LEAC) is possible which allows separation of ethoxylated surfactants under isocratic conditions. Isocratic elution is mandatory to enable quantitative determination of the different degrees of ethoxylation for a nonionic surfactant via universal detectors such as refractive index in combination with an evaporation light scattering detector (ELSD). For such quantification the full chromatographic separation of the single compounds is necessary, which is possible for binary nonionic surfactant mixtures but has not been demonstrated for complex mixtures such as agrochemical formulations [41-45]. Qualitative information in complex samples such as cleaning agents with mixtures of different nonionic surfactants can be provided via 2-dimensional liquid-chromatography with mass spectrometric detection [46]. Using either MS-MS or Time-of-Flight MS (ToF-MS) both techniques are more sensitive than for example ELSD or UV-detectors and enable identification via the (exact) molecular mass and/or specific fragments [31;38;47;48].

For anionic surfactants, such as sodium bis(2-ethylhexyl) sulfosuccinate, there are several methods known using RP-LC either coupled to UV-VIS-, ELSD, MS or due to the carried charge also electrochemical detectors [49-52]. Ionic surfactants in principle can also be analyzed using ion chromatography [53] or capillary electrophorese [54].

In this work RP-LC coupled to ToF-MS with exact mass measurement was used for analysis of both the anionic and the nonionic surfactants. Some instrument characteristics are described in the following. The coupling of LC to MS is the most powerful tool for the analysis of surfactants. Using ToF-MS with exact mass measurement further enables structure elucidation for yet unknown compounds and identification of known ones. For identification of by-products and characterisation of the surfactant, ToF-MS with exact mass measurement was the instrument of choice for this work.

Reversed phase liquid chromatography was chosen to ensure separation according to the length of the alkyl chain and the coupling to the MS was performed via atmospheric pressure chemical ionization (APCI),

electrospray ionisation (ESI) and atmospheric pressure photoionization (APPI). The set-up of these three ionisation devices is shown in Figure 9.

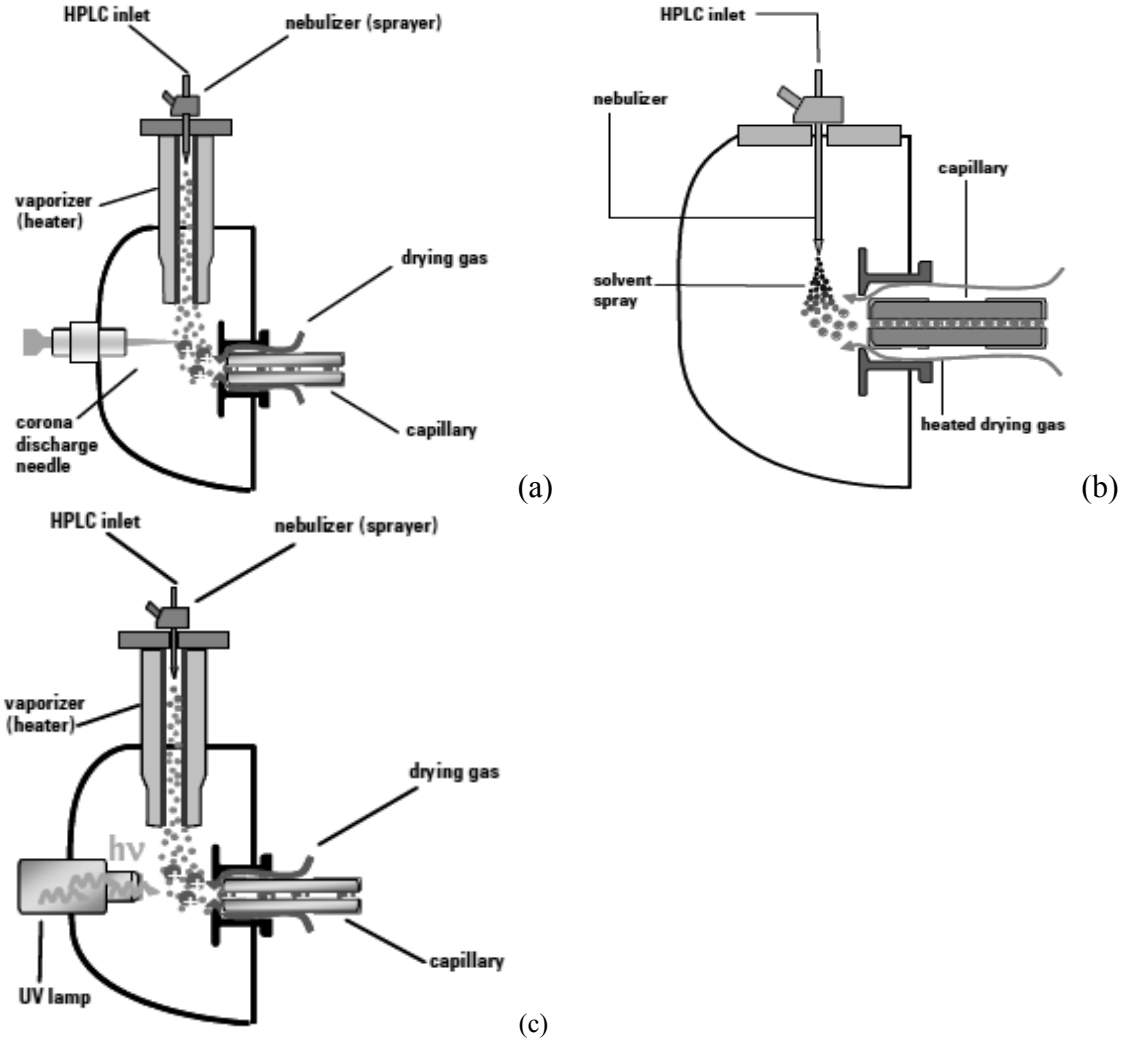


Figure 9: Schematic set-up of APCI (a), ESI (b) and APPI (c) [55]

In APCI the LC-eluent is nebulized with nitrogen gas into a heated tube. The eluent is evaporated and the analyte is carried by the gas flow through the column. There the analyte is ionized by a plasma of solvent molecules created by the discharge of the corona needle.

In ESI the eluent droplets are charged during the nebulization process at the tip of the nebulizing needle. In the spray cone, the so called Taylor-cone, the charge is transferred onto the surface of the solvent droplets as they form. As these are dried on their passage with a hot nitrogen stream, the charge is confined to the shrinking droplet surface, causing a further atomizing of the droplet. During this process the electric repulsion on the droplet surface is surpassing the surface tension and cohesion among the solvent molecules in the droplet. Finally, the charge is transferred to the analyte molecule itself.

In APPI the set-up of the nebulization process is the same as for ESI. The ionization however is carried out via a krypton UV-lamp emitting photons with an energy of about 10 eV. These are able to interact with molecules having conjugated π-systems such as aromatic rings only. For analytes without such π-systems incorporated modifiers like toluene have to be used, which then transfer the charge to the analyte [56].

The ionization depends on the chosen device. APCI is leading to more in-source fragmentation and less adducts for example for nonionic surfactants [57], ESI is producing more adducts and multiple charged entities, which is especially used for protein and polymer analysis. APPI can be very sensitive for aromatic compounds, however, it needs modifiers for analytes without conjugated π-systems.

The formed ions are guided into the MS via a series of orifices and electromagnetic lenses. These orifices are shaped in a way to ensure low pressure inside the MS-instrument, but also to allow entrance of ionized molecules. The lenses are creating a focused ion beam which is then accelerated into the time of flight tube by the ion pulser as shown in Figure 10.

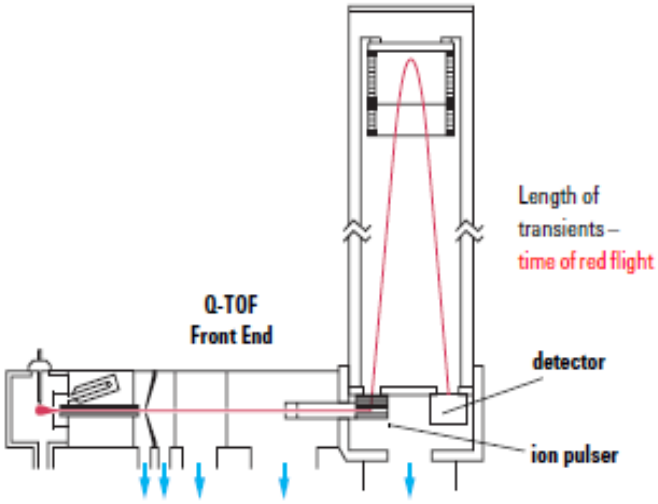


Figure 10: Scheme of a ToF mass spectrometer with highlighted ion flight path and length of transients, respectively [58].

The measurement is realized over the time of flight for different molecules. Every molecule is pushed with the same impulse and according to Equation 2 with given impulse p, time of travel t and the flight path the actual m/z-value for the respective ion is determined

$$m/z = p \cdot \frac{t}{s}$$

Equation 2

t: time of flight for the respective ion

m/z: m/z-value

s: flight path

p: pushing impulse

This kind of mass spectrometry depends on the accuracy of time measurement. The better the resolution for time measurement, the smaller differences between analyte masses can be resolved. Another important part is the accuracy of mass measurement. This delta is calculated according to Equation 3, and gives the relative difference between the exact and the actually measured mass [59;60].

$$\Delta ppm = \frac{m/z_{measured} - m/z_{exact}}{m/z_{exact}} \cdot 10^6$$

Equation 3:

Δppm : Relative delta value as parts per million between actual and measured mass

$m/z_{measured}$: measured mass

m/z_{actual}: actual exact mass

To achieve an acceptable accuracy the ToF-MS has to be mass calibrated daily and corrected during measurements against at least two reference masses, to compensate for differences in the extension of the flight tube caused by temperature fluctuations during the day. The ToF-MS used for this work is able to perform exact mass measurement with an accuracy below 1 ppm, which is often sufficient to determine the elemental formula for an organic molecule detected [59;60].

Besides the mass accuracy the MS has to be able to resolve the given m/z-signal well enough to distinguish it from other signals. Mass resolution is calculated according to Equation 4 using for Δm the full width at half peak maximum (FWHM) also graphically shown in Figure 11 [61;62].

$$R = \frac{m}{\Delta m}$$

Equation 4:

R: resolution

m: actual m/z-value

Δm : full width at half peak maximum (FWHM)

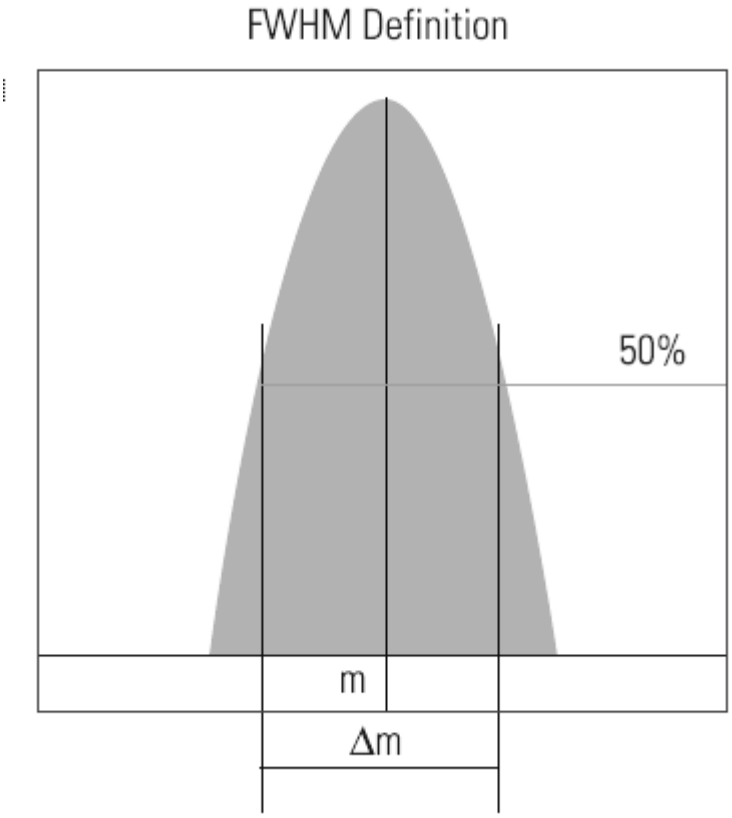


Figure 11: Definition of Δm at full width at half peak maximum (FWHM) [62]

The instrument used in this work achieved a resolution of 10.000 at FWHM for a mass signal at m/z 200, which was sufficient to perform exact mass measurements for this work.

1.5 Quality Control

The control of product quality is important for various areas and applications such as material control in construction or mechanical engineering, control of food quality, quality control of pharmaceutical and agrochemical products. These controls are necessary to avoid malfunction of products and in this course hazards to environment, operators and consumers of the final products.

To conduct these controls instrumental analyses with various scopes have been established. For material analysis such as for steel for construction there are different non-destructive techniques available, for example electrochemical testing [63], magnetic resonance [64] or ultra-sonic testing [65]. Food quality can be tested for example on hazardous or unwanted microorganism, which can be identified via specific DNA-sequences [66] or their metabolism products via liquid chromatography coupled to fluorescence detection [67]. Also the toxicologically relevant residues of agrochemical products in crops or pharmaceuticals used in livestock farming have to be analytically monitored in the raw product and in the processed food [68;69]. For this purpose mainly analytical methods using LC-MS [70] or LC-MS/MS [71;72] have been established.

Pharmaceutical and agrochemical products are commonly controlled for their content of the active ingredient(s) in order to avoid over or under dosing on the target. Furthermore, the functionality of the final products over time has to be controlled via storage tests. After the defined storage time, the content of active ingredient(s) and parameters such as viscosity, dispersity or homogeneity of the final product are tested [22;23].

1.6 Anti-Counterfeiting

Anti-counterfeiting is concerning the monitoring and the control of the origins of raw materials and products. This is necessary to avoid inferior quality or malfunction of the final product due to insufficient quality of the raw product(s). Anti-counterfeited products can cause economic damage for the product manufacturer and pose potential hazards to environment and consumer due to an altered choice of raw products. These raw products are often cheaper surrogates which have neither been investigated with regard to their compatibility when used in the product or on potential hazards nor been registered by the authorities.

There are various ways to identify counterfeited raw materials and products, such as specialized packing materials [73] or radio frequency identification (RFID) [74]. Furthermore, bulk analysis of products with spectroscopy techniques like near infrared (NIR) spectroscopy [75;76] or nuclear magnetic resonance (NMR)

spectroscopy [77] are used, which detect the spectroscopic fingerprint of a mixture. These fingerprints hold unique features enabling the distinction between the original and the counterfeited product. As these techniques are very sensitive to the chemical composition, they are at the same time very sensitive to non-chemical influences such as grain size, morphology etc. Therefore they require time consuming calibration and constant monitoring of these non-chemical features. Less sensitive to non-chemical influences are techniques focusing on the nature and content of the active ingredients using for example LC-MS analysis of the by-product content of the actual active ingredient in pharmaceutical products [78;79]. Amongst these the analysis of stable isotope ratios via isotope ratio mass spectroscopy (IRMS) is an important technique. The potential use of this feature for anti-counterfeiting has been demonstrated for the herbicide glyphosate [80] to distinguish between active ingredient of the original manufacturer and of different counterfeited sources. The described techniques are also applied to investigate the origin and nature of food raw products thus trying to identified faked beverages [81;82] or not labeled additions of synthetic ingredients instead of natural ones, such as caffeine of synthetic or natural origin [83].

1.7 Scope of the Thesis

In this work two commercially available surfactants commonly used in agrochemical products, sodium bis(2-ethylhexyl) sulfosuccinate (anionic) and TSP-16-ethoxylates (nonionic), are analytically characterized with regard to their main and by-products depending on their suppliers. Differences in by-product content between suppliers, their use for product identification in the final agrochemical formulation and their impact on the properties of the agrochemical formulation using the respective surfactant were investigated.

In **chapter 2** the focus is on the development of an analytical method for the analysis of the anionic surfactant sodium bis(2-ethylhexyl) sulfosuccinate, trade name Aerosol OT or AOT, and its two isomeric surface active mono esterified by-products. As both monoesters are used as surfactants in other applications, their contents may have potential influence on the properties of AOT and the agrochemical formulation using it. Analytical standards for both by-products were prepared and the method validated according to DIN 32645 for all three analytes using LC-ToF-MS with exact mass measurement.

In **chapter 3** the differences regarding the content of the monoesters as by-products were investigated for four different suppliers of AOT product with the analytical method developed in the previous chapter. The influence of these differences in content of monoesters on the properties of a model agrochemical formulation was

explored using storage tests. The differences in the by-product content of AOT product of different suppliers were statistically tested on their use as potential identifiers for anti-counterfeiting purposes in the raw product and in an agrochemical product.

Chapter 4 focused on the development of an analytical method for the quantitative characterization of the nonionic surfactant TSP-16-ethoxylates according to the content of its main and by-products. As a novel approach a combination of instrumental analysis via LC-ToF-MS with exact mass measurement and multivariate data analysis on the collected data was investigated as it is used in proteomics or metabolomics. Using this method possible differences in the main and by-product content of TSP-16-ethoxylates of four different suppliers and qualities were investigated and tested on statistical significance. Additionally, the use of these differences on supplier identification for anti-counterfeiting in the tristyrylphenol ethoxylates raw product and in the final agrochemical product using this nonionic surfactant was tested.

In **chapter 5** general conclusions on the results and findings in this work are given together with an outlook on the use of the developed techniques for future investigations.

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2. LC-MS Quantification of a Sulfosuccinate Surfactant in Agrochemical Formulations

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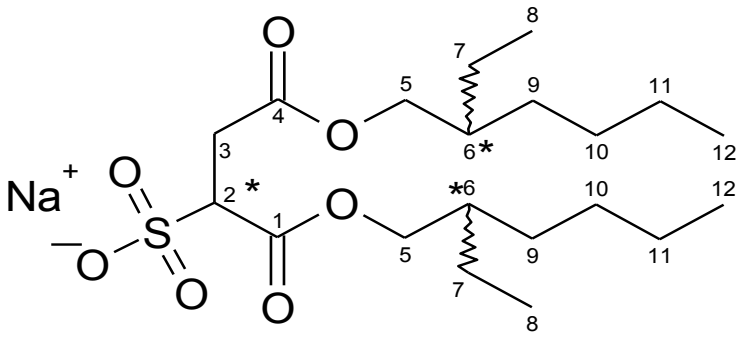
2.1 Abstract

Agrochemical products are mixtures of active ingredient(s) and inerts, which serve as dispersing or wetting agent or as emulsifiers. Varying qualities of these raw materials can have a significant impact on the properties of the final agrochemical product and so its quality has to be controlled. In this work sodium bis(2-ethylhexyl) sulfosuccinate (Aerosol OT or AOT) in commercial AOT products and its surface active isomeric by-products sodium 1-carboxy-3-[(2-ethylhexyl)oxy]-3-oxopropane-1-sulfonate and sodium 3-carboxy-1-[(2-ethylhexyl)oxy]-1-oxopropane-2-sulfonate were analyzed. A method using liquid chromatography coupled with Time-of-Flight mass spectrometry (LC-ToF-MS) with exact mass measurement was developed to quantify these molecules simultaneously. Both by-products were not commercially available and thus were synthesized as analytical standards for method validation. For this purpose, two regio-selective syntheses were developed. Validation was done according to DIN 32645 and recovery and precision for two different matrices were determined. Significant differences were observed in the by-product spectrum of real samples AOT products of three different suppliers. Their influence on the properties of an agrochemical can now be investigated, as a precise and accurate determination of the target analytes has been developed in this work.

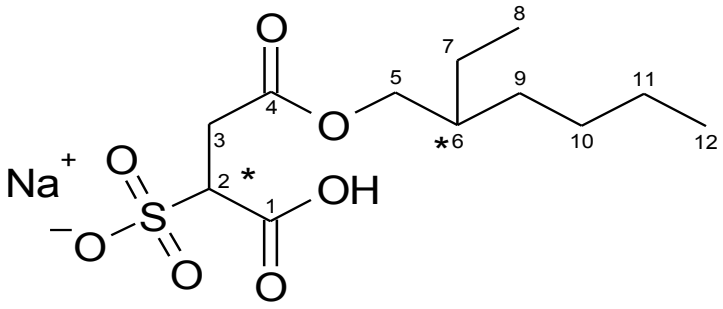
2.2 Introduction

Pesticide formulations are mixtures containing active ingredient(s) and surfactants, solvents, sticker, etc. The components of a formulation other than the active ingredient are called inerts. Typical representatives of pesticide formulations are emulsion concentrates (EC) or suspension concentrates [1]. In a formulation several inerts having different functionalities are used. They have to ensure the active ingredient's physico-chemical stability during storage. Inerts stabilize dispersions against sedimentation in suspension concentrates or prevent emulsion droplets in emulsion concentrates containing the active ingredient from agglomeration (syneresis) or coalescence (Ostwald ripening). They are responsible for the formulation's properties such as viscosity, dispersity or homogeneity. During application they assist with distributing the active ingredient(s) evenly over the target crop and to enhance the performance, e.g. by promoting its uptake by the plant. The selection of inerts has to consider potential interactions among inerts and/or with the active ingredient(s). Some effects such as on solubility may be anticipated, since they are either known or easily determinable for the chosen compounds, other effects may only be elucidated during storage tests [2;3]. Nevertheless, "to date, such a choice is made by trial and error procedure " [4] as setting up an agrochemical formulation [5;6].

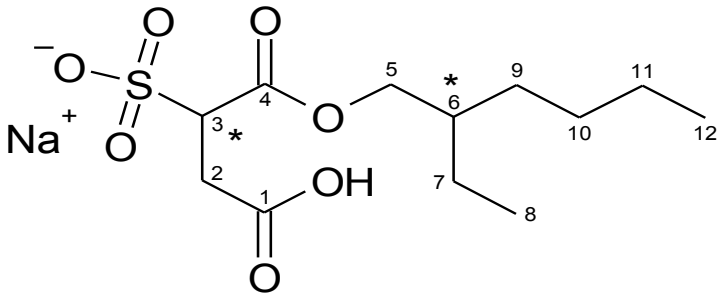
Changes in formulation composition potentially have an impact on the interactions among the inerts and may provoke unwanted behavior. Changes can involve an altered production process for an inert of one supplier or a change of suppliers for an inert. To avoid a negative impact on the formulation the inerts should be analytically monitored. This work's focus is on sodium bis(2-ethylhexyl) sulfosuccinate (i.e., Aerosol OT or AOT), (see Figure 5, **1**), which is an anionic surfactant commonly used in agrochemical formulations [7-9].



Structure of sodium bis(2-ethylhexyl) sulfosuccinate (1) (a)



Structure of sodium 1-carboxy-3-[(2-ethylhexyl)oxy]-3-oxopropane-1-sulfonate (2) (b)



Structure of sodium 3-carboxy-1-[(2-ethylhexyl)oxy]-1-oxopropane-2-sulfonate (3) (c)

Figure 12: Structural formulae of AOT (a) and monoesters 2 (b) and 3 (c) including their centers of chirality indicated by [*]

Through previous work it is known that commercially available AOT product is not pure, but contains two monoester sulfosuccinate isomers as by-products [10]. On the base of the synthetic route of pure AOT, it is reasonable to expect both regio-isomers monoester 2 and 3, shown in Figure 5, to be present in those products. These monoesters are also surface active and have been used in the past as wetting agents [6]. Changing their content might change the properties of the original wetting agent within the formulation and lead to unwanted side effects.

AOT product is supplied as solution of pure AOT in light naphtha solvent with a ratio of 64/36 (w/w) AOT/light naphtha solvent. Whereas the AOT content is specified and declared by the supplier, the contents of monoesters 2 and 3 are not routinely controlled and may vary according to the manufacturing process applied [11-13]. Taking into account afore mentioned potential problems in the final formulation, however, their content should be monitored. In cases where surfactants are either hardly degradable as in some halogenated surfactants or their metabolites are toxicologically relevant as for nonylphenolethoxylates, these were in particular investigated in environmental samples [14-16]. Pure AOT, however, has only been monitored as ecosystem indicator in the course of its use as dispersant after the “Deepwater Horizon” oil spill in the gulf of Mexico 2010, but has not been found toxicologically relevant so far [17-20]. Therefore, the analytical method developed in this work for AOT and monoester 2 and 3 was with the focus on product quality and control only.

Although there are a number of known methods to determine AOT [20-23] to the knowledge of the authors there is none to quantify AOT and its isomeric by-products –the monoesters 2 and 3- in one analytical method. The aim of this work was therefore to develop and validate such a method based on liquid chromatography coupled with Time-of-Flight mass spectrometry (LC-ToF-MS). Finally, characterization of AOT product delivered by different suppliers was performed.

2.3 Experimental Section

2.3.1 Chemicals and Reagents

High purity water was obtained by a Milli-Q-gradient A10 system (Millipore, Eschborn, Germany). Acetonitrile, methanol, formic acid and sodium bis(2-ethylhexyl) sulfosuccinate all of p.a. grade were purchased from Sigma Aldrich. Isomeric monoester 2 and 3 had to be prepared as they were not commercially available. Experimental conditions, method adaption and development for both monoesters are described in the Supplementary.

2.3.2 LC-MS Analysis

Reversed phase-liquid chromatography (RP-LC) was used to separate AOT and monoester 2 and 3. High purity water (Millipore) and methanol were used as LC eluents. In order to enhance retention of monoester 2 and 3 on a RP-column protonation of their carboxylate group had to be ensured. To that end, the pKa-value of the carboxylic acid group for each monoester was required. The pKa-value was known for monoester 3 (pKa = 5.2) [24], but had to be determined for monoester 2 experimentally (pKa = 4.0). Experimental details of pKa determination are given in the Supplementary. The pH of the eluents was then accordingly adjusted to pH 2.8 with 20 mmol formic acid/liter eluent. The sulfonic acid group of AOT and the monoester 2 and 3, however, still has a permanent charge which may compromise RP-HPLC separation and also impairs ionization efficiency in atmospheric pressure chemical ionization (APCI).

2.3.3 LC-MS-System

An Agilent 1200 SL HPLC coupled to an Agilent 6220 Accurate-Mass-TOF mass spectrometer with interchangeable dual-sprayer electrospray ionization (ESI) and APCI sources was used for LC-MS. All measurements were done on a Waters XBridge C18 (50 x 2.1 mm, 2.5 µm) column, which was chosen due to its good temperature and pH stability, to minimize signals in MS caused by column bleed [25].

For sample measurement a gradient was applied to ensure complete elution of matrix. Starting with 5% (v/v) methanol, raised to 95% in 6 min, hold for 3 min at 95%, decreased to 5% in 0.5 min and equilibration for 1.5 min at 5%. Total run time was 11 min with a flow of 0.7 mL/min and a column temperature of 55 °C. Flow was directed without split via the APCI source and with a split of 1:6 (MS:Waste) via the first sprayer needle of the dual-ESI source into the mass spectrometer. To realize the split an adjustable flow-splitter supplied by RESTEK was used equipped with resistors which enable a constant split ratio independent of changes in viscosity or pressure. Mass spectra were obtained in negative mode through the whole run. Every second a spectrum was obtained with 4925 transients per spectrum and a mass range of 105-1700 m/z. For the APCI source the parameters were 350 °C for gas temperature, 450 °C for vaporizer temperature, 8 L/min for dry gas, 30 psig nebulizer pressure and 4.5 µA corona current. For ESI the parameters were 350 °C for gas temperature, 8 L/min drying gas flow and 30 psig nebulizer pressure for both ESI sprayer needles of the dual-sprayer ESI source. For both sources capillary voltage was 3500 V, fragmentor voltage 100 V, skimmer voltage 60 V and octopole 1 RF Vpp 250 V.

Mass calibration was done for both sources with the corresponding calibration mixtures supplied by Agilent via the second sprayer of the dual-sprayer ESI source. Mass correction during analysis was handled on purine (neg.: $m \cdot z^{-1}$ = 119.036230 amu) and hexakis(1H,1H, 3H-fluoropropoxy)phosphazine (abbreviated: HP 921 (neg. +formate: $m \cdot z^{-1}$ = 966.000725 amu)). For analysis via the APCI source, a solution of both was delivered into the eluent after the LC unit via a tee with a flow of 0.2 mL/min. To manage the LC's pressure at the tee an additional Agilent isocratic HPLC pumping unit was used to deliver the recalibration mixture. For analysis via ESI the solution was delivered with a flow of 0.05 mL/min via the second sprayer needle of the dual-sprayer ESI source into the mass spectrometer.

2.3.4 Preparations of Standard and Sample Solutions

Stock solutions were prepared dissolving an equivalent amount of the respective analytes in a mixture of 50/50 (v/v) water and acetonitrile, both acidified with 100 mmol formic acid per liter solution, obtaining a concentration of 0.4 g/L. For preparation of the standard solutions the stock solutions were diluted to fit the concentration range 2 mg/L to 0.04 mg/L for the AOT and the monoesters.

For sample preparation of AOT raw product material 20 mg were diluted in 50 mL of 50/50 (v/v) acetonitrile/water acidified with 100 mM formic acid. The working solution for the measurement of AOT was diluted 1/1000 and for the measurement of monoester 2 and 3 it was diluted 1/20. For each sample five independently weighed replicates were measured.

The log mass solution was purchased by Agilent for both APCI- and ESI-source. For log masses a solution of Purine and HP 921 was prepared containing 1.0 µM Purine and 0.25 µM HP 921 in 95/5 (v/v) methanol/water. For measurement with APCI- and ESI-source a dilution of 1:100 was needed to avoid overloading the detector.

For testing the mass calibration during the analysis, a test sample containing molecules with known exact mass spanning the retention time window of the gradient analysis was analyzed at the beginning and the end of a test series. The composition of the test sample is given in Supplementary.

2.3.5 Data Analysis

The acquired scan data were either displayed as total ion chromatograms (TIC) or as extracted ion chromatograms (EIC) extracted on the exact molar masses of the analytes (m/z (AOT) = 421.2265 m/z; m/z(monoester 2 and 3) = 309.1013 m/z) and their A+1 and A+2 isotopic masses with a window of 100 ppm around each mass to account for potential mass divergences during the measurement.

Statistical tests were performed using Microsoft Excel. Five independently weighed replicates were measured for each production batch and all reported measurement results are averages of these five repetitive analyses. The respective standard deviation s divided by the square root of five is the standard uncertainty *u* of the average values, according to GUM [26]. For defining the expanded measurement uncertainty $U = k \cdot u$ a coverage factor of $k = 2.77$ was used. By this an interval around the results of a measurement was set that may be expected to encompass 95.0 % of the distribution of values that could reasonably be attributed to the measurement.

F-tests on variance were conducted on the replicates each sample and depending on its results an expanded paired t-tests or a t-test according to Welch was conducted with a level of significance of p = 0.05. To test whether the content of AOT in the investigated batches met the specified range of 62.5-66.0 % (w/w) a one-sided t-test with a p-value of 0.05 was conducted.

2.3.6 Validation

Validation of the developed method was done according to DIN 32645. Limits of linearity were defined by the linearity range of the used mass spectrometric detector and by the LOD for the analytes. The range of 0.04 mg/L to 2.0 mg/L was defined accordingly. The analytical parameters were calculated on basis of the linearity measurements according to DIN 32645.

Recovery and precision of the method were tested on two matrices spiked with analyte, to evaluate matrix effects on the analysis. The first matrix was the light naphtha solvent wherein dissolved AOT was purchased. The second matrix was an agrochemical formulation wherein AOT was commonly used as an inert. For spiking, AOT and monoester 2 were used at concentration levels 0.1 mg/L and 1.6 mg/L to represent both limits of the linearity region. Spiking was repeated 6 times to determine method precision. The precision at both concentration levels was then compared via an F-test to check on its homogeneity over the linear range.

2.4 Results and Discussion

2.4.1 Determination of AOT and both isomeric Monoesters 2 and 3

For method development a sample of AOT product was used, which contains pure AOT as well as the monoesters 2 and 3, to take potential interfering matrix effects into account. To achieve chromatographic separation the sample was at first analyzed with gradient elution, using water and methanol as eluents and APCI-MS for detection. Chromatographic separation, however, was not achieved under these conditions for the target analytes.

To increase chromatographic selectivity for both monoesters the eluents as well as the sample solutions were acidified with formic acid, in order to protonate the carboxylic group and hence making the whole molecule less polar.

Under these conditions the chromatograms shown in Figure 13 (a), diluted for detection of AOT, and Figure 13 (b), diluted for detection of monoester 2 and 3, respectively were obtained.

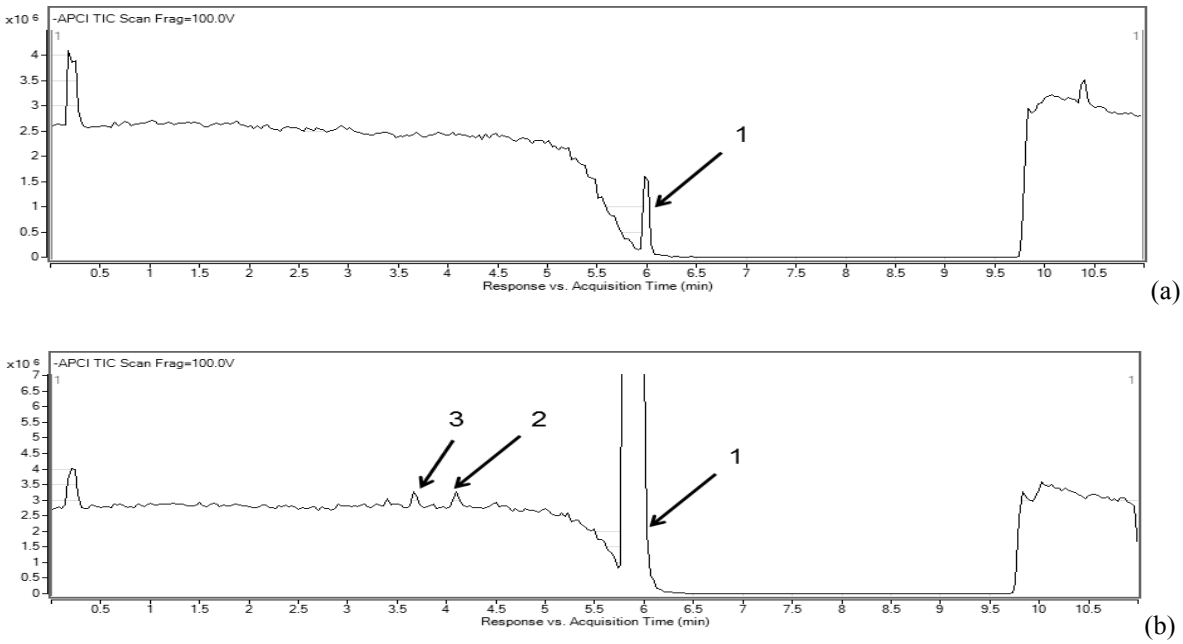
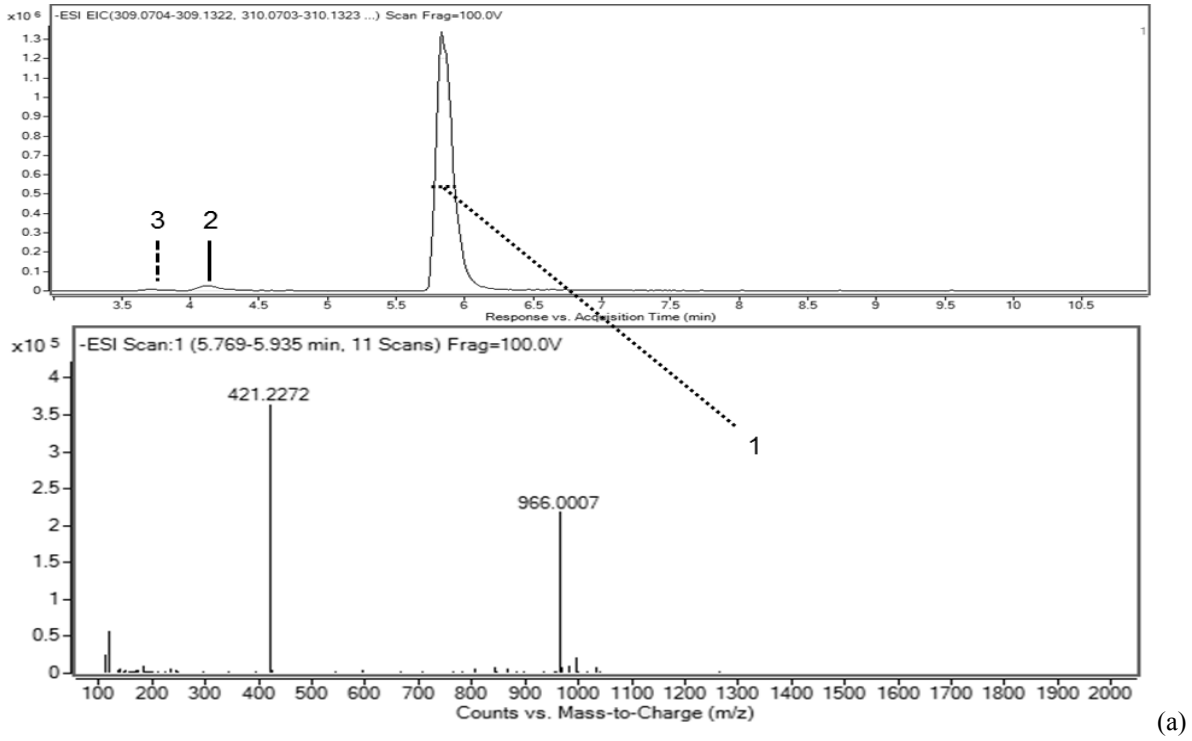


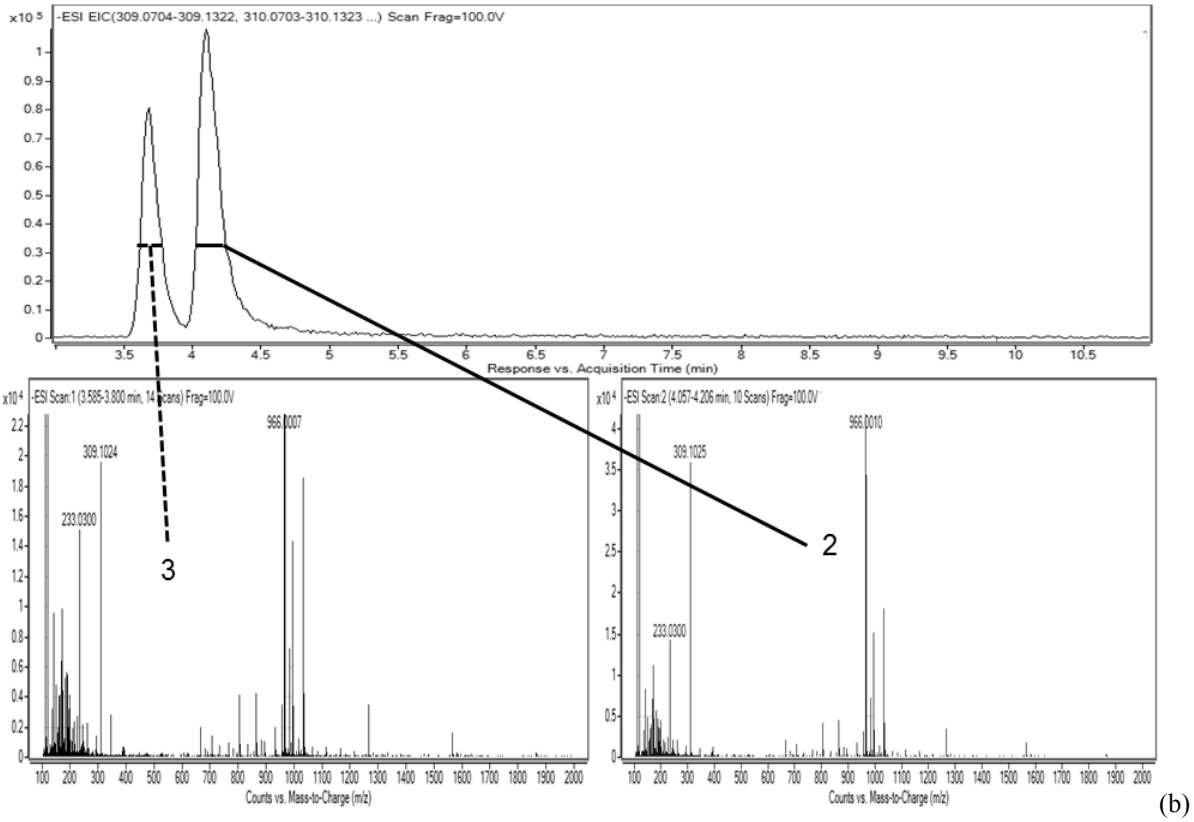
Figure 13: Total ion chromatogram (TIC) displaying the separation of AOT (a) and monoesters 2 and 3 (b) on RP-C18, using gradient elution with water and methanol as eluents acidified each with 20 mmol formic acid/liter, detected by APCI-ToF-MS

Although separation of the target compounds was achieved, ionization of AOT was not homogenous over the whole peak. This effect depended on the content of organic solvent in the mobile phase as shown in detail in the Supplementary. It is known that ionization performance in atmospheric pressure techniques is influenced by, among others, LC flow, eluent composition and pH-value [27-30]. Given that the eluent is needed as reactant gas for ionization in APCI, vaporizing the eluent too efficiently will decrease ionization [31]. Accordingly, gas flow was reduced in the next step to 4 L/min, but this led to incomplete evaporation of eluent with higher water content at the beginning of the gradient and contamination of the source, so that it had to be cleaned afterwards.

As vaporization performance could not be adjusted during the eluent gradient and the breakdown disturbed the detection of AOT, electrospray ionization (ESI) was tested instead, with the results shown in Figure 14 (a) for AOT and Figure 14 (b) for monoesters 2 and 3.



(a)



(b)

Figure 14: Extracted ion chromatogram (EIC) of the exact molar mass of AOT (a) and monoesters 2 and 3 (b) including their A+1 and A+2 isotopic pattern with a range of 20 ppm around each exact mass; displaying the separation of AOT and monoesters 2 and 3 with RP-C 18 gradient elution with methanol and water as eluents, detection via LC ESI-ToF-MS together with the mass spectrum of each compound

As the ionization performed homogenously throughout the whole gradient run, ESI was finally used as interface to the mass spectrometer for analysis. Chromatographic separation was achieved for AOT and monoesters 2 and 3, but not for their diastereoisomers. There were at least two centers of chirality in each target analyte, AOT and monoester 2 and 3, as indicated in Figure 5. Therefore there were at least two pairs of diastereoisomers possible for each target analyte.

Separation of other diastereoisomers should in principle be possible, but as in previous work for AOT [20-23] this was not aimed for, because different stereoisomeric configurations have little influence on its properties [32-34]. Monoester 2 and 3 were determined in analogous way, as little impact of different possible diastereoisomers on their properties as surfactants was expected either.

2.4.2 Determination of AOT and both isomeric Monoesters 2 and 3

Validation of the developed method was done according to DIN 32645. The validation parameters are given below in Table 1 and the results of recovery and precision in the two investigated matrices in Table 2. Additional results for linear range and the prediction interval are given in the Supplementary.

Table 1: Results of method validation for AOT and monoesters 2 and 3, containing linear range, linear regression, coefficient of determination (R), the method`s relative standard deviation (V_{x0}) and the limits of quantification (LOQ), capture (LOC) and detection (LOD)

	AOT	**monoester 2**	**monoester 3**
Linear range [mg/L]	0.15-2.0	0.11-2.0	0.17-2.0
R	0.9997	0.9998	0.9996
Method's relative standard deviation V_{x0} [%]	2.0	1.6	2.2
Limit of quantification [mg/L]	0.15	0.11	0.17

Table 2: Recovery and precision of AOT and monoester 2 for different matrices, id est light naphtha solvent and agrochemical formulation, on different concentration levels.

Naphtha solvent	**AOT**	**monoester 2**
Recovery c=1.6 mg/L light naphtha solvent [%]	99.9	99.4
Precision [%]	1.3	0.8
Recovery c=0.1 mg/L light naphtha solvent [%]	103.5	101.0
Precision [%]	1.7	1.5
F-Test (0.05)	Negative	Negative
Agrochemical formulation	**AOT**	**monoester 2**
Recovery c=1.6 mg/L formulation [%]	101.0	101.1
Precision [%]	1.0	1.4
Recovery c=0.1 mg/L formulation [%]	100.1	99.7
Precision [%]	1.6	1.6
F-Test (0.05)	Negative	Negative

All three analytes showed results in the evaluation of their analytical parameters within required limits in guidelines for validation of analytical methods such as SANCO 3030_99 for pre- and post-registration [35]. The achieved LOQ was significantly lower than reported in literature for the determination of sodium bis(2-ethylhexyl) sulfosuccinate, 13 mg/L [10] and 1 g/L [21] by non-mass spectrometric detection, but considerably higher than the 20 µg/L reported lately [20]. The goal of our method, though, was to identify and quantify the target analytes in the matrix of an agrochemical formulation. To that end, ToF-MS with exact mass measurement was used, as its advantage was its mass selectivity that allowed quantification even in the complex matrix of an agrochemical formulation (see Supplementary). Using MS-MS, however, as used in [20], which is usually by far more sensitive, was not necessary in this work, as the concentration of target analytes in the samples were high enough.

Moreover, it could be proven that complex matrices such as a light naphtha fraction or an agrochemical formulation with a mixture of surfactants, solvents and active ingredients did not negatively influence the analysis of the target analytes. The recovery for both matrices showed no loss of analyte. Precision was also good and homogenous over the monitored concentration range.

2.4.3 Comparison of three different Suppliers of AOT Product

The validated method was finally used to analyze the content of AOT and monoesters 2 and 3 in AOT product of different suppliers. Samples of AOT product from three suppliers A, B and C were analyzed for their content of AOT and the monoesters 2 and 3. For each sample five independently weighed replicates were measured. The corresponding values for each single measurement were displayed in Supplementary. Shown in Table 3 are the average values of these replicate measurement including the expanded measurement uncertainty for each value calculated according to GUM [26]. The resulting extracted ion chromatograms of monoester 2 and 3 are shown below [supplier A: Figure 15 (a), supplier B: Figure 15 (b), Supplier C: Figure 15 (c)].

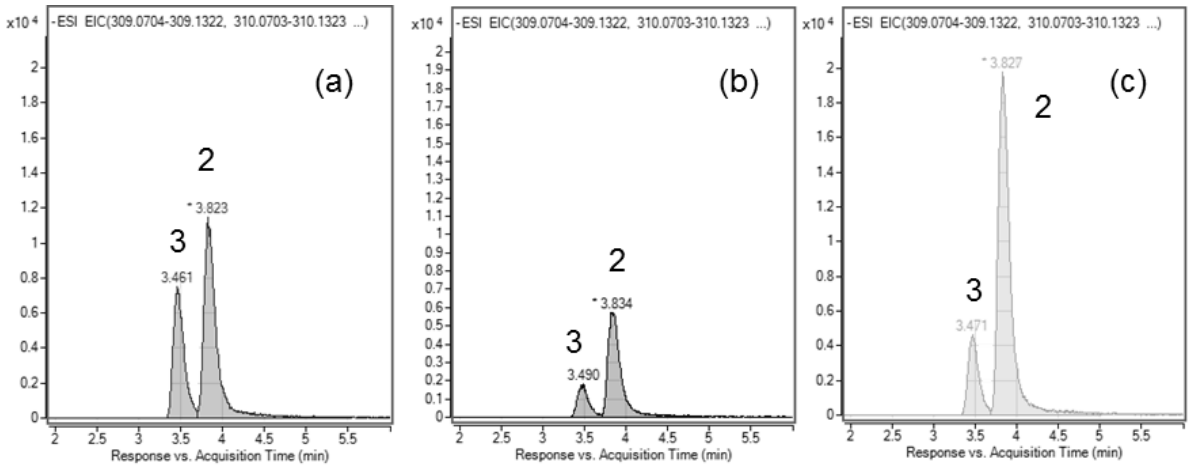


Figure 15: Extracted ion chromatogram (EIC) of the exact molar mass of monoester 2 and 3 including their A+1 and A+2 isotopic pattern with a range of 20 ppm around each exact mass showing varying monoesters‘ content for AOT product from three different suppliers. The results for supplier A are shown in (a), for supplier B in (b) and for supplier C in (c)

Table 3: Content of AOT, monoester 2 and 3 in three different suppliers of AOT product. Analysis of five independently weight samples each batch number averaged. The expended measurement uncertainty is calculated according to GUM [26] encompassing 95% of the distribution of values

	Supplier A	**Supplier B**	**Supplier C**	**Specified content (w/w)**
w(AOT) [%]	63.0±1.2	65.8±0.7	61.4±1.1	62.5-66.0
w(monoester 2) [%]	1.3±0.02	0.8±0.01	3.2±0.06	not specified
w(monoester 3) [%]	0.7±0.02	0.2±0.004	0.7±0.02	not specified

A one-sided t-test with a level of significance of p = 0.05 was conducted to determine whether the content of AOT was within the specified concentration range, 62.5-66.0 % (w/w), in the commercial product. The observed

p-values were p = 0.42 (Supplier A), p = 0.52 (Supplier B) and p = 0.05 (Supplier C). As all values were higher or equal than the level of significance p = 0.05, it was shown, that the content of AOT was within the error margin of the specified value for all three suppliers.

To analyze, if the content of each target analyte is significantly differing between the measured samples of the three suppliers paired t-tests were conducted with a level of significance of p = 0.05. The observed p-values of the paired t-tests are shown in Table 4, the calculation steps are given in Supplementary.

Table 4: Observed p-values of the paired t-test on the content of AOT and monoester 2 and 3 in AOT product. Paired groups are formed by the three suppliers of AOT product A, B and C, resulting in the test groups A/B, A/C and B/C with a level of significance of p = 0.05.

Paired groups	AOT	Monoester 2	Monoester 3
A/B	0.01	1.5E-06	5.6E-07
A/C	0.1	2.8E-07	0.07
B/C	1.0E-03	8.8E-08	4.1E-06

The difference in content of AOT was not significant for the suppliers. The content of the by-products, monoester 2 and 3, however, was significantly different between suppliers A, B and C with the only exception of monoester 3 between supplier A and C.

Monoesters 2 and 3 might interfere with the complex mixture of surfactants in an agrochemical product, as they have surface active properties as well and had been applied as wetting agents in the past [6]. As preliminary results showed differences in physico-chemical properties of agrochemical formulations containing AOT product of different suppliers, analytical methods became necessary to determine the content of AOT and monoester 2 and 3 in raw material and formulation samples, respectively.

2.5 Conclusion

A method was developed to chromatographically separate pure AOT (sodium bis(2-ethylhexyl) sulfosuccinate) and its by-products –monoester 2 and 3 (sodium 1-carboxy-3-[(2-ethylhexyl)oxy]-3-oxopropane-1-sulfonate (2) and sodium 3-carboxy-1-[(2-ethylhexyl)oxy]-1-oxopropane-2-sulfonate (3))– and to analyze them via a coupled MS (ToF) with exact mass measurement. Validation was carried out according to DIN 32645 and proved the method to work not only for analytical standards but also for complex matrices.

As the content of monoester 2 and 3 differed significantly in AOT product of three different suppliers and preliminary results showed differences in physico-chemical properties of agrochemical formulations containing AOT product of these suppliers, this offers interesting starting points for future work.

Using these differences in the by-products spectrum of AOT product for identification of counterfeited agrochemicals, might provide another direction of research. A precise and accurate determination of AOT and monoesters 2 and 3 in agrochemical products is needed and has not been available so far.

2.6 Acknowledgement

The authors gratefully acknowledge the department of formulation technology at Bayer CropScience for the financial support and the analytical team for technical and intellectual support. We also acknowledge the support by the research department of Bayer CropScience on providing time and laboratory equipment for the synthesis and structure elucidation of monoester 2 and 3.

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3. Composition of commercial AOT Surfactant Products and its Effects on an Agrochemical Formulation

3.1 Abstract

Sodium bis(2-ethylhexyl) sulfosuccinate (Aerosol OT or AOT) is a commercially available surfactant commonly used in agrochemicals. Besides the principal diester surfactant, commercial AOT product contains two surface-active isomeric monoester by-products, which may influence the surfactant's overall properties. This work investigates whether the purity of the surfactant affects its ability to stabilize an agrochemical formulation. The concentrations of the diester and two monoester impurities in batches of commercial AOT product from several suppliers were determined quantitatively by liquid chromatography–mass spectrometry. The tested batches showed different contents of the monoesters. Samples of a model agrochemical formulation containing AOT product formed more sediment during storage when the content of monoesters in the surfactant was high. The supplier of a commercial AOT product could be traced by analysis of the monoester content of either the raw product or the aged agrochemical formulation.

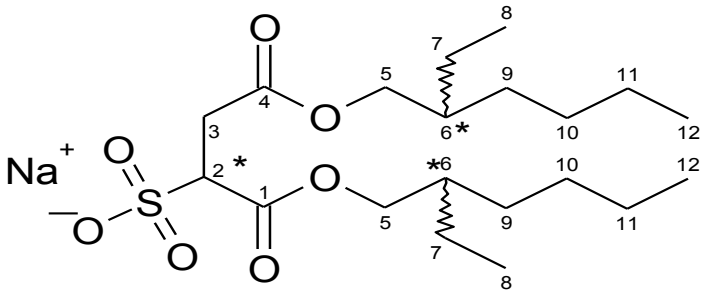
3.2 Introduction

High levels of surfactants are common in domestic products used for personal care and cleaning, and they are also common in industry, e.g., in agrochemical products [1; 2]. The active ingredients of agrochemical formulations are generally mixed with additives, such as surfactants and solvents. Surfactant additives disperse the active ingredients homogeneously throughout the formulation and stabilize it physically and chemically. They facilitate the application of the active ingredients by ensuring their even distribution over the area of application, thus avoiding over- or under dosing; and they also aid in the uptake of the active ingredient by the target crop or species [3; 4]. These various tasks require different surfactants in different situations. The chosen surfactant or combination of surfactants must complement the mixture of other components without inducing unwanted effects during production or storage, such as sedimentation, agglomeration, or crystallization [5].

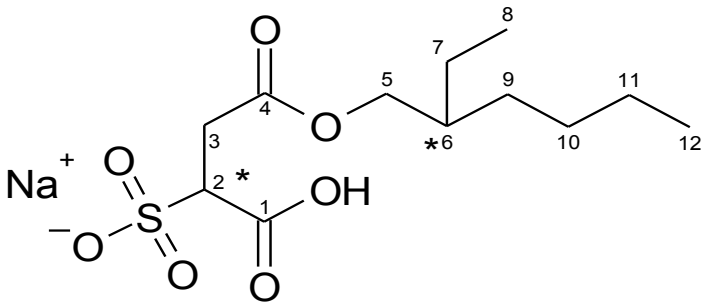
Once a formulation has been developed, it must be registered and approved before it can be sold commercially. Registration requires the formulation and its components to be evaluated with regard to their safety and their adverse effects to human health and the environment [6–8]. A registered formulation has a fixed composition, which must not be changed, although chemically identical substitutions are permitted, to allow the raw materials to be obtained from different suppliers. The interchangeability of components from different sources is necessary

not only for legal compliance, but also to ensure the formulation behaves consistently. Changes may affect the production of the product or the product itself, which may lead to unwanted changes in its properties. Therefore, quality control of the raw materials is necessary. Furthermore, differences among the products of different suppliers, where observed, could also assist in the investigation of counterfeiting, by allowing a supplier to be identified by the by-product spectrum of the product. Such techniques have been used to identify fake perfumes [9] and whiskey [10], and counterfeit pharmaceutical products have been identified by the nature and content of their active ingredients [11; 12].

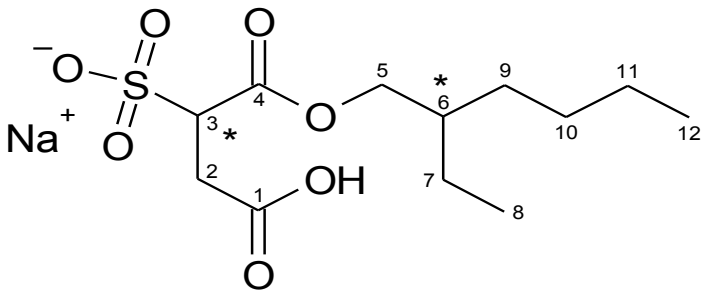
In this study, we investigated the composition of commercial Aerosol OT (AOT) products that are commonly used as surfactants in agrochemicals, and the influence that variations of the product's composition have on the stability of an agrochemical formulation. Commercially available AOT products contain as their major constituent sodium bis(2-ethylhexyl) sulfosuccinate (i.e., pure AOT) (Figure 5a). The surfactant, together with surface active isomeric by-products, labeled here "monoester 2" (Figure 5b) and "monoester 3" (Figure 5c), is solvated in light aromatic naphtha. These other substances in the product may influence the overall properties of AOT product.



(a) Sodium bis(2-ethylhexyl) sulfosuccinate (AOT)



(b) Sodium 1-carboxy-3-[(2-ethylhexyl)oxy]-3-oxopropane-1-sulfonate (monoester 2)



(c) Sodium 3-carboxy-1-[(2-ethylhexyl)oxy]-1-oxopropane-2-sulfonate (monoester 3)

Figure 16: Structures of (a) AOT, (b) monoester 2, and (c) monoester 3. Centers of chirality are indicated by *.

A method to quantify AOT and monoesters 2 and 3 was developed in a previous work, which examined the different contents of the monoesters in AOT products from different suppliers [13]. This work investigates whether these observed differences are characteristic of the corresponding suppliers and whether they affect the properties of a formulation containing AOT products.

3.3 Experimental

3.3.1 Chemicals and Reagents

High-purity water was obtained with a Milli-Q-gradient A10 system (Millipore, Eschborn, Germany). Acetonitrile, methanol, formic acid, and sodium bis(2-ethylhexyl) sulfosuccinate, all of per analysis grade, were from Sigma Aldrich. Reversed-phase liquid chromatography was used with high-purity water and methanol as eluents. The eluent pH was adjusted with 20 mmol formic acid per liter eluent.

Commercially available AOT products were sourced from four suppliers (8–16 different production batches from each). The contents of AOT and monoesters 2 and 3 were analyzed in 50 mL samples from each batch. The suppliers are labeled A–D (see Table 6). Batches from supplier A came from two different production sites: one in Germany (supplier A1) and one in Spain (supplier A2). The different batches from these different sites are labeled a-1 to a-8 for supplier A1 and A-1 to A-5 for supplier A2.

3.3.2 Liquid Chromatography–Mass Spectrometry

The analytical method is described in detail in our previous work [13]. The settings used are briefly given here. An Agilent 1200 SL HPLC instrument was used coupled via dual-sprayer electrospray ionization (ESI) to an Agilent 6220 Accurate-Mass-TOF mass spectrometer. All measurements were made on a Waters XBridge column (50 × 2.1 mm, 2.5 µm), chosen for its good temperature and pH-value stability minimizes signals on mass spectrometry caused from column bleed [14].

A gradient elution was applied for sample measurement. The initial 5% (v/v) methanol was increased to 95% in 6 min, with subsequent column flushing and equilibrating; the total run time was 11 min with a flow rate of 0.7 mL/min and a column temperature of 55 °C. Flow was directed with a split of 1:6 (MS:waste) via the first sprayer needle of the dual-ESI source into the mass spectrometer. Mass spectra were obtained in negative mode throughout the whole run with a rate of one spectrum per second and a mass range of 100–1700 m/z. The ESI parameters were a gas temperature of 350 °C, drying gas flow of 8 L/min, and nebulizer pressure of 30 psig for both ESI sprayer needles of the dual-sprayer ESI source.

The acquired scan data were either displayed as a total ion chromatogram (TIC) or as an extracted ion chromatogram (EIC) extracted on the exact molar masses of the analytes (m/z(AOT) = 421.2265 amu; m/z(monoester 2 and 3) = 309.1013 amu) and their A+1 and A+2 isotopic masses, with a window of 100 ppm around each mass to account for potential mass axis divergence during measurement.

Mass calibration was conducted with a mass range calibration mixture (Agilent) via the second sprayer of the dual-sprayer ESI source. Mass correction during analysis was made on purine (neg.: m/z = 119.036230 amu) and hexakis (1H, 1H, 3H- fluoropropoxy)phosphazine; abbreviated: HP 921 [neg. +formate: m/z = 966.000725 amu]). A solution of both was delivered constantly into the mass spectrometer at a flow rate of 0.05 mL/min during the analysis via the second sprayer needle of the dual-sprayer ESI source.

3.3.3 Preparations of Standard and Sample Solutions

All standard and stock solutions were prepared using a mixture of 50/50 (v/v) water and acetonitrile acidified with formic acid to a final concentration of 100 mM acid in the mixture. Stock solutions were prepared by dissolving the respective analytes at 0.4 g/L in the 50/50 (v/v) water/acetonitrile mixture. Standard solutions were prepared from the stock solutions by dilution to final concentrations of the monoesters and the AOT of 0.04 mg/L to 2 mg/L.

Each raw AOT product sample was analyzed by dissolving a 20 mg portion in 50 mL of solvent mixture. The working solution for the measurement of AOT was diluted 1:1 000; for the measurement of the monoesters, it was diluted 1:20.

AOT was analyzed in formulation and sediment samples using 20 mg samples in 50 mL of solvent mixture. The working solution for the measurement of AOT was further diluted 1:200; for the measurement of the monoesters, it was further diluted 1:4.

A mass calibration solution (Agilent) for the ESI source was applied according to the instructions of the supplier.

A mass solution (Agilent) for the correction of the mass calibration of the TOF instrument during analysis was used for the ESI source. It contained 1.0 µM purine and 0.25 µM HP 921 in 95/5 (v/v) methanol/water. A dilution of 1:100 was needed to avoid overloading the detector. Mass calibration during analysis was tested using a test sample containing reference compounds of known exact masses spanning the retention time window of the gradient analysis. The test sample was analyzed at the beginning and the end of a test series. Further information on the test samples is given in the Supplementary.

3.3.4 Storage Tests

A model formulation a non-aqueous suspension was made using AOT product from suppliers A, B, and D; these AOT products represented the minimum (B), average (A), and maximum (D) contents of the monoesters. The model formulation was constituted as listed in Table 5. All ingredients other than AOT were kept identical, to ensure that the results of the storage test were due only to variations in AOT. To simulate the storage conditions of an agrochemical product, the samples were stored in 5 L high-density polyethylene bottles in a climate cabinet at 24 °C for six months [15–17].

Table 5: Composition of the model agrochemical formulation

Raw material	**Content [%] (w/w)**
Active ingredient	15
AOT	19
Dispersing agent (nonionic)	9.0
Emulsifier 1 (nonionic)	10
Emulsifier 2 (nonionic)	15
Hydrophobically modified clay	1.0
Buffer	3.0
Hydrophobic solvent	28

3.3.5 Statistical Data Evaluation

Statistical tests were performed using Microsoft Excel and script programmed in R, a language and environment for statistical computing and graphics [18].

3.4 Results and Discussion

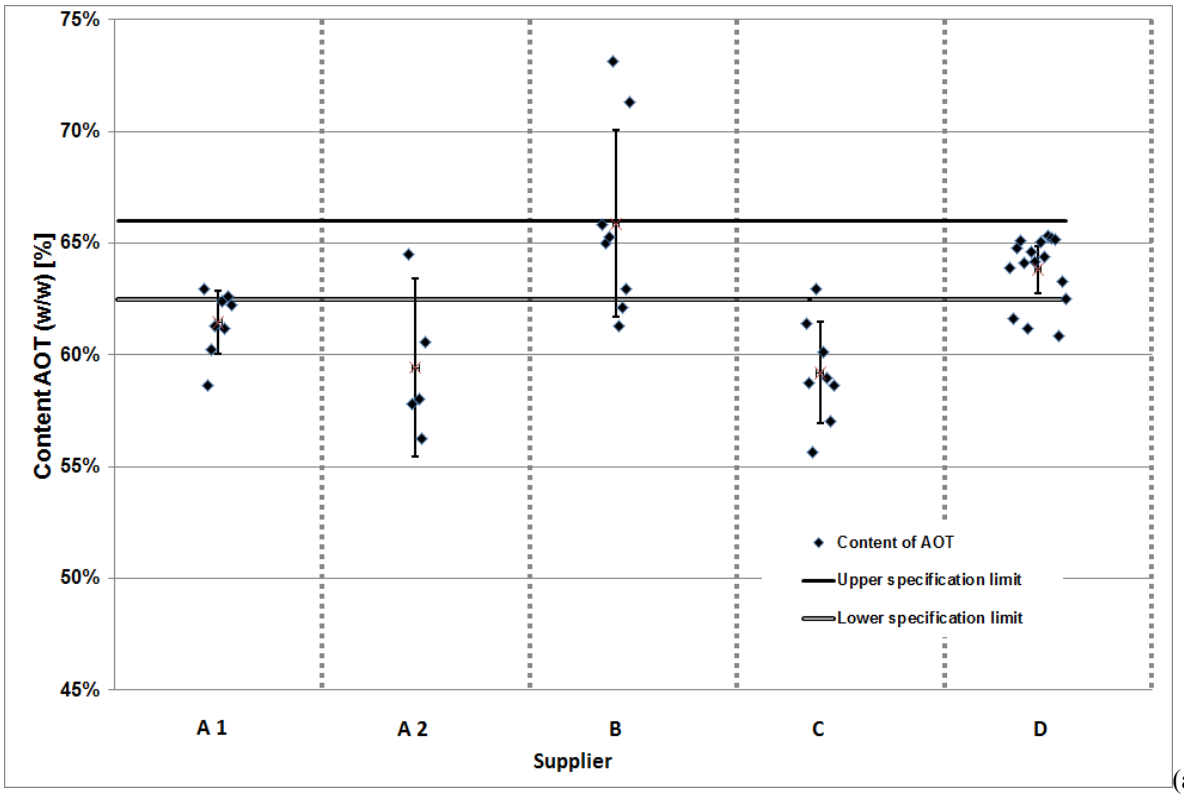
3.4.1 Contents of AOT and Monoesters 2 and 3 in Batches of AOT Product from various Suppliers

Preliminary work revealed AOT product to vary among the suppliers in its contents of monoesters 2 and 3 [13]. These variations were further investigated here to determine the consistency among batches from a given supplier and the differences among suppliers: 8–16 batches were acquired from each of four suppliers. The contents of AOT and monoesters 2 and 3 listed in Table 2 are average values of five replicate analyses of a sample from each of the batches (full results in the Supplementary). The table also lists the different AOT product batches from each supplier and their respective production sites.

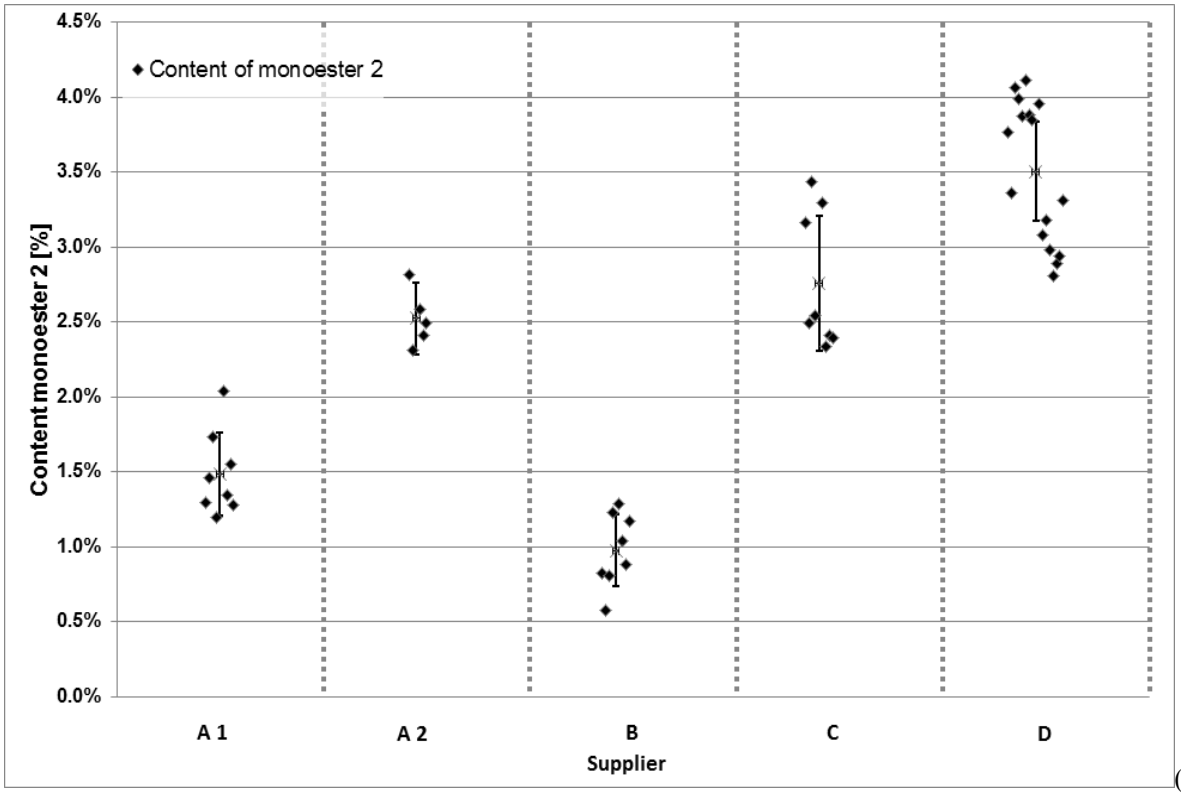
Table 6: Average contents of AOT and monoesters 2 and 3 in batches of AOT product from different suppliers and production sites. Average values are listed with 95% confidence intervals.

Supplier	Batch No	Production site	AOT (w/w) [%]	Monoester 2 (w/w) [%]	Monoester 3 (w/w) [%]
A1	a-1 to a-8	Germany	62 ± 1.4	1.5 ± 0.3	0.7 ± 0.1
A2	A-1 to A-5	Spain	59 ± 4.0	2.5 ± 0.2	1.9 ± 0.2
B	B-1 to B-8	USA	66 ± 4.1	1.0 ± 0.2	0.3 ± 0.1
C	C-1 to C-8	Germany	59 ± 2.3	2.8 ± 0.5	0.8 ± 0.3
D	D-1 to D-16	Germany	64 ± 1.1	3.5 ± 0.3	2.2 ± 0.2

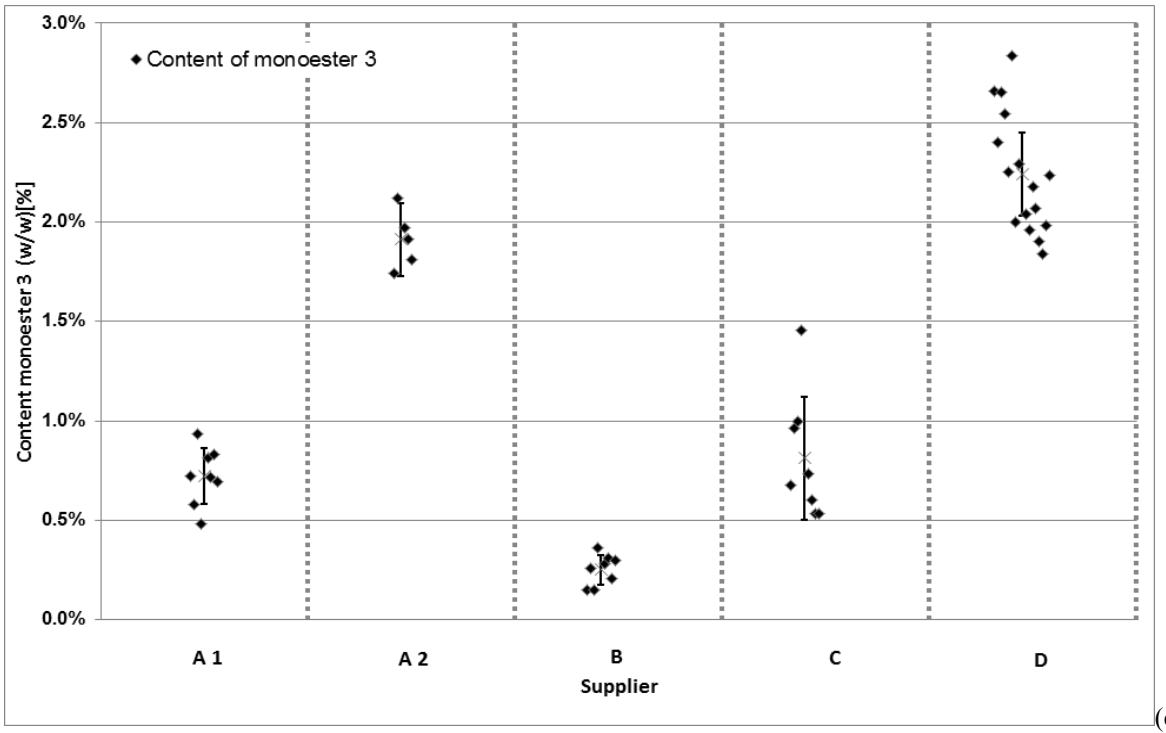
The variations among the different batches are visualized in Figure 17, which plots the average contents in each batch of AOT, monoester 2, and monoester 3. Figure 17 (a) shows the measured range of AOT contents in the commercial samples plotted against the range specified by the suppliers (62.5%–66.0% w/w).


75%
70%
65%
60%
55%
50%
45%
Content AOT (w/w) [%]
Content of AOT
Upper specification limit
Lower specification limit
A 1
A 2
B
C
D
Supplier

(a)


4.5%
4.0%
3.5%
3.0%
2.5%
2.0%
1.5%
1.0%
0.5%
0.0%
Content monoester 2 [%]
Content of monoester 2
A 1
A 2
B
C
D
Supplier

(b)



(c)

Figure 17: Contents of (a) AOT, (b) monoester 2, and (c) monoester 3 in different batches of AOT product from four different suppliers. Each data point is the average value of five replicate analyses. The averages of the batches from each individual supplier are plotted together with their 95% confidence intervals. The range of AOT contents (62.5%–66.0% w/w) specified by the suppliers is marked by horizontal lines in (a). The data sets of all suppliers were statistically tested against one another with paired t tests of the significant differences between their means (Table 7).

Table 7: Observed p-values for paired t-tests comparing the average contents of AOT, monoester 2, and monoester 3 for the individual suppliers with one another. Values of $p < 0.05$ (italicized) denote significant differences between the suppliers, and values of $p < 0.01$ (underlined) denote highly significant differences.

		A 1	**A 2**	**B**	**C**
A 2	AOT	0.7			
	Monoester 2	$\underline{3 \times 10^{-5}}$			
	Monoester 3	$\underline{6 \times 10^{-5}}$			
B	AOT	0.1	0.08		
	Monoester 2	$\underline{0.005}$	$\underline{1 \times 10^{-6}}$		
	Monoester 3	$\underline{3 \times 10^{-5}}$	$\underline{1 \times 10^{-5}}$		

		A 1	**A 2**	**B**	**C**
C	AOT	0.2	0.9	*0.02*	
	Monoester 2	0.0001	0.2	10×10^{-6}	
	Monoester 3	0.5	4×10^{-5}	0.005	
D	AOT	*0.02*	0.2	0.7	0.004
	Monoester 2	6×10^{-11}	2×10^{-11}	4×10^{-13}	0.005
	Monoester 3	6×10^{-13}	*0.01*	2×10^{-14}	1×10^{-6}

As displayed in Figure 17, the content of AOT does not deviate significantly from the specified limits, with the exception of supplier C. Table 7 shows that in no case is the difference between the AOT contents of two suppliers highly significant. However, the contents of monoesters 2 and 3 are highly significantly different in nearly all cases, except the pair A2/C ($p = 0.2$, not significant) for monoester 2 and the pairs A1/C ($p = 0.5$, not significant) and A2/D ($p = 0.01$, significant) for monoester 3.

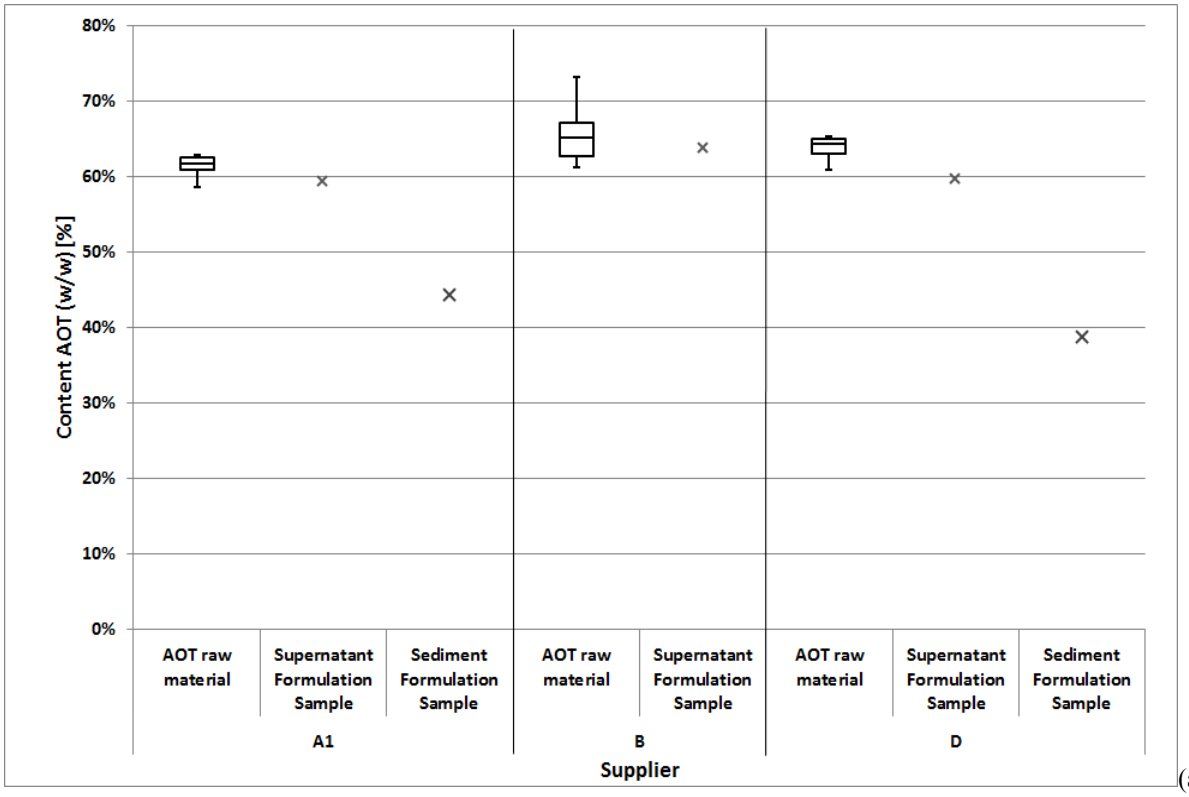
To ascertain whether these differences affect the properties of agrochemical formulations made using AOT product, storage tests of a model agrochemical formulation were performed. Formulations were made using the AOT product from suppliers A1, B, and D, which represent low (B), medium (A1), and high (D) contents of the monoesters in the products.

The stored samples differed in their sedimentation behavior after six months at room temperature. Once decanted, the samples containing the AOT product of supplier A1 and supplier D showed visible sediment, with the latter showing more sediment than the former. However, the formulation containing the AOT product of supplier B was free of sediment. (Pictures of the decanted samples are included in the Supplementary.)

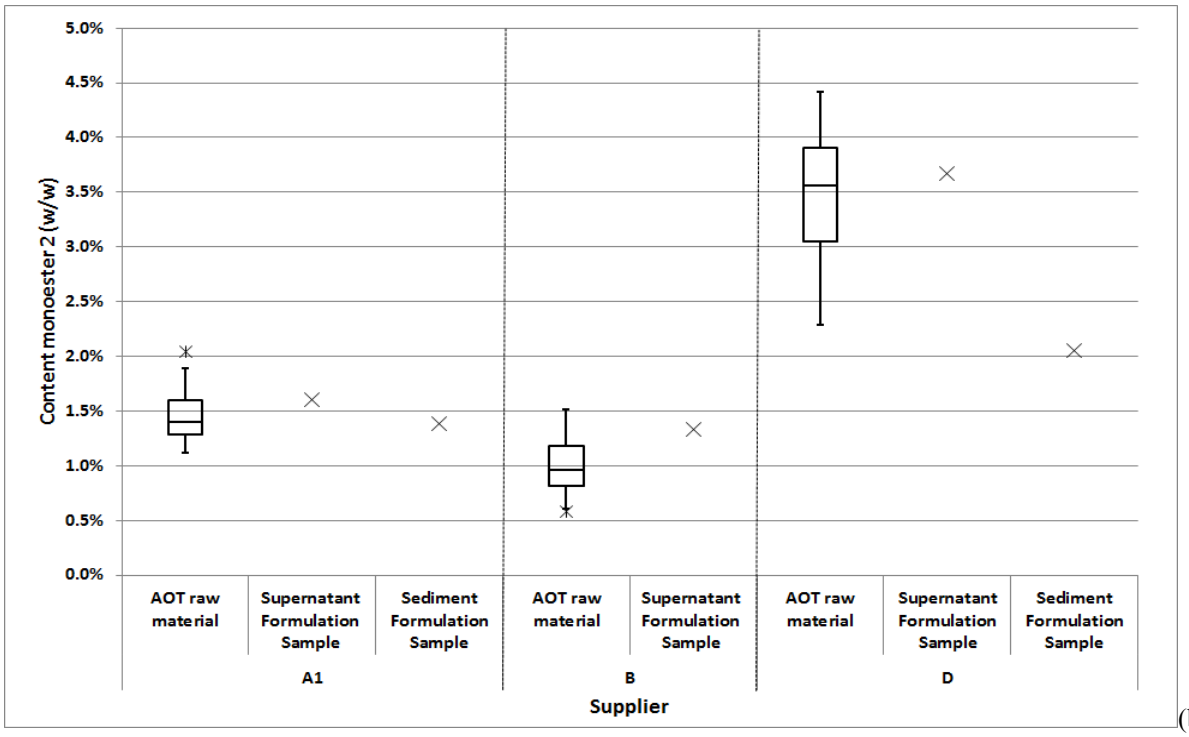
Both sediment and supernatant were analyzed for their contents of AOT and monoesters 2 and 3 to investigate whether the sedimentation was related to one of the target analytes. The results, given as percentage compositions of the AOT product used in the formulation, are listed in Table 8 and visualized in Figure 18. Each value is the average of five replicate analyses, given with its 95% confidence interval. For comparison, in each graph, the corresponding results for the production batches of the AOT product are also given as box and whisker plots

Table 8: Contents of AOT, monoester 2, and monoester 3 in supernatants and sediments, given as percentage compositions of commercial AOT used in the formulation. Formulation samples containing AOT product from supplier A1, B, or D were stored for six months at room temperature. Each value is the average of five replicates analyses, given together with its 95% confidence interval.

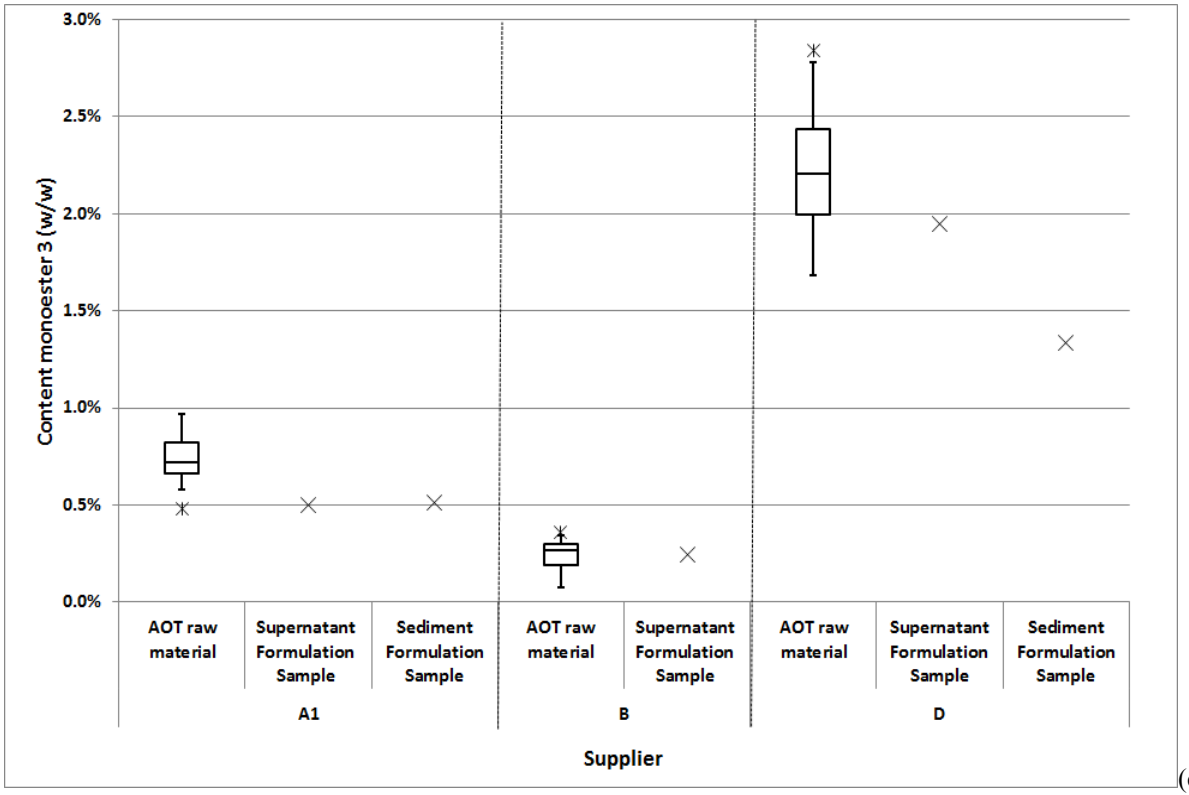
Supplier/ Batch	Phase	w(AOT) [%]	w(Monoester 2) [%]	w(Monoester 3) [%]
A1	Supernatant	59 ± 0.09	1.6 ± 0.09	0.5 ± 0.002
	Sediment	45 ± 2	1.4 ± 0.08	0.5 ± 0.01
B	Supernatant	63 ± 1	1.3 ± 0.008	0.2 ± 0.003
D	Supernatant	59 ± 1	3.7 ± 0.06	1.9 ± 0.06
	Sediment	39 ± 3	2.0 ± 0.1	1.3 ± 0.08



(a)



(b)



(c)

Figure 18: Contents of AOT (a), monoester 2 (b), and monoester 3 (c) in the supernatant and sediment of a model agrochemical formulation containing AOT product from supplier A1, B, or D after storage for six months at room temperature. Each value is the average of five replicates, given together with its 95% confidence interval. For comparison, the corresponding values for the production batches of AOT product product are shown as box and whiskers plots.

The formation of sediment during the storage of the model formulation depended on the supplier of the AOT product. The sample containing AOT product from supplier B, which contained the lowest concentrations of monoesters 2 and 3, had no visible sediment, whereas the samples containing AOT product from suppliers A1 and D showed increasing amounts of sediment. The sediment, when observed, contained less AOT than did the corresponding AOT product; the two monoesters showed similar decreases only for the formulation containing AOT product from supplier D. The supernatants, however, each retained levels of all three analytes similar to those of the corresponding AOT raw products. The main part of the sediment was not soluble in organic solvent or in water; neither was it combustible in a Bunsen flame. Therefore, it was deemed to be mainly inorganic, consisting of the hydrophobically modified clay used in the model formulation.

These results indicate that the monoesters may have destabilized the dispersions during ageing, because the amount of sediment appeared to correlate with the monoester content of the AOT raw products. To understand the ageing process of the model formulation, a freshly prepared formulation was centrifuged. The resulting sediment contained a disproportionately high amount of AOT (data given in Supplementary), unlike that formed by ageing, which contained less AOT than did the supernatant. This indicates that AOT adsorbed to the dispersed particles (such as the hydrophobically modified clay) in the formulation, thus aiding the stabilization of the dispersion. As the monoesters themselves are surface active—they have been used as wetting agents [19]—it is likely that they competed with AOT for the free surfaces of the particles in the dispersion. This competition would result in less AOT adsorbing to the dispersed particles, which would lead to the particles being less stable, and thus to their sedimentation during ageing [1, 5]. This sediment would consequently be depleted of AOT, as observed here in the aged model formulation after storage. Although this is a plausible explanation, no direct experimental proof is provided here. Further evidence could be sought through investigation of the competitive adsorption of the different surface-active components of the AOT products on the dispersed particles. Such an analysis was not conducted here, because the focus was on the analytical characterization of the AOT product in complex mixtures rather than on isolated surfactant–adsorbent systems.

To minimize the factors influencing the findings, all components in the formulation, except the specific AOT product, were unvaried. However, besides the composition of the AOT product, the subject of this and previous work, the contents of inorganic anions and cations might also influence the properties of the model formulation. Therefore, these were also investigated with ion chromatography using the raw AOT product (data shown in Supplementary). All ions were present at relatively consistent levels in all the samples, suggesting that there was no supplier-specific influence in this regard on the model formulation system.

Another potentially influential variable was the solvent in which the AOT product was delivered. The solvent content is specified by the data sheets of the suppliers. It is classified as light aromatic naphtha solvent, which mainly consists of C9–C10 di-alkyl- and tri-alkyl benzenes obtained as a fraction from the cracking of crude oil. Therefore, its composition depends on the process as well as on the origin of the crude oil [20–22]. The solvent of the AOT product makes up 8% of its total content, and in the model formulation, it may influence the critical micelle concentrations of the surfactants and their distribution in the different phases. The batches of AOT product from suppliers A-1, C, and D showed variations in their contents of benzene derivatives, but no major differences in the composition of the solvent were observed (see the Supplementary). These variations in solvent composition were not expected to lead to the observed supplier-specific sedimentation in the agrochemical formulations. Nevertheless, they may still influence the sedimentation process and should not be neglected in future investigations of the sedimentation process.

Several papers are dedicated to the analysis [13; 23–28] and properties [1; 28–33] of AOT in various media and its uses, but the findings observed here, which might be attributable to the interactions of AOT with its monoesters, have not been discussed elsewhere to the knowledge of the authors. However, these results suggest that the main and by-product spectra of surfactants from different suppliers must have similar chemical compositions to ensure the consistent behavior of complex mixtures containing surfactants, such as agrochemical formulations.

3.4.2 Statistical Evaluation of the Contents of AOT, Monoester 2, and Monoester 3 with regard to their use for product identification

As described above, there were substantial differences in the contents of monoesters 2 and 3 in the AOT products from different suppliers. These differences could be used as signatures for product identification. To test whether the contents of the monoesters and/or of AOT were sufficiently different to allow identification of the different suppliers, linear discriminant analysis was conducted on the dataset (described in detail in the Supplementary). This showed that the differences among all the samples were mainly attributable to the contents of the monoesters. The relatively low variation in the content of AOT played only a minor role.

Accordingly, the different production batches of AOT products clustered well with respect to their contents of monoesters 2 and 3. The best separation of the different clusters was achieved with localized discriminant

analysis (Figure 19) [34, 35]. Of the 45 batches considered, only three were misallocated (red letters), corresponding to an error rate of about 7%.

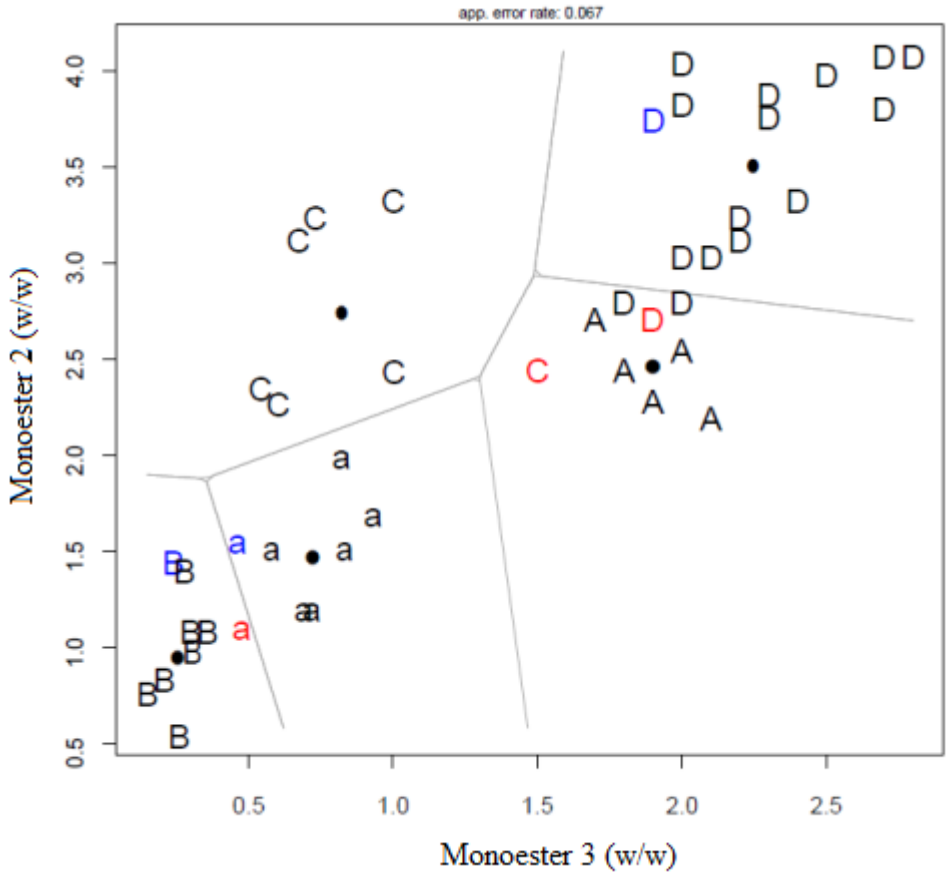


Figure 19: Raw AOT product (black) and the supernatant samples from the storage test (green) displayed in a partition plot resulting from a localized discriminant analysis. Red data points are misclassified. Samples from batches from supplier A1 are designated "a", and those from supplier A2 "A". Black dots correspond to the mean of the respective data set for each supplier.

The AOT products from the two production sites of supplier A were sufficiently different for the batches from the Spanish site to resemble more closely those of supplier D than those from the German site of the same supplier. The monoester content of the AOT product in the supernatant of each aged sample matched that in the corresponding raw AOT product. These results indicate that this clustering can also correctly assign the AOT product used in a formulation to its corresponding supplier and that aging the formulation does not undermine this assignment.

The clustering observed in Figure 19 might be useful in identifying the supplier of the AOT product used in an unknown agrochemical formulation. It could also be used to identify counterfeit products, if the composition and the supplier of the AOT product used in the original formulation are known.

Identification of counterfeit products by the nature and content of their active ingredient(s) has been demonstrated for pharmaceutical products [11; 12]. The use of the by-product spectrum of a formulation additive for the detection of a counterfeit would constitute an additional technique to those already existing, such as specialized packing materials [36], radio frequency identification (RFID) [37], bulk analysis of products with, for example, NIR [38; 39] or NMR [40], which all have their limitations. Packing material and RFID labels can be faked; spectrometric techniques require time-consuming calibration procedures, and the obtained spectra can be very sensitive to nonchemical influences, such as grain size, morphology, etc. Therefore, the chemical analysis of the specific by-product spectra of subcomponents might provide an attractive additional tool in the fight against counterfeit products.

3.5 Conclusion

Substantial differences were observed in the quality of commercially available AOT surfactant products. While the tested samples all mainly consisted of sodium bis(2-ethylhexyl) sulfosuccinate (i.e. AOT) dissolved in light aromatic naphtha solvent, significantly different contents of the surface-active by-products “monoester 2” and “monoester 3” were found among the AOT products from four different suppliers. Samples of a model agrochemical formulation made using the different AOT products aged differently: storage tests revealed that an increased content of monoesters in the AOT product used correlated with increased sedimentation during storage. There are several papers dedicated to the analysis [13; 23–28] and properties [28–33] of AOT in various media and its uses, but little has been published on the interactions of AOT with its monoesters in complex mixtures such as agrochemical formulations. Although the fundamental behaviors of mixtures of different surfactants are well understood [5; 19], predicting their interactions in complex mixtures such as agrochemical formulations is shown here to require more research. However, the results presented should extend our understanding of such processes. The analysis of the by-product spectra of surfactants might also contribute to the development of a more robust approach to agrochemical formulations, based on the understanding that surfactants with similar by-product patterns will display similar behaviors and properties.

This work demonstrates that changing the supplier of a formulation additive, such as AOT, may adversely affect the stability of the formulation because of minor variations in the additive’s by-product spectrum. Investigation of the underlying process causing the observed instability, which was not addressed here, could constitute further work in this area.

Finally, we have demonstrated that the contents of monoesters can be used to identify the supplier of an AOT product in a model agrochemical formulation. Therefore, the by-product spectra of formulation additives might be useful in the identification of the origins of agrochemical products in anticounterfeiting investigations, complementing the established methodologies [11, 12, 36–40]. The applicability of such identification, using by-product spectra, to other surfactant products with much broader by-product spectra, such as nonionic surfactants, warrants investigation.

3.6 Acknowledgements

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4. Analytical Characterization and Comparison of Tristyrylphenol Ethoxylates used in Agrochemical Formulation

4.1 Abstract

The technical nonionic surfactant TSP-16-ethoxylates (Tristyrylphenol ethoxylates), is no single defined molecule but contains a polymeric distribution with an average of 16 EO units. In order to analyze differences in the EO number distribution of various suppliers and thus to specify more precisely the required quality for the use in agrochemical formulations, an analytical method was developed using LC-ToF-MS with exact mass measurement in combination with multivariate data analysis. This method enables a fast and comprehensive characterization and comparison of commercially available TSP-16-ethoxylates of different suppliers and qualities.

Significant differences were found in composition and content of by-products among the suppliers. These were based on the content of the different styrenated phenol ethoxylates, such as mono-, di-, tri-, and tetrastyrylphenol ethoxylates and on the content of mono- and distyrylphenol copolymerized propoxylates-ethoxylates. These differences were utilized to identify the respective supplier in the raw material as well as formulated in a model agrochemical formulation using a combination of principle component analysis and hierarchical clustering.

4.2 Introduction

As shown in previous work, a small variation in the by-product spectrum of surfactants can have a significant impact on the physico-chemical properties of agrochemical products [1]. Therefore characterization and control of the quality of these surfactants is necessary. Whether differences in the composition of a surfactant of different suppliers have an impact on the properties of an agrochemical product, is usually tested by storage stability tests. Effects on the physico-chemical properties of the agrochemical product are depending on long-term processes, manifesting for example in phase separation or viscosity changes. They cannot be accelerated in the same manner as a chemical process, such as the degradation of an active ingredient, to save time in development of the formulation [2;3]. A life-time or shelf-life of at least two years is mandatory for an agrochemical formulation, in order to gain a registration in most countries in the world [4-6]. In consequence, storage tests have to cover at least two years at ambient conditions. This is time consuming, especially, if one or more additional suppliers for a specific surfactant have to be registered. In addition, the surfactant has to be

continuously monitored during the life cycle of an agrochemical product to avoid potential problems in the physical-chemical stability induced by changes in the quality of the surfactant.

The focus of this work is to develop a fast and reliable method to characterize a nonionic surfactant of different suppliers and qualities according to its main and by-product spectrum. Investigated were tristyrylphenol (TSP) ethoxylates with an average degree of polymerization of 16 ethylene oxide units (EO), in the following abbreviated with TSP-16-ethoxylates. The molecular structure is displayed in Figure 20.

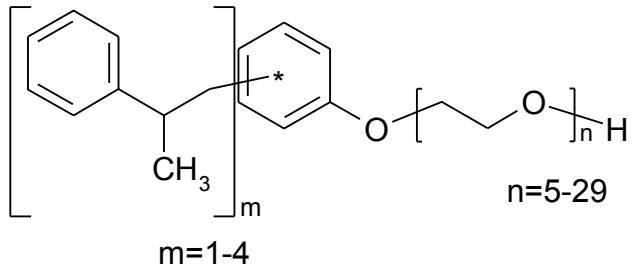


Figure 20: Structure of commercially available tristyrylphenol (m=3) with an average number of ethylene oxide units of n = 16.

TSP-16-ethoxylates are widely used as emulsifiers in agrochemical products and are purchased without additional solvent as liquid [7-10]. The distribution of the ethoxylates depends primarily on the reaction conditions during polymerization and on the acidity of the hydroxyl functionality which undergoes polymerization. For phenol derivatives, such as the TSP, the acidity of the hydroxyl group (pKa (TSP) = 11.0) ensures that no residual phenol is left after polymerization as by-product [11-14]. The polymerization on this kind of educt results in a Poisson-like distribution of ethoxylates, which leads to a complex composition of the final commercial product [15].

Several methods for the analysis of poly ethylene glycol or alkoxide, fatty acid ethoxylates, respectively, with liquid chromatography coupled to mass spectrometry have been published. A separation according to the degree of ethoxylation can be achieved via normal phase-liquid chromatography (NP-LC) [16] or hydrophilic liquid interaction chromatography (HILIC) [17] whereas the separation according to the hydrophobic group is achieved via reversed phase liquid chromatography (RP-LC) [18-21] Another possibility for a separation according to alkyl chain and polyether chain length is liquid exclusion adsorption chromatography (LEAC). Here, separation of the hydrophobic group is conducted according to liquid adsorption chromatography (LAC) and separation of the polyether chain according to size exclusion chromatography (SEC) [22-26]. Also the separation of complex mixtures of different alkoxylates via 2-dimensional liquid-chromatography has been shown [27]. For detection of the ethoxylated entities universal detectors like the evaporation light scattering detector (ELSD) have been

succeeded by mass spectrometry (MS) using either MS-MS or Time-of-Flight (ToF), as these are more sensitive and enable identification via the (exact) molecular mass and/or specific fragments [19;28-30].

Although there has been some research dedicated to characterization of nonionic surfactants in various matrices including agrochemical formulations [31], there has been no method to the knowledge of the authors for compound specific characterization and comparison of TSP-16-ethoxylates of different suppliers. Therefore, it was the aim of this work to develop such a method using reversed-phase liquid-chromatography coupled to a Time-of-Flight mass spectrometer with exact mass measurement in combination with targeted multivariate data analysis considering all main components in TSP-16-ethoxylates.

4.3 Experimental

4.3.1 Chemicals and Reagents

High purity water was obtained by a Milli-Q-gradient A10 system (Millipore, Eschborn, Germany). Methanol and ammonium formiate both of p.a. grade were purchased from Sigma Aldrich. Hexanophenone for internal standard was supplied by Sigma Aldrich with a purity of 99%. 9 to 10 different production batches each from 3 suppliers and two different product qualities for one of the suppliers of TSP-16-ethoxylates were purchased and their spectrum of nonionic surface-active compounds was analyzed. For each batch an amount of at least 25 mL was available. In Table 9, the TSP-16-ethoxylates batches and the respective production sites are listed for each supplier. The suppliers are indicated with A-C. Supplier B has two different product qualities. The refined product quality is indicated with “B1” and the single production batches with an upper case “B”. The technical product quality is indicated with “B2” and the single production batches with a lower case “b”.

Table 9: Investigated suppliers, qualities and production batches of TSP-16-ethoxylates. The refined quality of supplier B is indicated as “B1” and the technical product with “B2”. The corresponding production batches are indicated with upper case “B” for the refined quality and with lower case “b” for the technical product.

Supplier	**A**	**B1**	**B2**	**C**
Batch No.	A-1 to A-10	B-1 to B-9	b-1 to b-10	C-1 to C-10

4.3.2 LC-MS Analysis

An Agilent 1200 SL HPLC coupled to an Agilent 6220 Accurate-Mass-TOF mass spectrometer with interchangeable dual-sprayer electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources and an Agilent 1200 SL HPLC coupled to a Thermo Orbitrap Q-exactive equipped with an atmospheric pressure photo ionization (APPI) source was used for LC-MS measurements. All measurements were done on a Phenomenex Kinetex (50 x 3.0 mm, 2.6 µm) column, which was chosen due to its good separation capacity, while allowing higher flow rates due to larger particles, compared to HPLC columns with full porous particle. Thus, accelerated separation was used, in order to separate the various functionalized and non-functionalized poly ethylene and copolymerized poly propylene and poly ethylene glycols contained in TSP-16-ethoxylates.

Reversed phase-liquid chromatography (RP-LC) was used to separate the different poly ethylene glycols and copolymerized poly propylene and ethylene glycols contained in TSP-16-ethoxylates according to their degree of polymerization. High purity water (Millipore) and methanol were used as LC eluents. Both eluents were modified with 5 mM of ammonium formate, in order to promote the formation of $[M+(NH_4)]^+$ -adducts in the mass spectrometer, thus facilitating the interpretation of the obtained mass spectra especially for ESI. For the analysis of the different components in commercially available TSP-16-ethoxylates gradient elution was chosen.

For identification an extended gradient was chosen starting with 20% (v/v) methanol, raised to 97.5% in 12 min, hold for 3 min at 97.5%, decreased to 20% in 0.5 min and equilibration for 3.5 min at 20%. Total run time was 18 min with a flow of 1.0 mL/min and a column temperature of 50 °C.

For comparison of the different suppliers of TSP-16-ethoxylates a shortened gradient elution was used starting with 20% (v/v) methanol, raised to 97.5% in 6 min, hold for 3 min at 97.5%, decreased to 20% in 0.5 min and equilibration for 1.5 min at 20%. Total run time was 11 min with a flow of 1.0 mL/min and a column temperature of 50 °C.

Flow was directed without split via the APCI and APPI source and with a split of 1:6 (MS:Waste) via the first sprayer needle of the dual-ESI source into the mass spectrometer. To realize the split a QuickSplit adjustable flow-splitter (Restek), was used equipped with resistors which enable a constant split ratio independent of changes in viscosity or pressure. Mass spectra were obtained in positive mode through the whole run.

For the ToF-instrument, the high resolution mode with 4 GHz recording frequency was chosen resulting in an average resolution of about 10000 full width at half peak maximum (FWHM). Two spectra every second were obtained with 4959 transients per spectrum and a mass range of 105-3200 m/z. For the APCI source the parameters were 350 °C for gas temperature, 450 °C for vaporizer temperature, 8 L/min for dry gas, 30 psig

nebulizer pressure and 4.5 µA corona current. For ESI the parameters were 350 °C for gas temperature, 8 L/min drying gas flow and 30 psig nebulizer pressure for both ESI sprayer needles of the dual-sprayer ESI source. For both sources capillary voltage was 3500 V, fragmentor voltage 100 V, skimmer voltage 60 V and octopole 1 RF Vpp 250 V.

For the Orbitrap instrument a resolution of 30000 FWHM and a collector time of 200 ms were chosen with a mass range of 200-4000 m/z. The parameters of the APPI source were 150 °C for capillary temperature, 450 °C for vaporizer temperature, sheath gas flow rate of 50 psig and 10.0 eV photon energy for the krypton lamp.

Mass calibration on the ToF was done for the APCI and ESI source with the corresponding calibration mixtures supplied by Agilent via the second sprayer of the dual-sprayer ESI source. Mass correction during analysis was handled on purine (ionization in positive mode forming a proton adduct with m/z = 121.050873 amu) and hexakis(1H,1H, 3H-fluoropropoxy)phosphazine (abbreviated: HP 921 (ionization in positive mode forming a formate adduct with m/z = 922.009798 amu)).

For analysis via the APCI source, a solution of both was delivered into the eluent after the LC unit via a tee with a flow of 0.2 mL/min. To manage the LC pressure at the tee an additional Agilent 1100 isocratic HPLC pumping unit was used to deliver the recalibration mixture.

For analysis via ESI the solution was delivered with a flow of 0.1 mL/min via the second sprayer needle of the dual-sprayer ESI source into the mass spectrometer.

Mass calibration on the Orbitrap was done with Pierce PN 88322, the corresponding calibration mixture, supplied by Thermo Scientific. Mass correction during the measurement was not necessary, according to the producer, as the mass calibration on this type of instrument is stable enough [32].

4.3.3 Preparations of Standard and Sample Solutions

For the preparation of all standard and stock solutions and dilution steps a mixture of 50/50 (v/v) water and methanol was used. For determination of the linear range a sample of production batch A-1 of supplier A was taken.

Linearity Range

For determination of the linearity range a stock solution of tristyrylphenol with 16 EO units was prepared dissolving an equivalent amount of TSP-16-ethoxylates (Batch A-1) in the mixture of water and methanol, obtaining a concentration of 0.6 g/L. This stock solution was further diluted 1:9 (v/v) obtaining a concentration

of 0.06 g/L. For preparation of the standard solution this intermediate stock solution was diluted to fit the concentration range 30 mg/L to 0.1 mg/L. As no analytical standard for TSP with 16 EO units was available all given values were calculated based on the weighed amount of TSP-16-ethoxylates.

A stock solution of the internal standard hexanophenone for determination of linearity was prepared dissolving an equivalent amount of the hexanophenone in the mixture of water and methanol obtaining a concentration of 0.2 g/L. For preparation of the standard solution the stock solution was diluted to fit the concentration range 100 mg/L to 1 mg/L.

Preparation of Sample Solutions

Stock solution of the internal standard hexanophenone for spiking of the samples was prepared dissolving an equivalent amount of the hexanophenone in the mixture of water and methanol obtaining a concentration of 0.6 g/L. A volume of 0.1 mL of this stock solution was added to every sample after its final dilution step obtaining a concentration of 60 mg/L of internal standard.

For the analysis of TSP-16-ethoxylates in product batches, 40 mg of the sample was dissolved in 20 mL of the solvent mixture of water and methanol. The working solution was then diluted 1:100.

For the analysis of TSP-16-ethoxylates in agrochemical formulation samples 30 mg of the sample were diluted in 20 mL of the solvent mixture. The working solution was then diluted 1:10.

Preparation of Mass Calibration Solution

The mass calibration solution was purchased from Agilent for ESI-source and applied according to the instructions of the supplier. The solution for mass correction during the analysis was purchased from Agilent for both APCI- and ESI-source. For mass correction a solution of Purine and HP 921 was prepared containing 1.0 µM Purine and 0.25 µM HP 921 in 95/5 (v/v) methanol/water. For measurements with the ESI source a dilution of 1:100 was needed to avoid overloading of the detection unit. For testing the mass calibration during analysis, a test sample containing standards with known exact masses spanning the retention time window was analyzed at the beginning and the end of a test series. The composition of the test sample is given in the Supplementary.

4.3.4 Formulation Sample

Samples of four model formulations containing TSP-16-ethoxylates of supplier A, B1, B2 and C were prepared according to the composition shown in Table 10. These formulation samples were then analyzed according to the method developed in this work in order to investigate whether the detection and identification of respective suppliers of TSP-16-ethoxylates was possible in the given matrix of the formulation.

Table 10: Table of composition of the model agrochemical formulation

Raw material	**Content [%] (w/w)**
Active ingredient	23
TSP-16-ethoxylates	2.5
Dispersing agent (nonionic)	10
Emulsifier 1 (nonionic, functionalized PEG)	15
Emulsifier 2 (nonionic, functionalized PPG-PEG-co-polymer)	9.0
Clay	0.1
Acid	0.4
Solvent	40

4.3.5 Data Analysis

Data extraction was performed with Agilent Mass Hunter and data analysis with Agilent Mass Profiler Professional. The acquired scan data were displayed for TSP-16-ethoxylates as EIC with the range of m/z 500 to 921 and the range of m/z 930 to 3200 or, for hexanophenone, as the exact molar mass of the $[M+(H)]^+$ adduct with an exact mass of m/z 177.1274 and its A+1 and A+2 isotopic masses with a window of 100 ppm around each mass to account for potential mass divergence during the measurement. The mass range of m/z 921 to 930 was left out intentionally, because in this range the mass signal of HP 921 is detected that was used for mass calibration.

The acquired scan data of the three replicate measurements of each production batch were at first subjected to a targeted compound search using the molecular-feature-extraction (MFE) algorithm of the Agilent Mass Hunter software with a mass tolerance of 10 ppm and a threshold of 2000 counts signal height. For the targeted

compound search a custom made data base of exact masses was used containing a wide range of different derivates of styrylphenol ethoxylates and copolymerized propoxylates-ethoxylates. The ammonium adducts $[M+(NH_4)]^+$ of the respective compounds were searched for. The data base is used in the csv-format and the corresponding data is given in the Supplementary. This first compound extraction was fast screening using a peak finding algorithm for the respective masses to reduce findings for marginal compounds. For a comprehensive data extraction a second extraction step was employed next.

The results of the first extraction step were exported to Agilent Mass Profiler Professional software where the results of all repetition analyses and batches were binned according to the suppliers and qualities to one data file. The obtained data files for each of the suppliers and qualities contained all compounds found in the first step with annotation and retention time, except those occurring only once, which were removed in this step to eliminate marginal compounds.

For the second, exhaustive extraction all samples were reanalyzed with a so called find-by-formula (FBF) algorithm in the Agilent Mass Hunter software searching for the compounds identified in the first step at their respective retention times in all samples to minimize false-negative findings. The set deviation from the calculated exact mass was 20 ppm and ± 0.5 min from the expected retention time. The single charged $[M+(NH_4)]^+$ and the double charged $[M+2(NH_4)]^{2+}$ ammonium adducts of the respective m/z-values of the molar mass of each compound were searched for. They were then summed up to one peak in an extracted ion chromatogram (EIC). This peak was integrated and the obtained peak area was used as quantitative information for the respective compound.

The obtained data set was restricted to the linear range determined consecutive to each sequence of measurements. All compounds were removed that had insufficient signal-to-noise ratios (SNR<20:1) for quantification

The data set confined to the linear range was then exported to Agilent Mass Profiler Professional software where the peak areas of all compounds of each analysis were normalized according to a standard procedure of the software to the peak area of the internal standard as shown in Equation 5.

$$A_{nor} = \log_2 A_x - \log_2 A_{Istd}$$

Equation 5

A_{nor}: Area value normalized

A_x: Area value before normalization

A_{Istd}: Area value internal standard

The normalized data set was then subjected to principle component analysis and hierarchical clustering.

4.3.6 Validation

Linearity was defined by the linearity range of the used mass spectrometric detector and by the LOQ for the analytes. The linear ranges of 30 mg/L to 0.1 mg/L of TSP with 16 EO units (referring to the weighted amount of TSP-16-ethoxylates) and 100 mg/L to 1 mg/L of the internal standard hexanophenone were defined accordingly.

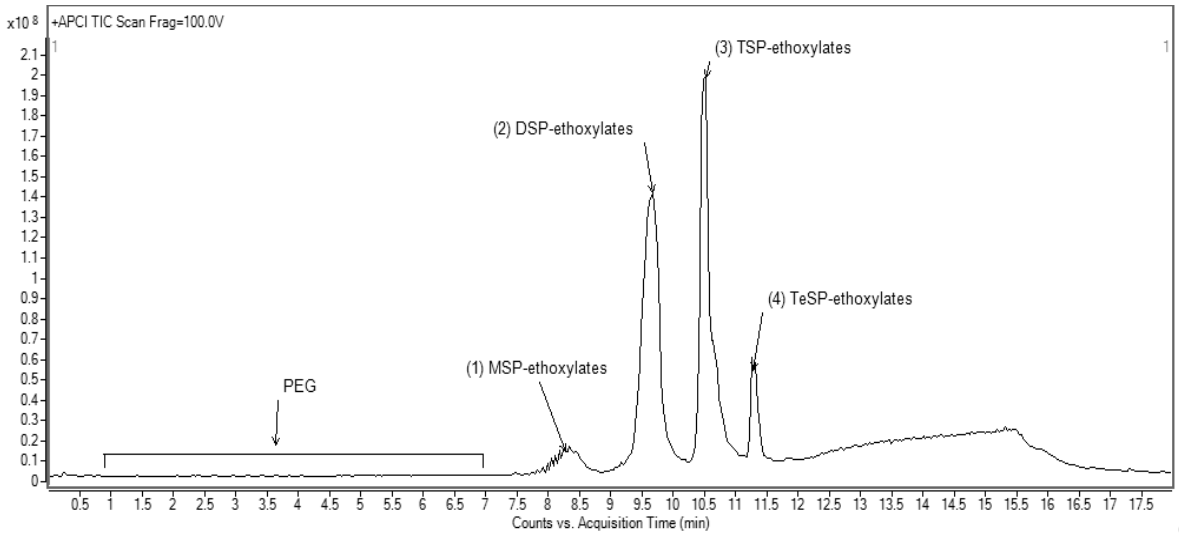
Precision was determined on three repetition analyses at a level of 60 mg/L for the internal standard and 40 mg/L for TSP (16-EO units). The LOQ was defined as a signal-to-noise ratio of at least 20:1 which was calculated via the height of the respective analytes.

The specificity of the method was ensured not only by using exact mass measurements for identification and extraction of each investigated component in TSP-16-ethoxylates, but also by using the retention time windows in which the different styrenated phenol ethoxylates were eluted under the given chromatographic conditions. Thereby, false positive hits were as much reduced as possible.

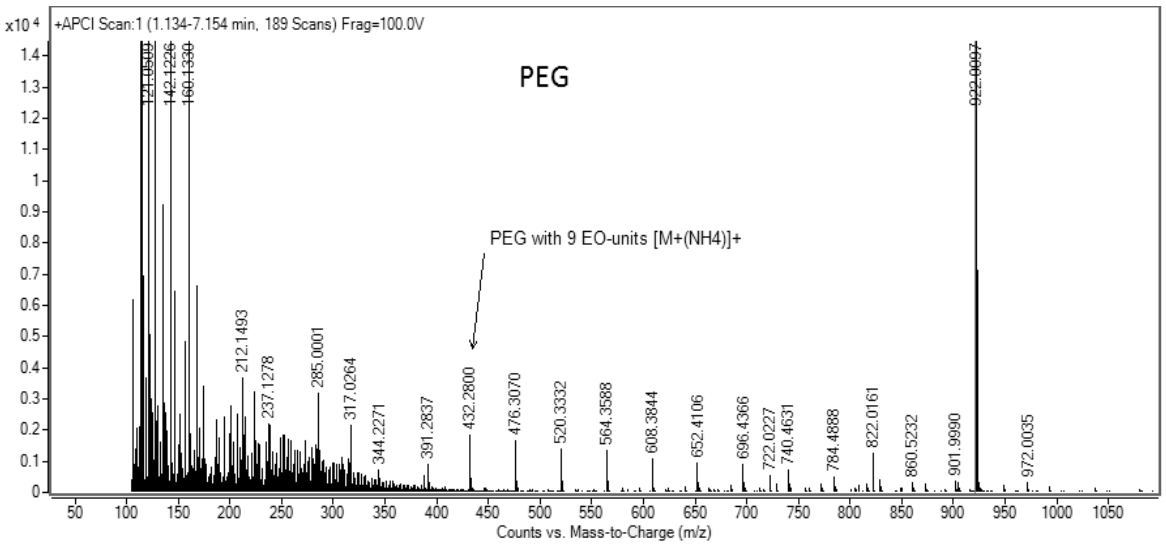
4.4 Results and Discussion

4.4.1 Method Development

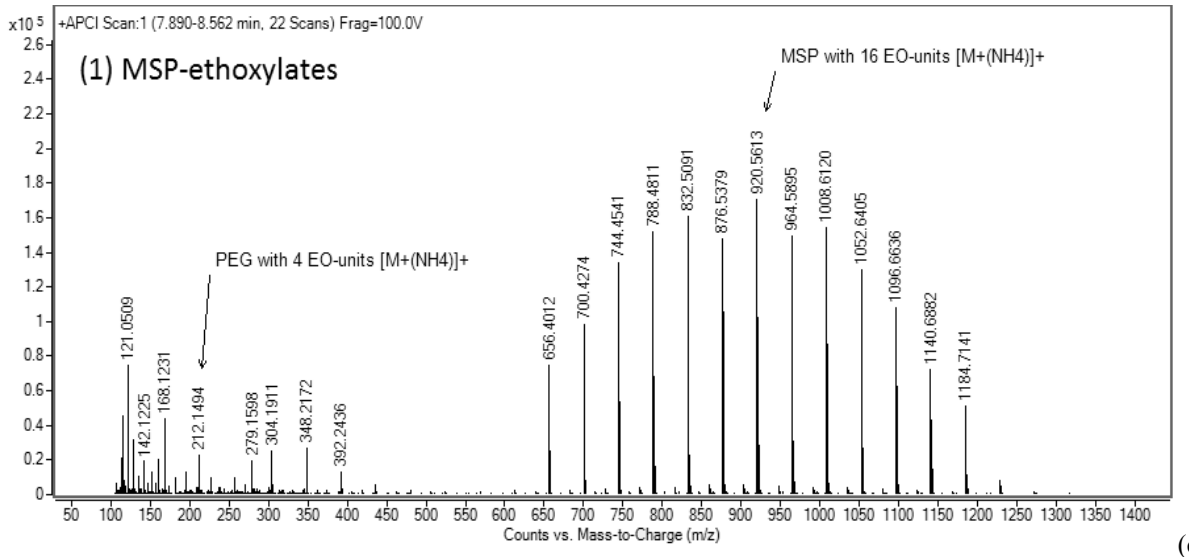
For method development a sample of commercially available TSP-16-ethoxylates was used. For analysis reversed phase liquid chromatography (RP-LC) coupled via atmospheric pressure chemical ionization (APCI) to a Time-of-Flight mass spectrometry (ToF-MS) with exact mass measurement was used. The latter was necessary to enable identification of the single ethoxylated entities in the sample. The coupling of APCI was chosen in the first place as the target analytes are nonionic molecules. Chromatographic separation according to the functional groups via reversed phase liquid chromatography was achieved as shown in Figure 21 (a) together with the mass spectra of the identified peaks in Figure 21 (b) for polyethylenglycol (PEG), in Figure 21 (c) for monostyrylphenol (MSP), in Figure 21 (d) for distyrylphenol (DSP), in Figure 21 (e) for tristyrylphenol (TSP) and in Figure 21 (f) for tetrastyrylphenol (TeSP) ethoxylates.


x10 8
+APCI TIC Scan Frag=100.0V
(3) TSP-ethoxylates
(2) DSP-ethoxylates
(4) TeSP-ethoxylates
(1) MSP-ethoxylates
PEG
Counts vs. Acquisition Time (min)

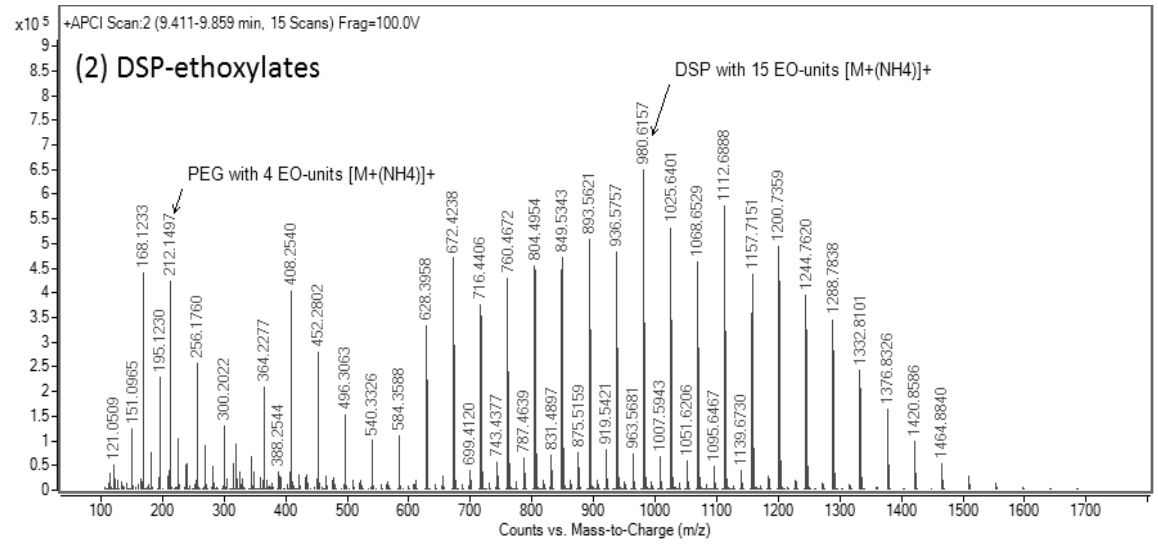
(a)


x10 4
+APCI Scan:1 (1.134-7.154 min, 189 Scans) Frag=100.0V
PEG
PEG with 9 EO-units [M+(NH4)]+
Counts vs. Mass-to-Charge (m/z)

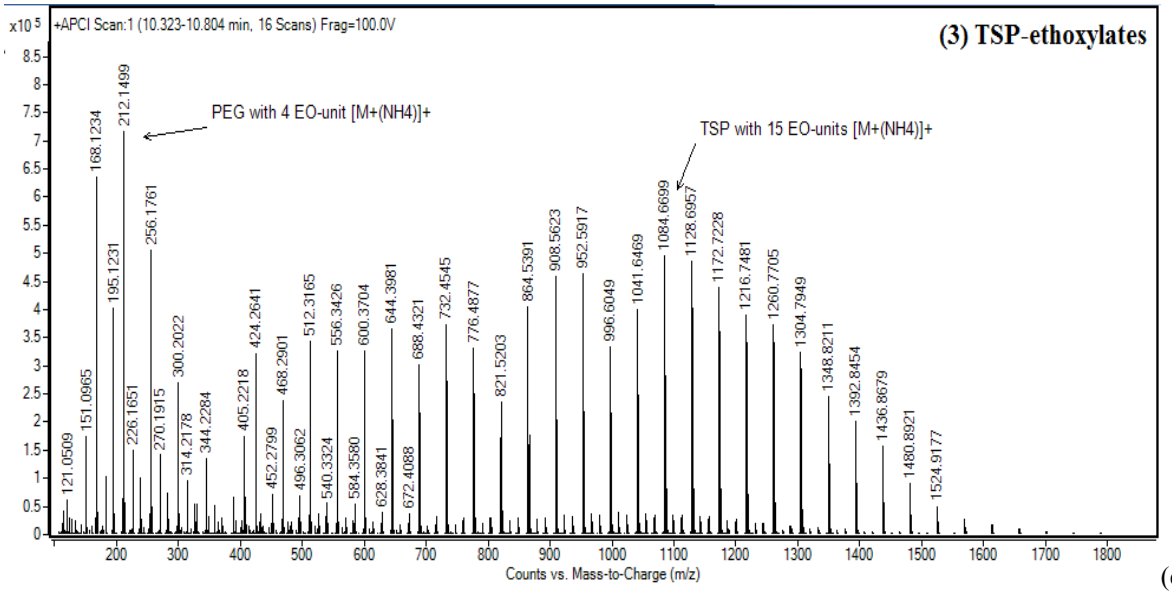
(b)


x10 5
+APCI Scan:1 (7.890-8.562 min, 22 Scans) Frag=100.0V
(1) MSP-ethoxylates
MSP with 16 EO-units [M+(NH4)]+
PEG with 4 EO-units [M+(NH4)]+
Counts vs. Mass-to-Charge (m/z)

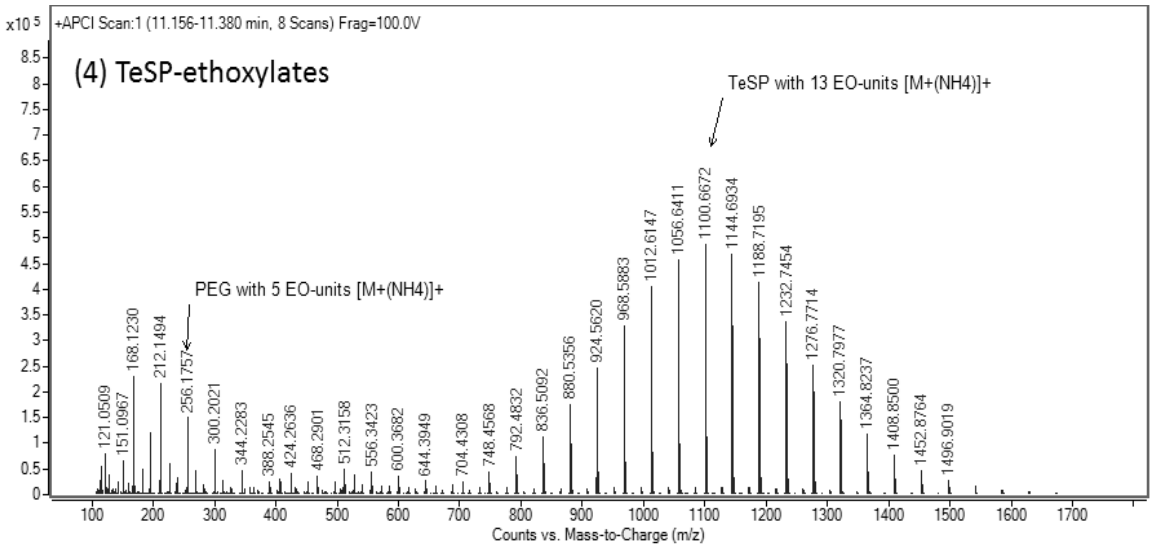
(c)



(d)



(e)



(f)

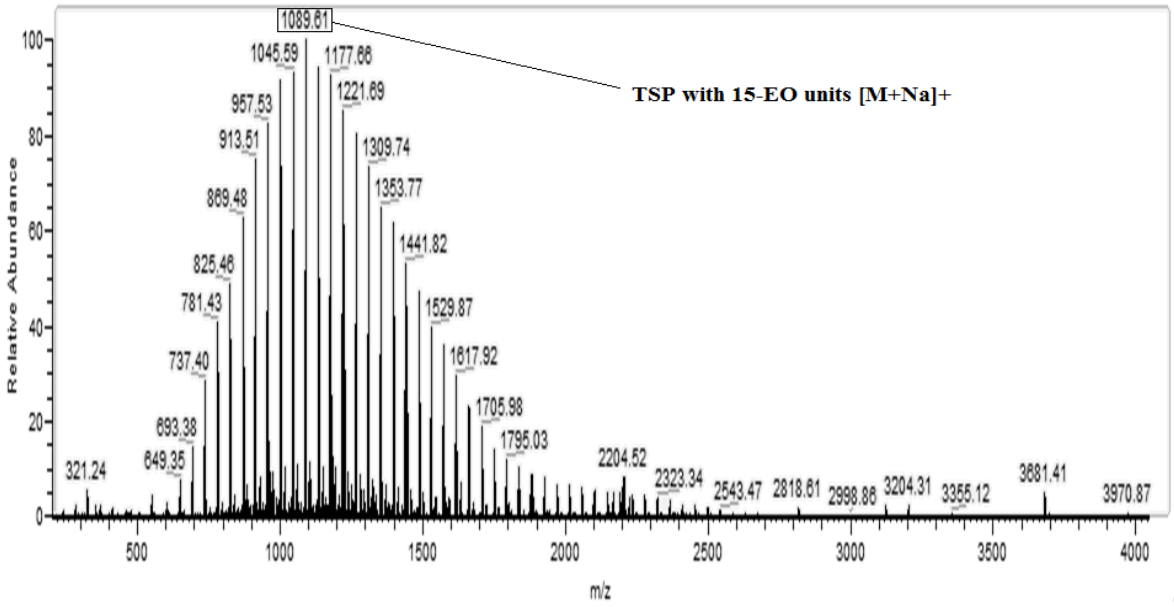
Figure 21: Chromatographic separation of commercial available TSP-16-ethoxylates with a C18 RP-LC coupled via APCI in positive mode to a ToF-MS with exact mass measurement. Indicated are PEG, (1) MSP-, (2) DSP-, (3) TSP- and (4) TeSP ethoxylates in Figure 21 (a). The mass spectra of the identified

peaks are displayed in Figure 21 (b) for polyethylenglycol (PEG), in Figure 21 (c) for monostyrylphenol ethoxylates (MSP), in Figure 21 (d) for distyrylphenol (DSP), in Figure 21 (e) for tristyrylphenol (TSP) and in Figure 21 (f) for tetrastyrylphenol (TeSP).

For identification of the different molecules a coupling to a (ToF-MS) with exact mass measurement was used. Entities of MSP (1), DSP (2), TSP (3) and TeSP (4) ethoxylates were identified and separated according to the degree of styrenation. Furthermore, entities of PEG were identified, eluted between t_R=1.0 min and t_R=7.0 min. As there are numerous possibilities for the analysis of PEG and as the focus of this work is on the characterization of the main component, i.e., the actual surfactant, analysis of PEG was not elaborated further. The identified m/z are $[M+(NH_4)]^+$ adducts, due to the composition of the eluent, which has been modified with 5 mM of NH_4COOH.

The mass spectra of the different styrylphenol ethoxylates derivates obtained by APCI also show PEG with a range of 3 (m/z =168.1230 amu; $[M^+(NH_4)]^+$) to 8 (m/z =388.2545 amu; $[M^+(NH_4)]^+$) EO units. As PEG originating from the sample has been chromatographically separated at the beginning of the gradient, the observed PEG within the peaks of MSP-, DSP-, TSP- and TeSP-ethoxylates, respectively, were caused by in-source degradation of the polyether chain during ionization, as described in literature [33]. As a consequence of the distribution each of the styrylphenol ethoxylate derivates is discriminated to shorter chain lengths and the original distribution cannot be retraced. Reducing vaporization temperature or corona current did not improve the result, so APCI was considered unsuitable for determining the actual distribution of EO chain length in nonionic surfactants.

Therefore, the ionization performance of the target analytes was tested on two further ionization devices for the coupling of liquid chromatography and mass spectrometry, APPI and ESI in positive ionization mode. Exemplarily, the mass spectrum for the peak of TSP-ethoxylates was used for comparison with the results shown in Figure 22 (a) for APPI and in Figure 22 (b) for ESI.



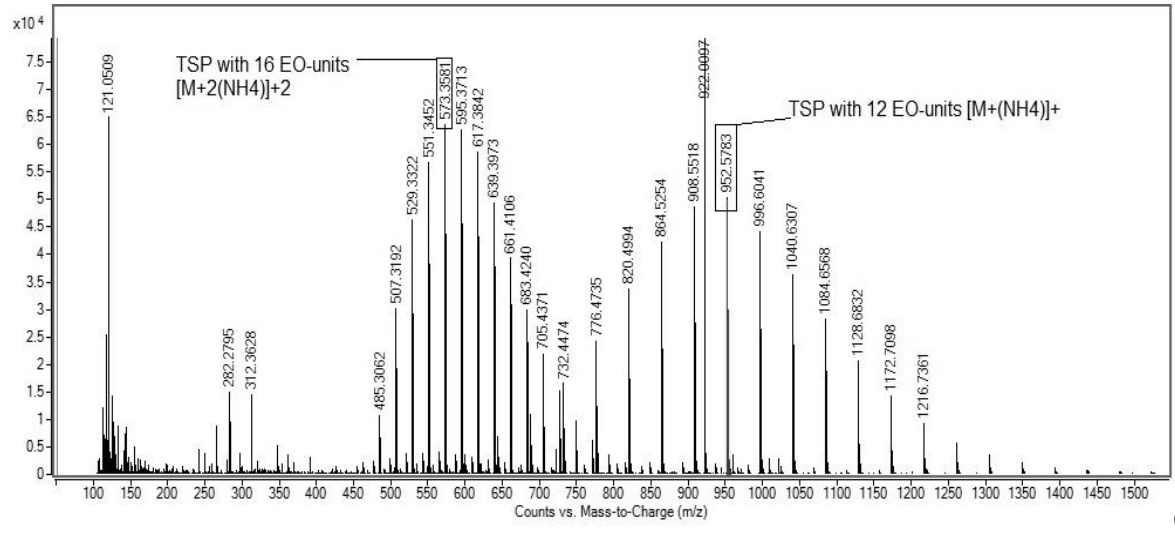


Figure 22: Ionization behavior of TSP-ethoxylates ionized by APPI (a) and ESI (b). In each case the of TSP-ethoxylates is shown. For each experiment the same elution conditions with water and methanol as mobile phase, plus 5 mM ammonium formiate each eluent were chosen. For ESI (b) an Agilent 6220 ToF-MS with exact mass measurement and for APPI (c) a Thermo Orbitrab Q-exactive had been used.

As shown there are substantial differences in the ionization behavior of the different TSP-16-ethoxylates between the investigated types of ionization devices. The spectrum obtained by APPI showed the different TSP ethoxylates as almost t-distributed, without apparent degradation products except for the signal at 321.24 amu corresponding to DSP. All entities are detected with their molar masses as the dominant signal being a [M+Na]+ adduct. The mean of the distribution, however, is at TSP ethoxylate with 15 EO units and not at TSP with 16 EO units as expected for TSP-16-ethoxlyates. Because of its softer mode of ionization APPI leads to less in-source degradation than observed for APCI, though possibly discriminating entities with higher EO chain length during ionization [34].

The spectrum obtained by ESI showed two clusters of signals which corresponded to single- and double-charged entities of the TSP ethoxylates molar masses. Degradation products, such as PEG for APCI, were not observed. The double charged state of the TSP-ethoxylates is favored for entities with longer chain length and is ranging from TSP with 12 EO units (m/z =485.3062 amu; [M+2(NH4)]+2) to TSP with 27 EO units (m/z =815.5037 amu; [M+2(NH4)]+2), whereas the single charged entities are ranging from TSP with 4 EO units (m/z=600.3961 amu; [M+(NH4)]+) to TSP with 26 EO units (m/z =1568.9413 amu; [M+(NH4)]+). For some entities both single- and double-charged masses are detected, so that the spectrum has to be deconvoluted in the end for analysis.

Although APPI and ESI are performing comparably on TSP-16-ethoxylates, TSP with an average chain length of 40 EO units is analyzed next, to determine if both techniques of ionization are applicable for nonionic surfactants with higher degree of ethoxylation. Again the spectrum is taken for the chromatographic peak of TSP ethoxylates, with the results shown in Supplementary. For ESI only the molar masses of TSP-ethoxylates are detected as single- to fourfold-charged entities without apparent in-source degradation of the ions through ionization. For APPI, however, a complex spectrum with a wide variety of mass signals is received, which can only partly be assigned to TSP ethoxylates like the highest mass signal to TSP ethoxylate with 33 EO units. Apparently ionization of TSP ethoxylates is limited with APPI to entities with a shorter EO chain, resulting in fragmentation of entities with a longer EO chain.

As their actual composition can be determined without discrimination during the ionization process ESI is chosen as coupling of LC to ToF-MS to characterize TSP-16-ethoxylates of different suppliers according to their degree of styrenation and ethoxylation.

4.4.2 Method for the Quantitative Determination

Internal Standard for Quantification

As there is no analytical standard available to quantify the different components in commercial TSP-16-ethoxylates, an internal standard was used to compensate for variations in the performance of the LC-MS instrument, variation in the sample composition and enabling comparison of TSP-16-ethoxylates between different suppliers. Hexanophenone was chosen as internal standard because it is easily available, not co-eluting with the target analytes (see Figure 23) and has a comparable detector response as the target analytes as shown in the following. For the quantitative comparison of different suppliers of TSP-16-ethoxylates the gradient was shortened in order to save analysis time in comparison to the gradient used for identification of the single compounds in TSP-16-ethoxyltes as shown in Figure 21 (a).

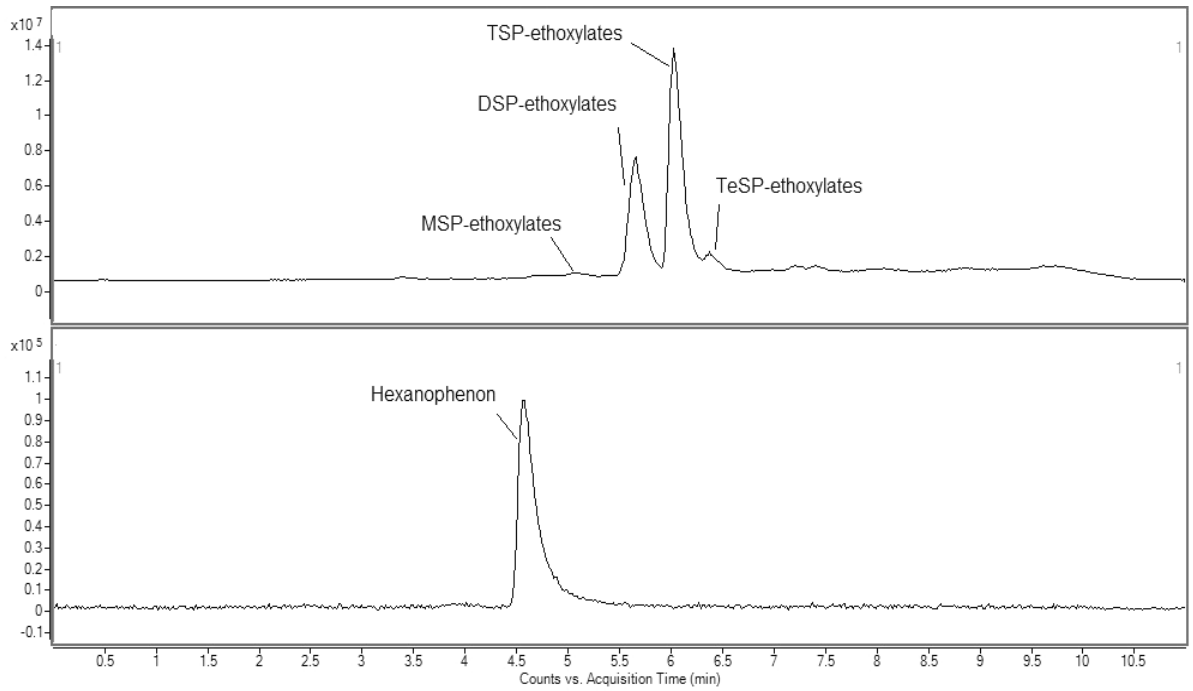


Figure 23: Usage of hexanophenone as internal standard for the quantification of the styrenated phenol ethoxylates contained in TSP-16-ethoxylates. Hexanophenone, shown in lower the figure, is not co-eluting with the target analytes, MSP-, DSP-, TSP- and TeSP-ethoxylates, shown in the upper figure. The shortened gradient is still sufficient to separate the different styrenated phenol ethoxylates.

As shown the different styrenated phenol ethoxylates are still separated well enough and the overlapping of the peaks of TSP- and TeSP-ethoxylates can be accepted, because identification and extraction of the single ethoxylate entities is ensured via the detection with ToF-MS and exact mass measurement.

Linear Range

The linear ranges for both internal standard and target analytes were defined based on the linearity range of the used mass spectrometric detector and by the LOQ for the analytes which had been defined at a signal-to-noise ratio of at least 20:1 to ensure acceptable quantification. As representative for the target analytes, TSP ethoxylate with 16 EO units was chosen, as it is the most abundant component in the investigated TSP-16-ethoxylates. The results for the linear range and the relative standard deviation of the method for both analytes are shown in Table 11 together with the precision of 3 repetition analyses at a level of 60 mg/L for the internal standard and 40 mg/L for TSP with 16-EO units. The linearity plots and the EICs of both analytes at the corresponding limit of quantification (LOQ) are given in the Supplementary.

Table 11: Linear range and the relative standard deviation of the method for the analytes TSP with 16 EO units and hexanophenone, together with the precision of 3 repetition analyses at a level of 60 mg/L for the internal standard and 40 mg/L for TSP with 16 EO units and the LOQ.

	TSP with 16 EO units	**Hexanophenone**
Linear range [mg/L]	0.3-33.2	1.2-122.0
R	0.9997	0.9993
Relative standard deviation of the Method V_{x0} [%]	3.0	2.6
Precision [%]	2.5	1.9
LOQ [mg/L]	0.3*	6.0

*calculated based on the weighted amount of TSP-16-ethoxylates (Supplier A, batch 1)

Both hexanophenone and TSP with 16 EO units show a comparable linear range and response for the LC-ToF-MS with ESI. Based on the ionization behavior of TSP with 16 EO units, a linear response for the other TSP ethoxylates as well as for MSP, DSP and TeSP ethoxylates is assumed. For every measurement the linear range is defined beforehand and only those compounds within this range are normalized against the content of internal standard and used for comparison of the different suppliers of TSP-16-ethoxylates.

Nevertheless, determination of the exact distribution of different entities in TSP-16-ethoxylates was not possible, as the ionization yield of each of the ethoxylates is depending on the EO chain length and its functionalization. As the aim of this work is the *relative* comparison of different suppliers of TSP-16-ethoxylates and not an absolute quantification of the single components this limitation is acceptable.

4.4.3 Comparison of TSP-16-ethoxylates of different Suppliers and Qualities

For comparison of different suppliers of TSP-16-ethoxylates three different suppliers and two different product qualities for one of the suppliers were compared with respect to their relative content of nonionic surfactants using the method developed in this work. To this end, data analysis techniques used for example in proteomics [35-37] or forensics [38-40] have been utilized, where data sets containing multiple components in each sample are analyzed on significant variations among samples and the compound(s) responsible for it. For this work

complementary principle component analysis (PCA) and hierarchical clustering (HCA) were used as recommended by Boyd [41] and Want [42].

Accordingly, from each of the suppliers and qualities at least 9 different production batches were purchased and analyzed in order to account for variations in the production processes. For each batch three replicate analyses were performed to account for possible instrument variations. The analytical raw data of all analyses were subjected to a 2-step targeted data mining approach using the data base composed in this work. The PCA was conducted then on the complete data set with three repetition analyses of each sample, in order to visualize the error of the analytical method. The PCA was performed on conditions, i.e. the PCA was performed on the different samples and not on the compounds identified in the samples [43]. The results of the PCA are displayed in Figure 24.

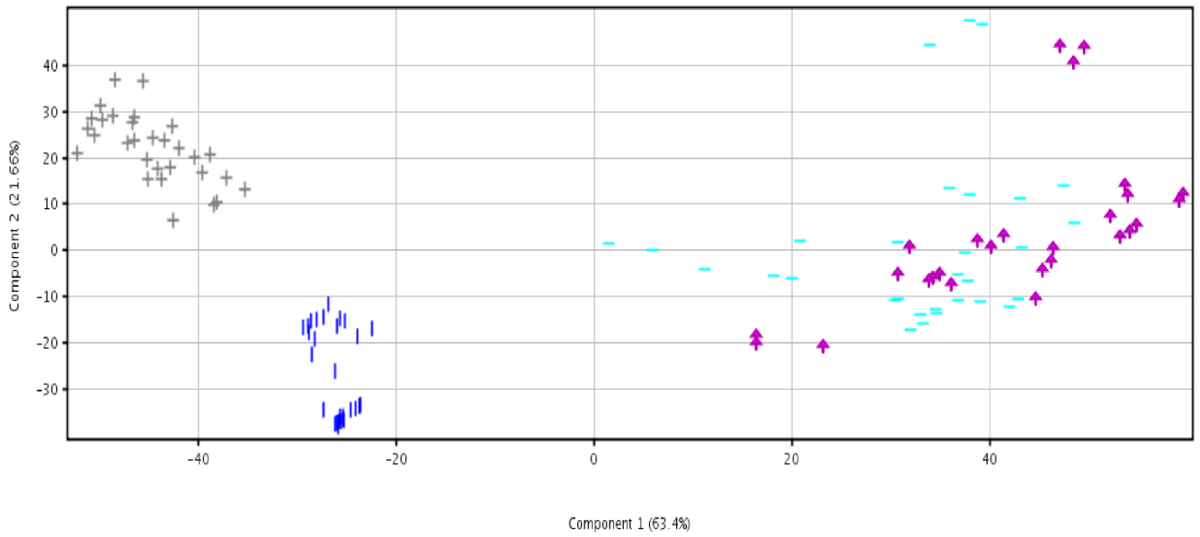


Figure 24: PCA of the data sets from supplier A (Cross), B1 (Arrow), B2 (Horizontal Bar) and C (Vertical bar). The results of 3 repetition analysis each production batch of TSP-16-ethoxylates of the investigated suppliers were used for this PCA.

The variations in the data set are mainly explained by the first two components, as displayed in Figure 24. Distinct clusters were formed, which correspond to the respective suppliers A (Cross), B (B1: Arrow; B2: Horizontal Bar) and C (Vertical Bar). The data points corresponding to the different qualities B1 and B2, however, are overlapping and form a combined cluster. The variations in the extracted data sets of the suppliers A, B and C are big enough to result in distinct clusters in PCA. Conversely, the variations between the production batches of each supplier and between the replicate analyses for each batch are significantly smaller than between the different suppliers of TSP-16-ethoxylates. As the production batches of each of the suppliers cover at least four different production campaigns and a time span of three to four years, respectively, the observed differences can be viewed as systematic and not random. To elucidate on which compounds the

observed variations in the PCA are based on, their score in direction of the 2 components is plotted as well and shown in Figure 25.

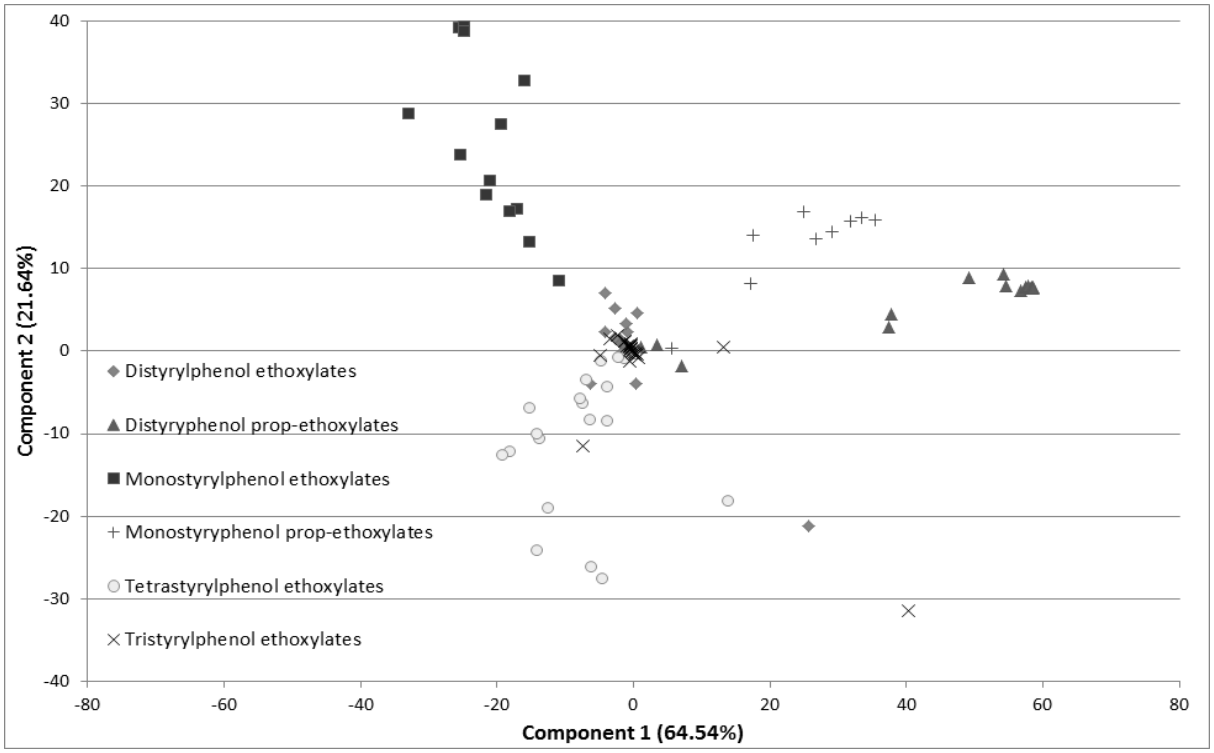


Figure 25: Loading of each compound of MSP-, DSP-, TSP- TeSP ethoxylates and MSP- and DSP-copolymerized-propoxylates-ethoxylates for both components obtained by the PCA on conditions as shown in Figure 24.

The results shown in Figure 25 indicate that the variation in component 1 is mainly explained by the content of the copolymerized-propoxylated-ethoxylated compounds of MSP (Plus) and DSP (Triangle). The variation in component 2 is explained by the content of ethoxylated compounds of TeSP (Circle) and MSP (Square). The content of ethoxylated compounds of TSP and DSP, however explain none of the variations in component 1 or 2 which led to the clustering observed in Figure 24. According to these results supplier A and C are differentiated according to their content of MSP and TSP-ethoxylates and supplier B separated due to its content of MSP- and DSP- copolymerized-propoxylates-ethoxylates. The HCA was performed combined on the suppliers, as well as, on the compounds in each data set in order to analyze which compounds were responsible for the variations between the suppliers and qualities, respectively. As the results of the PCA had shown little variation originating from the analytical method, the results of the three repetition analyses of each batch were averaged. The clustering arrays of the compounds are numbered and marked with brackets within the displayed HCA in Figure 26. These single compounds in these arrays are given in Supplementary.

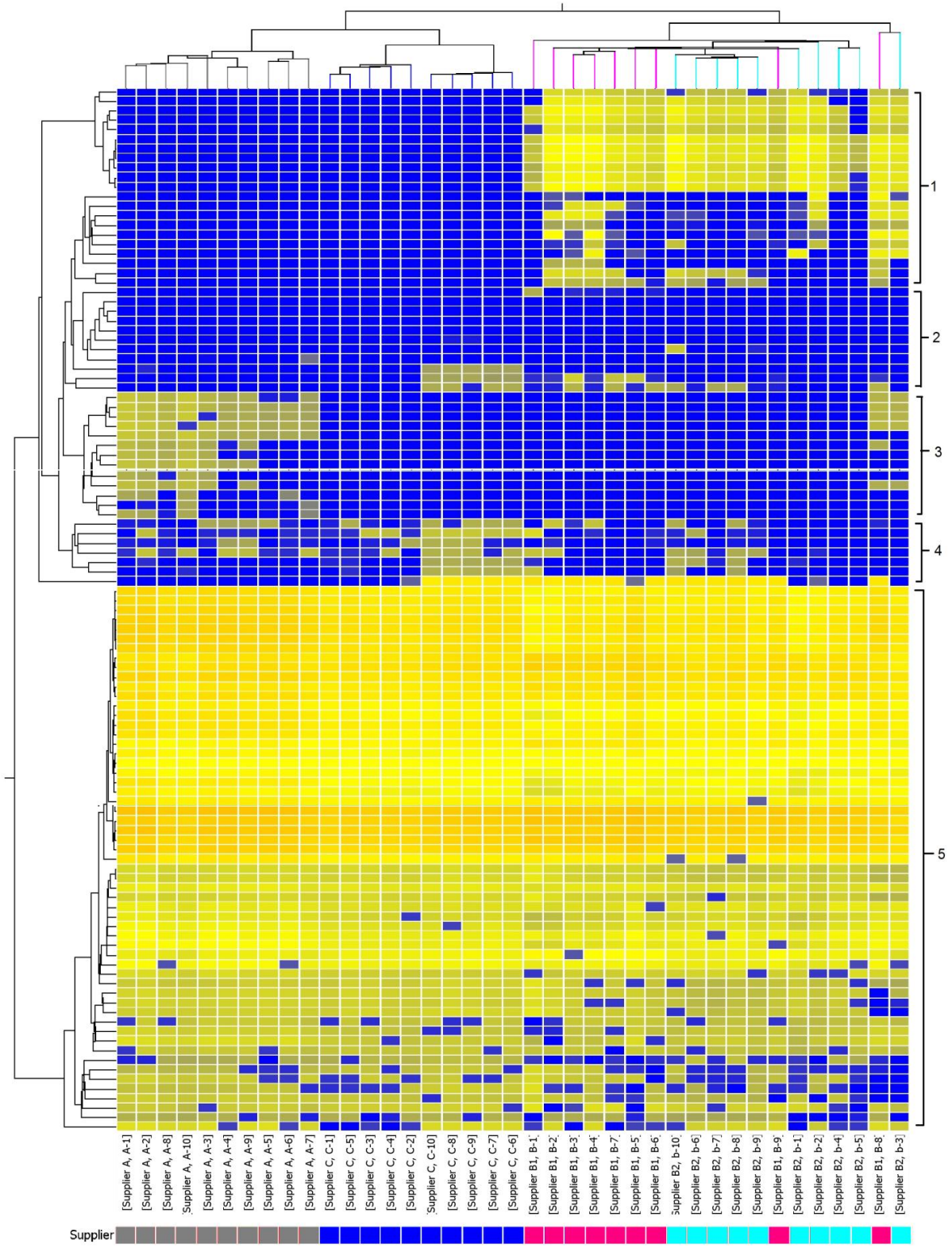


Figure 26: Combined hierarchical clustering of the samples (x-axis) and the compounds (y-axis) detected in the samples of supplier A (grey), B1 (light blue), B2 (violet) and C (dark blue). Each sample is the average of 3 repetition analyses. The content of a compound in the analyzed sample is coded via a colored rectangle in the column beneath the respective sample. The color ranges from deep blue, compound not detected, over yellow, compound as abundant as internal standard, to red, compound with the maximum

content. Numbered and marked with brackets are those arrays of compounds which are responsible for the observed clustering of samples according to their suppliers and qualities. The single compounds are listed in Supplementary

The HCA confirmed the clustering obtained in the PCA. The dendrogram displayed in the top of Figure 26 is forming three main clusters marked in color in the bottom line starting with cluster 1 containing the samples of supplier A (grey) than cluster 2 containing those of supplier C (dark blue) and finally cluster 3 for containing both qualities B1 (light blue) and B2 (violet) of supplier B.

The actual compounds responsible for the observed clustering are given in y-axis and are marked with numbered arrays. Array 1 lists the compounds that distinguish Supplier B, B1 and B2 from the other suppliers. Samples of supplier B contain copolymerized propoxylates-ethoxylates of MSP and DSP, which are not detected in the samples of supplier A and C. These compounds may be explained as contamination originating from the production of copolymerized propoxylates-ethoxylates of TSP which are also produced by supplier B. Based on their contents of ethoxylates or copolymerized propoxylates-ethoxylates of MSP, DSP, TSP and TeSP, a differentiation, however, was not possible between the two qualities of supplier B, B1 and B2. There is at least no difference in quality between B1 and B2, regarding their content of nonionic-surfactants.

Supplier A and C are mainly differentiated by their content of MSP-ethoxylates (array 3), but also to some extend by the content of TeSP-ethoxylates (array 4). MSP-ethoxylates are detected in all samples of supplier A, whereas for supplier C and B (B1 and B2) these compounds are only present in few samples. TeSP-ethoxylates are present for some samples of supplier C whereas they are absent for all samples of supplier A. The arrays 2 and 5, which contain DSP-, TSP- and TeSP-ethoxylates, show little or no contribution to the observed clustering of samples. There is hardly any variation in the content of these compounds in the samples of all suppliers. These findings correlate with those of the PCA, where mainly the content of MSP-ethoxylates and of copolymerized propoxylates-ethoxylates MSP and DSP and to some extend TeSP-ethoxylates were responsible for the variations between the different suppliers, resulting in the observed clustering.

The methodology combining instrumental analysis and multivariate data analysis was successfully transferred for the characterization and differentiation of TSP-16-ethoxylates from different suppliers based on their content of styrenated phenol ethoxylates. The content of contaminants (supplier B1/B2) and the content of MSP- and TeSP-ethoxylates (supplier A and C) were differing between the suppliers, whereas the content of main components, DSP- and TSP-ethoxylates, was comparable. The differences in the content of the surface-active entities in commercial TSP-16-ethoxylates of the suppliers are significant, however, in the properties of

agrochemical formulations no differences were observed for different qualities neither during preparation of the formulation nor during or after storage tests (data not shown).

There are several methods published using LC-MS for the characterization of nonionic surfactants in various matrices, among others in agrochemical formulations. Characterization of nonionic surfactants according to hydrophobic chain and degree of ethoxylation using LEAC as proposed by Trathnigg [22-26] was only demonstrated in absence of matrix compounds and so cannot be applied for complex matrices such as agrochemical formulations. Another standard approach is using matrix assisted laser desorption ionization (MALDI) ToF-MS for characterization of nonionic surfactants [44]. The mass spectra obtained by MALDI-ToF-MS, however, are not easy to interpret, especially the more compounds are detected. In the case of the variations observed in the analyzed TSP-16-ethoxylates this could result in very complex and hardly interpretable mass spectra.

Another approach for the determination of nonionic surfactants in complex matrices was shown using GCxGC or LCxLC coupled to MS [27;29]. These enable identification of the respective surfactants in the samples, however, lack quantitative information and multivariate data analysis needed for comparison of different samples. A characterization of nonionic surfactants in an agrochemical formulation, namely octylphenol and nonylphenol ethoxylates has been shown by Meisen et al. [31] using a combination of different techniques with the focus on quantification of the total amount of surfactant. For that investigation, however, a combination of different techniques in different analysis steps was necessary, using at first RPLC for fractionation of the target analytes, which were then analyzed on their distribution of ethoxylates via NPLC followed by a consecutive identification of the prior fractionated nonionic surfactant via GC-MS and MALDI-ToF-MS. This is very time consuming and laborious especially for a large number of samples.

The method developed in this work, using LC-ToF-MS with exact mass measurement in combination with multivariate data analysis, offers a fast and comprehensive semi-quantitative comparison of a nonionic surfactant of different suppliers, such as TSP-16-ethoxylates. The characterization obtained by this method can aid the formulation chemist in comparing different suppliers for one surfactant and thus aiding his choice.

4.4.4 Statistical Evaluation of the Results on the Content of the Components in TSP-16-ethoxylates on their Use for Product Identification

There are substantial differences regarding the content of the main components, MSP-, DSP-, TSP- and TeSP-ethoxylates, and the by-products, copolymerized propoxylates-ethoxylates of MSP and DSP, in commercial TSP-16-ethoxylates. These differences are specific for the analyzed suppliers A, B and C and could be possibly further used for product identification. Therefore, model formulation samples were prepared containing TSP-16-ethoxylates of each supplier and quality (A, B1, B2, C). These samples were then analyzed with the new method in order to test if they were assigned correctly to their suppliers using both PCA and hierarchical clustering. In the following, the results of the PCA (Figure 27) and of the hierarchical clustering (HCA) (Figure 28) are shown. For the HCA only the clustering according to the samples were of interest and so the clustering in y-axis of the compounds is shown only compressed.

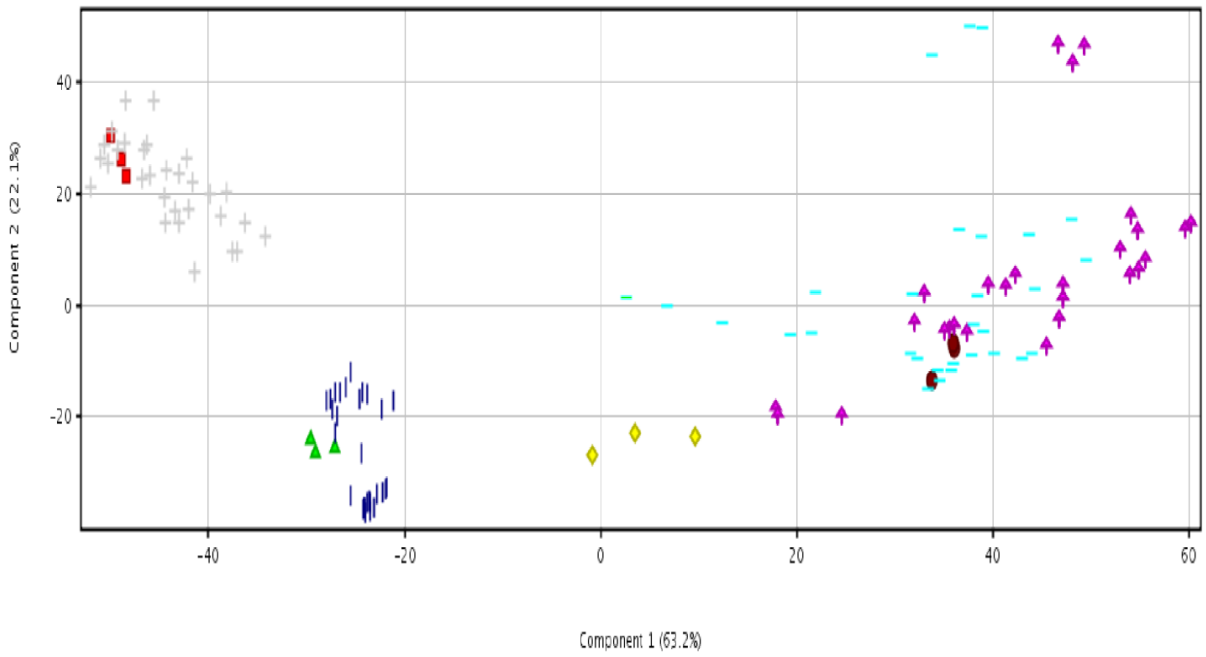


Figure 27: PCA of the data sets from supplier A (Cross), B1 (Arrow), B2 (Horizontal Bar) and C (Vertical bar) together with the data of the formulation samples containing TSP-16-ethoxylates of supplier A (Square), B1 (Diamond), B2 (Circle) and C (Triangle). For the PCA the whole data set was taken including the 3 repetition analysis each production batch and formulation sample.

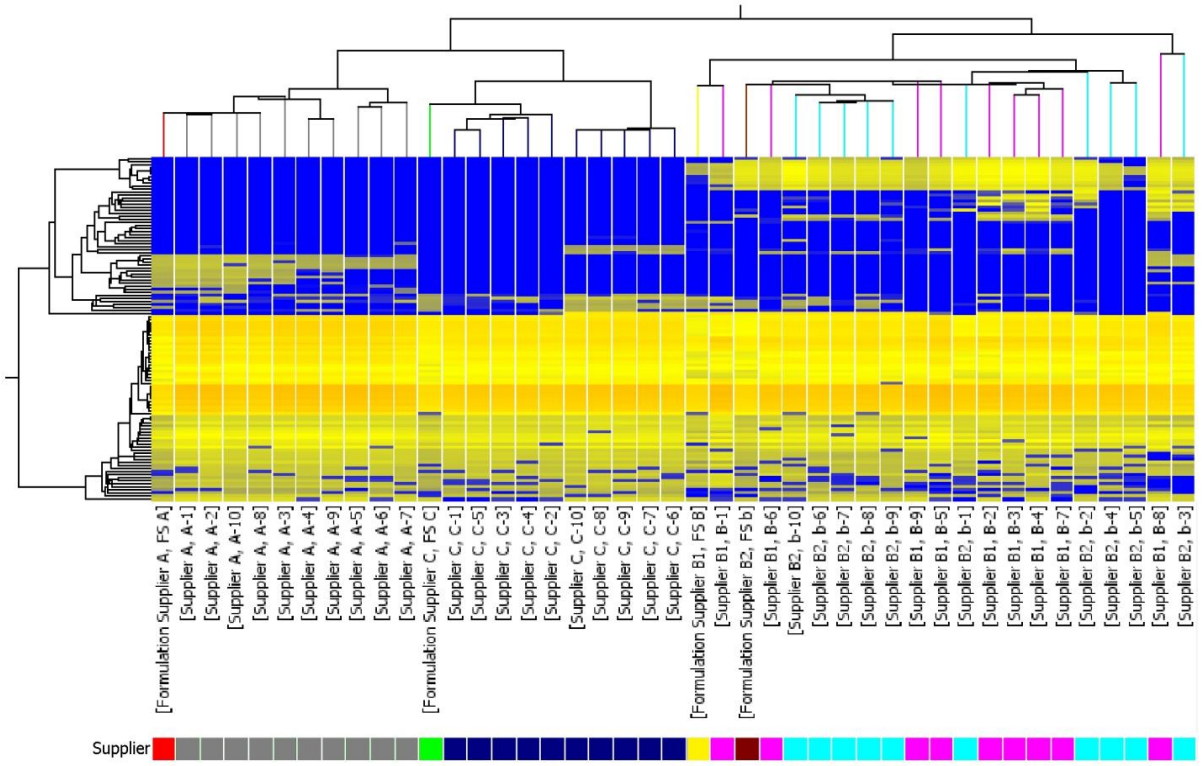


Figure 28: Combined hierarchical clustering of the samples (x-axis) and the compounds (y-axis) detected in the samples of supplier A (grey), B1 (magenta), B2 (turquoise) and C (blue) together with sample of formulation containing TSP-16-ethoxylates of Supplier A (red), B1 (yellow), B2 (brown) and C (green). Each sample is the average of 3 repetition analyses. The content of a compound in the analyzed sample is coded via a colored rectangle in the column beneath the respective sample. The color ranges from deep blue, compound not detected, over yellow, compound as abundant as internal standard, to red, compound with the maximum content.

As demonstrated, both data analysis techniques are correctly assigning the TSP-16-ethoxylates in the model formulation to their corresponding supplier. For the PCA all four formulation samples are identified in their corresponding supplier cluster. They are all group within the clusters of their suppliers, as shown in the top dendrogram. The linkage of the formulation samples to a sample of the corresponding supplier cluster in the hierarchical clustering was formed for all four samples at least two levels lower than the linkage of the respective supplier cluster. The assignments to the corresponding suppliers displayed in Figure 28 are thus reasonable. The developed method combining instrumental analysis and multivariate data mining enables the identification of a supplier of TSP-16-ethoxylates, without apparent matrix interference even though another functionalized PEG, an ethoxylated alcohol, had been used as well in the chosen model formulation.

This is only possible because the reliable identification and quantification of the single compounds used for differentiation of the suppliers is ensured by the combination of chromatographic separation and detection via

exact mass measurement. Albeit this method is highly selective for its target analytes, interferences caused by the matrix were observed for agrochemical formulations containing terminal sulfated or phosphated TSP-ethoxylates and/or copolymerized propoxylates-ethoxylates of TSP besides TSP-16-ethoxylates (see Supplementary). Formulation using a combination of TSP-16-ethoxylates and another TSP-ethoxylates derivate are not widely spread and so this interference can be accepted. Nevertheless, further investigations should test the possibility for a correction of the observed interferences.

For agrochemical formulations containing TSP-16-ethoxylates without other nonionic surfactants functionalized with TSPs, identification of the supplier of TSP-16-ethoxylates for anti-counterfeiting purposes via the method described in this work would be possible. Analyzing the chemical composition of a subcomponent of an agrochemical formulation might provide an additional tool to established techniques of anti-counterfeiting, such as specialized packing material [45], Radio Frequency Identification (RFID) [46], bulk analysis of products via for example NIR [47;48] or NMR [49]. The chemical composition of the whole product or one of its subcomponents can hardly be retraced with these techniques. This chemical composition, however, can be highly significant for identification of counterfeited products. Although the spectroscopic techniques, NIR and NMR, are also sensitive to the chemical composition, they are at the same time very sensitive to non-chemical influences such as grain size, morphology etc. Therefore they require time consuming calibration and constant monitoring of these non-chemical features. In this regard, the developed method is more robust and additionally allows tracing of the chemical features more easily.

4.5 Conclusion

A fast and comprehensive semi-quantitative method for the characterization of surface active TSP-16-ethoxylates (tristyrylphenol ethoxylates with an average number of 16 EO units) using LC-ToF-MS with exact mass measurement combined with multivariate data analysis was developed. The method allows the determination of the main components which were identified as monostyrylphenol (MSP), distyrylphenol (DSP), tristyrylphenol (TSP) and tetrastyrylphenol (TeSP) ethoxylates. It was possible to quantify the single ethoxylated entities in the sample normalized against an internal standard and to subject the result to multivariate data analysis for analytical characterization and comparison of the different TSP-16-ethoxylates.

From the results of the multivariate data analysis the single ethoxylated entities could be retraced, It was shown that there are substantial differences in the composition of commercial TSP-16-ethoxylates supplied by four different producers and available in up to two qualities, with respect to their content of MSP-, DSP-, TSP- and TeSP ethoxylates, but not with respect to their number and distribution of ethylene oxide units. These differences

could be successfully used to predict the corresponding supplier of TSP-16-ethoxylates in an agrochemical model formulation. Therefore, using the by-product spectrum of formulation additives might provide an interesting alternative for identification of the origin of agrochemical products in anti-counterfeiting.

For future work the usability of the method for product characterization of other ethoxylated surfactants could be investigated, thus aiding the formulation chemist on the suitable choice for this class of surfactants, reducing the need for long term storage tests. Finally, the method could be adapted to more sophisticated mass spectrometers like the Q-Exactive using the Orbitrap-technology to obtain higher mass resolution and so better performance regarding the identification of the single compounds. For some analytes and matrices the resolution of the ToF-MS used in this thesis is not sufficient to resolve the analyte m/z-signal from nearly isobaric analyte or matrix signals. This has been stressed out by Marshall et al [50] for the use of high-resolution MS for petroleum analysis. In context of this work such a highly complex composition of analytes and matrix, respectively, would be represented by copolymerized propoxylates-ethoxylates tristyrylphenol, which have variations in regard to the number of styrenes, the degree of propoxylation and ethoxylation.

4.6 Acknowledgement

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5. General Conclusion and Outlook

Analysis of surfactants and their properties has been widely investigated, mainly in pure form to determine their physical-chemical properties such as water solubility, cloud point concentration or critical micelle concentration. Analysis of surfactants is furthermore necessary in environmental samples [1-3], but also for controlling and monitoring of the composition and content of by-products in the technical product. There are still open questions regarding the purity of surfactants and its influence on the properties of the surfactant in complex mixtures, like an agrochemical formulation. Also the usability of differences in the content of by-products for product identification has potential for research. There are analytical methods known for quantification of the target analyte sodium bis(2-ethylhexyl) sulfosuccinate (anionic) [4;5], but none for quantifying it together with its isomeric mono esterified surface active by-products in an agrochemical formulation. For the other target analyte tristyrylphenol ethoxylates with an average number of 16 ethylene oxide units (TSP-16-ethoxylates; nonionic), there are analytical methods known to quantify it either as sum parameter [2;6;7] or via the different ethoxylated entities. These methods have only been demonstrated to be suitable for pure surfactants [8-12]. The quantification according to degree of ethoxylation in complex matrices such as agrochemical formulations has only been shown by Meisen et al. [13] with an offline combination of LC for separation according to hydrophobic group and degree of ethoxylation and identification via GC-MS and MALDI-ToF-MS.

For analytical characterization according to the content of main- and by-products in a single step, analytical methods have been developed in this thesis using a LC-ToF-MS with exact mass measurement in combination with multivariate data analysis in case of the TSP-16-ethoxylates.

With these methods significant differences were found between different suppliers of both investigated surfactants, sodium bis(2-ethylhexyl) sulfosuccinate and TSP-16-ethoxylates with regard to the content of by-products. For the investigation of differences in the by-product content in TSP-16-ethoxylates, an analytical method using LC-ToF-MS with exact mass measurement in combination with multivariate data analysis was developed. With the developed method TSP-16-ethoxylates of three different suppliers and two different qualities for one supplier were analyzed. For both surfactants the differences in the content of by-products were significant for the respective suppliers and production qualities. Only the two different qualities of tristyrylphenol ethoxylates showed no significant differences regarding the content of ethoxylated compounds.

Identification of the surfactants based on the content of their by-products was possible in the raw product and beyond that also formulated in an aged agrochemical formulation. For the identification of bis(2-ethylhexyl) sulfosuccinate there were no interferences observed, for the identification of TSP-16-ethoxylates interferences

are possible whenever other derivates of tristyrylphenol ethoxylates or copolymerized ethoxylates-propoxylates were used alongside in the agrochemical formulation. All these derivates partly contained the same styrenated ethoxylates as by-products which were used for the identification of TSP-16-ethoxylates. Formulation using a combination of TSP-16-ethoxylates and another TSP-ethoxylates derivate are not widely spread and so this interference can be accepted. Nevertheless, further investigations should test the possibility for a correction of the observed interferences.

As demonstrated, identification of the supplier of the surfactant in agrochemical formulations could be utilized for anti-counterfeiting. In this thesis identification has only been demonstrated for a few artificially prepared formulation samples but not for real counterfeited ones. Accordingly, further tests should be conducted for known counterfeited samples using sodium bis(2-ethylhexyl) sulfosuccinate or TSP-16-ethoxylates to confirm the correct classification. Nevertheless, the chosen approach using the defined by-product content of a sub-component in an agrochemical product, would offer an additional tool in anti-counterfeiting.

Finally, the found difference in content of by-product had in case of sodium bis(2-ethylhexyl) sulfosuccinate an impact on the storage behavior of a model agrochemical formulation containing it. A model agrochemical formulation containing the surfactant with raised content of by-products showed sedimentation after half a year of storage at 24°C in a climate cabinet, whereas the formulation containing the surfactant with lower content of by-product stayed dispersed after storage. To rule out the influence of other parameters, exactly the same model formulation had been chosen for all trials. Moreover, there were no significant differences regarding the content of inorganic ions or the composition of the organic solvent containing sodium bis(2-ethylhexyl) sulfosuccinate. Based on the results presented in this thesis an interference of the mono esterified by-products on the sorption-desorption equilibrium on the dispersed hydrophobically modified particles in the formulation is proposed, because the monoesters are surface active as well and have been used as wetting agents in the past. This type of surfactant adsorbs rapidly on new surfaces, thus competing with the surfactant determined to stabilize the particles in of the dispersion. This hypothesis, however, has not been proven so far. For further clarification it would be useful to determine an adsorption isotherm for both monoesters on the used dispersed particles, to characterize the interaction. Furthermore, a storage test with stepwise increasing content of by-product could be carried out to determine the actual detrimental concentration.

For future research the usability of the developed method for characterization and comparison of TSP-16-ethoxylates should be tested on other ethoxylated or copolymerized propoxylated-ethoxylated surfactants, thus aiding the formulation chemist on the suitable choice for this class of surfactants, reducing the need for long term

storage tests. Furthermore, the applicability of the presented approach should be proven for the analytical characterization of surfactants/analytes with a comparable, broad composition of main- and by-products as the tristyrylphenol ethoxylates, such as condensed naphthalene sulfonate (Trade name: Morwet D425; Akzo Nobel). Finally, the method could be adapted to more sophisticated mass spectrometers like the Q-Exactive using the Orbitrap-technology to obtain higher mass resolution and so better performance regarding the identification of the single compounds. For some analytes and matrices the resolution of the ToF-MS used in this thesis is not sufficient to resolve the analyte m/z-signal from nearly isobaric analyte or matrix signals. This has been stressed out by Marshall et al [14] for the use of high-resolution MS for petroleum analysis. In context of this work such a highly complex composition of analytes and matrix, respectively, would be represented by copolymerized propoxylates-ethoxylates tristyrylphenol, which have variations in regard to the number of styrenes, the degree of propoxylation and ethoxylation. .

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6. Supplementary

6.1 General Introduction

No supplements

6.2 LC-MS Quantification of a Sulfosuccinate Surfactant in Agrochemical Formulations

Redrafted from "Glaubitz J, Schmidt TC (2013) LC-MS Quantification of a Sulfosuccinate Surfactant in Agrochemical Formulation s Chromatographia 76:1729-1737", Copyright © Springer-Verlag 2011. The final publication is available at http://link.springer.com.

6.2.1 Determination of the pKa Value of Monoester 2 and 3

The pKa values were determined via a pH controlled titration with HCl. 0.15 M of analyte, obtained in the synthesis of monoester 2 and 3 in this work, was dissolved in 1/10 (w/w) methanol/water and then analyzed. The required amount of titrant against the pH value and the pH against the distribution of ion species [%] is shown in Figure S 1 for monoester 2. The pKa value of monoester's 3 carboxylic acid group, however, was already known in literature and so had not to be determined [1].

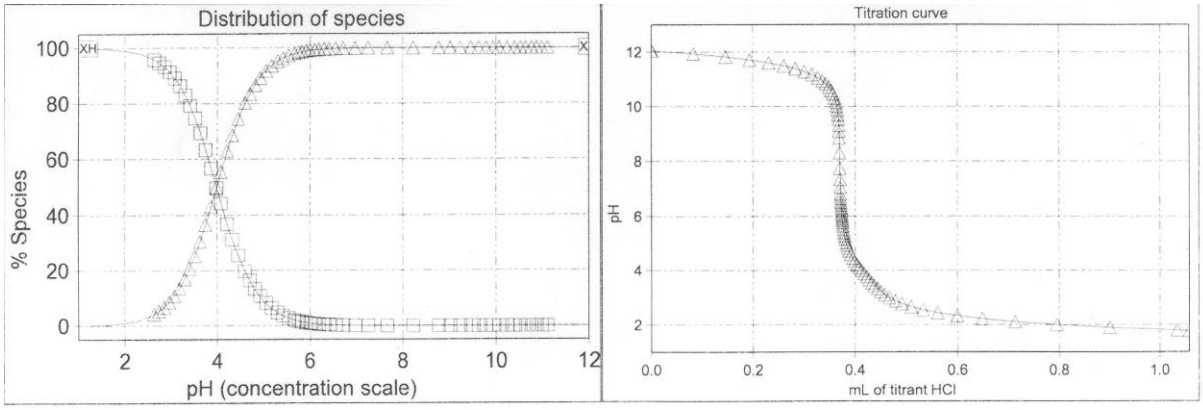


Figure S 1: Amount of titrant against the pH value and pH value against the distribution of ionic species as obtained in the determination of the pKa value of the carboxylic acid group of monoester 2

6.2.2 Sample for Testing on Mass Calibration of ToF-MS

The retention times and exact masses for the compounds in the test sample for checking on mass calibration of the used ToF-MS are given in Table S 1.

Table S 1: Retention time and exact masses for compounds in the test sample for checking on mass calibration

Compound	t_R [min]	Exact mass [m/z]
Imidacloprid	2.0	254.0450
Thiacloprid	2.5	252.0236
Tebuconazole (1.Isomer)	4.3	307.1451
Triadimenol	4.6	295.1088
Tebuconazole (2.Isomer)	4.9	307.1451
Distyrylethoxylate-5-EO	5.8	522.2981
Distyrylethoxylate-6-EO	5.8	566.3244
Distyrylethoxylate-7-EO	5.8	610.3506
Distyrylethoxylate-8-EO	5.8	654.3768
Distyrylethoxylate-9-EO	5.8	698.4030
Distyrylethoxylate-10-EO	5.8	742.4292
Distyrylethoxylate-11-EO	5.8	786.4554
Distyrylethoxylate-12-EO	5.8	830.4816
Distyrylethoxylate-13-EO	5.8	874.5079
Distyrylethoxylate-14-EO	5.8	918.5341
Distyrylethoxylate-15-EO	5.8	962.5603
Distyrylethoxylate-16-EO	5.8	1006.5865
Distyrylethoxylate-17-EO	5.9	1050.6127
Distyrylethoxylate-18-EO	5.9	1094.6389
Distyrylethoxylate-19-EO	5.9	1138.6651
Distyrylethoxylate-20-EO	5.9	1182.6914
Distyrylethoxylate-21-EO	5.9	1226.7176
Distyrylethoxylate-22-EO	5.9	1270.7438
Distyrylethoxylate-23-EO	5.9	1314.7700
Distyrylethoxylate-24-EO	5.9	1358.7962
Distyrylethoxylate-25-EO	5.9	1402.8224
Distyrylethoxylate-26-EO	5.9	1446.8486
Distyrylethoxylate-27-EO	5.9	1490.8749
Distyrylethoxylate-28-EO	5.9	1534.9011
Distyrylethoxylate-29-EO	5.9	1578.9273
Distyrylethoxylate-30-EO	5.9	1622.9535
Nonylphenolethoxylate-5-EO	6.6	440.3138
Nonylphenolethoxylate-6-EO	6.3	484.3400
Nonylphenolethoxylate-7-EO	6.2	528.3662
Nonylphenolethoxylate-8-EO	6.2	572.3924
Nonylphenolethoxylate-9-EO	6.2	616.4186
Nonylphenolethoxylate-10-EO	6.2	660.4449
Nonylphenolethoxylate-11-EO	6.2	704.4711
Nonylphenolethoxylate-12-EO	6.2	748.4973
Nonylphenolethoxylate-13-EO	6.2	792.5235
Nonylphenolethoxylate-14-EO	6.2	836.5497
Nonylphenolethoxylate-15-EO	6.2	880.5759
Nonylphenolethoxylate-16-EO	6.2	924.6022
Nonylphenolethoxylate-17-EO	6.2	968.6284
Nonylphenolethoxylate-18-EO	6.2	1012.6546
Nonylphenolethoxylate-19-EO	6.2	1056.6808
Nonylphenolethoxylate-20-EO	6.2	1100.7070

Supplementary

Compound	t_R [min]	Exact mass [m/z]
Nonylphenolethoxylate-21-EO	6.2	1144.7332
Nonylphenolethoxylate-22-EO	6.2	1188.7594
Nonylphenolethoxylate-23-EO	6.2	1232.7857
Nonylphenolethoxylate-24-EO	6.2	1276.8119
Nonylphenolethoxylate-25-EO	6.2	1320.8381
Nonylphenolethoxylate-26-EO	5.9	1364.8643
Nonylphenolethoxylate-27-EO	5.9	1408.8905
Nonylphenolethoxylate-28-EO	5.9	1452.9167
Nonylphenolethoxylate-29-EO	5.9	1496.9429
Nonylphenolethoxylate-30-EO	5.9	1540.9692
Tristyrylethoxylate-5-EO	5.9	626.3607
Tristyrylethoxylate-6-EO	5.9	670.38695
Tristyrylethoxylate-7-EO	5.9	714.4132
Tristyrylethoxylate-8-EO	6.5	758.4394
Tristyrylethoxylate-9-EO	5.9	802.4656
Tristyrylethoxylate-10-EO	5.9	846.4918
Tristyrylethoxylate-11-EO	6.0	890.5180
Tristyrylethoxylate-12-EO	6.0	934.5442
Tristyrylethoxylate-13-EO	6.0	978.5705
Tristyrylethoxylate-14-EO	6.0	1022.5967
Tristyrylethoxylate-15-EO	6.0	1066.6229
Tristyrylethoxylate-16-EO	6.0	1110.6491
Tristyrylethoxylate-17-EO	6.0	1154.6753
Tristyrylethoxylate-18-EO	6.0	1198.7015
Tristyrylethoxylate-19-EO	6.0	1242.7278
Tristyrylethoxylate-20-EO	6.0	1286.7540
Tristyrylethoxylate-21-EO	5.9	1330.7802
Tristyrylethoxylate-22-EO	5.9	1374.8064
Tristyrylethoxylate-23-EO	5.9	1418.8326
Tristyrylethoxylate-24-EO	5.9	1462.8588
Tristyrylethoxylate-25-EO	5.9	1506.8850
Tristyrylethoxylate-26-EO	5.8	1550.9113
Tristyrylethoxylate-27-EO	5.8	1594.9375
Tristyrylethoxylate-28-EO	5.8	1638.9637
Tristyrylethoxylate-29-EO	5.8	1682.9899
Tristyrylethoxylate-30-EO	5.8	1727.0161

6.2.3 Synthesis of Monoester 2 and 3

Experimental

Synthesis of Monoester 2

1st Step: Synthesis of 2-ethylhexanyl-maleic acid

An equimolar amount of maleic anhydride and 2-ethyl-hexanol was stirred for 3 h at 90 °C. After cooling to room temperature, the reaction mixture was partitioned between a mixture of 50/50 (v/v) of methyl tert-butyl ether (MTBE) and 1 N NaOH until no more reaction product was visible in the MTBE phase. The pH of the aqueous phase was adjusted with 1 N HCl (pH-value ~ 1) before extraction with dichloromethane. The combined organic phase was then dried with $MgSO_4$ and filtered. After evaporation of the organic solvent, the product was obtained as oil with a yield of 81.6 %.

The product's ^{1}H-NMR spectrum was obtained in DMSO and matches that in literature [2]. (Found: δ_H (DMSO) 0.85 (3 H, t, *J* 7.3, CH_3), 0.87 (3 H, t, *J* 5.9, CH_3), 1.25 (8 H, m, CH_2), 1.60 (H, m, CH), 4.02 (2 H, dd, *J* 3.9, *J* 10.1, CH_2O), 6.33 (H, d, *J* 12.0 *CH*=CH) 6.38 (H, d, *J* 12.0, CH=C*H*)).

2nd Step: Synthesis monoester 2

525 mmol of sodium bisulfite was dissolved in 300 mL water and purged with argon for 30 min. Then 420 mmol of 2-ethylhexanyl-maleic acid were added and the mixture was heated to reflux for 24 h under an atmosphere of argon. The completeness of the reaction was verified by thin layer chromatography (TLC) (eluent 2:1 (v/v) ethyl acetate/cyclohexane) showing the absence of maleic acid's double bond. After evaporation of the solvent under reduced pressure, the reaction mixture was extracted repeatedly with a mixture of 80/20 (v/v) methanol/water. The extracts were combined and the solvent evaporated. The remaining solid was then washed with diethyl ether and dried under vacuum. The product was obtained as white crystals with a yield of 60.2 %. The proposed structure of the synthesized monoester 2 was confirmed by ^{1}H-NMR, ^{13}C-NMR, 2d-HMBC, 2d-HMQC, ^{13}C-COSY, 2d-^{13}C-^{13}C-Inadequate and was in line with literature values [2]. The positioning of the sulfonic acid group is determined by the 2d-^{13}C-^{13}C-Inadequate measurement. (Found: δ_H (50/50 (v/v) ACN-d_6/D_2O) 0.87 (3 H, t, *J* 7.4, CH_3), 0.89 (3 H, t, *J* 6.6, CH_3), 1.34 (8 H, m, CH_2), 1.64 (H, m, CH), 2.94 (H, dd, $J_{2,3}$ 4.5, $J_{3,3}$ 17, C*H*HCO), 3.06 (H, dd, $J_{2,3}$ 10.6, $J_{3,3}$ 17, CH*H*CO), 4,00 (H, dd, $J_{2,3}$ 4.5, $J_{3,3}$ 10.6, $CHSO_3Na$), 4.07 (H, m, OCH_2); δ_C (50/50 (v/v) ACN-d_6/D_2O) 13.02, 13.07 (C8), 16.30 (C12), 25.17, 25.19 (C11), 25.92, 25.93 (C7), 31.06 (C10), 32.33, 32.38 (C9), 37.35, 37.36 (C3), 40.84, 40.89 (C6), 67.40 (C2), 70.95, 70.98 (C5), 175,50 (C1), 176.7 (C4)). Quantification of synthesized monoester 2 via NMR yields a purity of 90.9 %.

Supplementary

Synthesis of Monoester 3

216 mmol of pure AOT was dissolved in 1.5 L of 50/50 (v/v) water/isopropanol followed by the addition of 320 mmol of NaOH. The mixture was stirred for 24 h at room temperature until no starting material was visible by LC-MS. The reaction mixture was then evaporated to dryness. The product was obtained as white crystals with a yield of 82.5%.

The proposed structure of the synthesized monoester 3 was confirmed by ^{1}H-NMR, ^{13}C-NMR, 2d-HMBC, 2d-HMQC, ^{13}C-COSY. The sulfonic acid group was located at C3, as the CH_2-group in the ester side-chain showed long-range coupling to C1 in 2d-HMBC, and so CH_2 had to be direct neighbor of the carboxylic-group at C1. Found: δ_H (50/50 (v/v) ACN-d_6/D_2O) 0.87 (6 H, m, CH_3), 1.34 (8 H, m, CH_2), 1.66 (H, m, CH), 2.9

4 (H, dd, $J_{2,3}$10.6, $J_{3,3}$ 15.4, C*H*HCO), 3.06 (H, dd, $J_{2,3}$ 12, $J_{3,3}$ 15.4, CH*H*CO), 4.00 (H, dd, $J_{2,3}$ 4.2, $J_{3,3}$ 10.6, $CHSO_3Na$), 4.13 (H, m, OCH_2); δ_C (50/50 (v/v) ACN-d_6/D_2O) 13.00, 13.10 (C8), 16.30 (C12), 25.20 (C11), 26.00 (C7), 31.00, 31.10 (C10), 32.30, 32.40 (C9), 38.45, 38.60 (C3), 40.90, 40.95 (C6), 67.85 (C2), 71.60 (C5), 172.80 (C4), 176.0 (C1)). Quantification of synthesized monoester 3 via NMR yields a purity of 25.0% and a content of 71.0% sulfosuccinic acid.

Chemicals and Reagents

All chemicals used for synthesis were purchased by Sigma Aldrich in p.a. grade. Dimethylsulfone (w = 99.9%) was used as NMR standard for quantifying the isomeric monoester 2 and 3 after synthesis for their use as analytical standard. 5 mg dimethylsulfone and 20 mg isomeric monoester were diluted in 5 mL of 50/50 (v/v) deuterated acetonitrile/water and then ^{1}H-NMR was measured.

Characterization via LC-MS and NMR

^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker 600 MHz. Before measurement analytes were diluted in 50/50 (v/v) deuterated acetonitrile/water. Mass spectra were recorded using electrospray ion source on a 6130 Agilent Quadrupole mass spectrometer coupled with an Agilent 1290 HPLC-system.

Results and Discussion

For validation of a new analytical method, analytical standards are needed. There were no analytical standards commercially available for monoester 2 and 3, so they had to be prepared. There are two different methods described in literature for synthesizing each isomer, monoesters 2 and 3 selectively [1;2] as shown in Figure S 2.

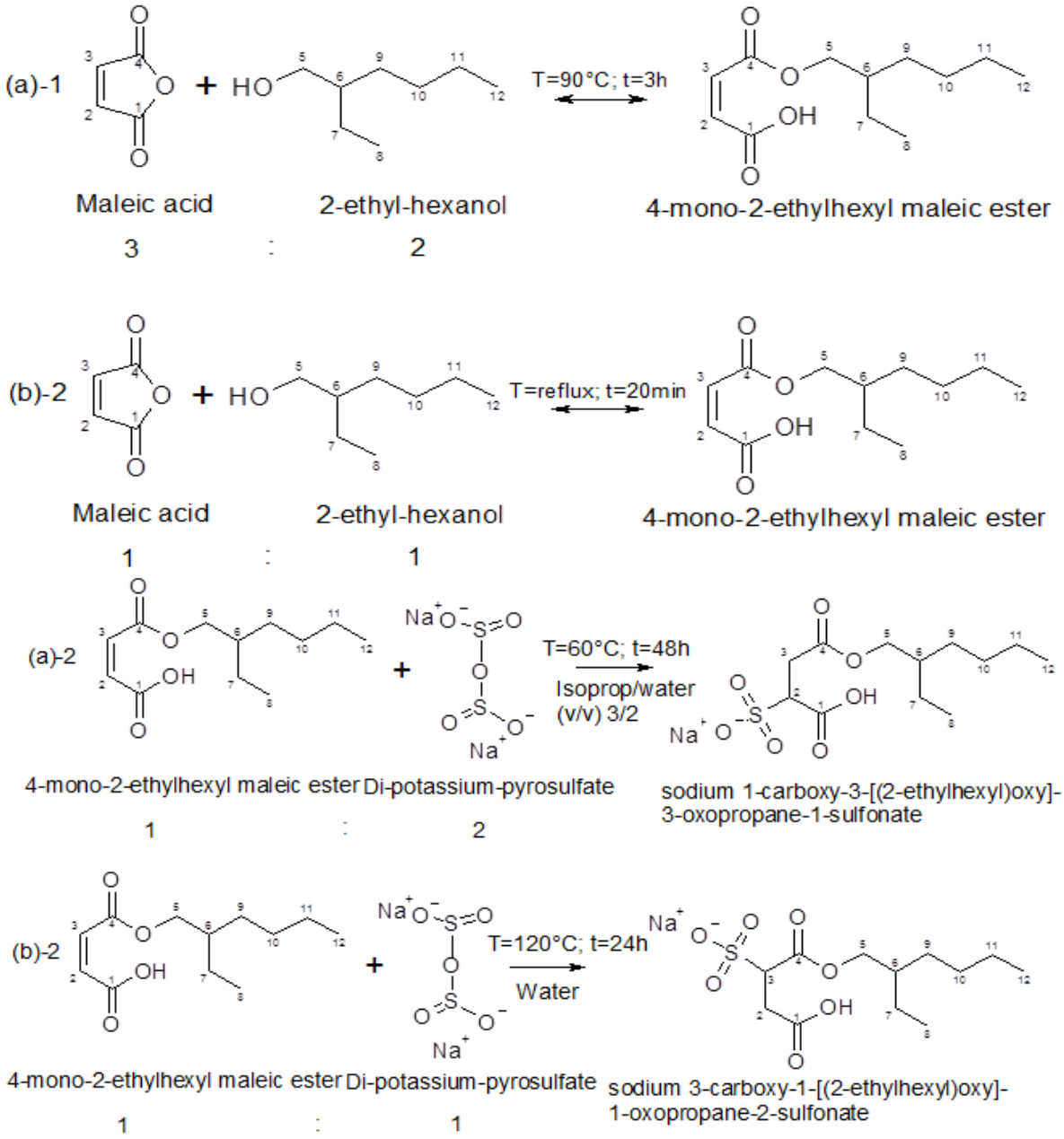


Figure S 2: 2-step regio-isomer selective synthesis for monoester 2 (a) and 3 (b) according to literature [1;2]

Both syntheses were conducted to reproduce literature results for small batches. The analysis results of the products with LC-MS are shown in Figure S 3.

Supplementary

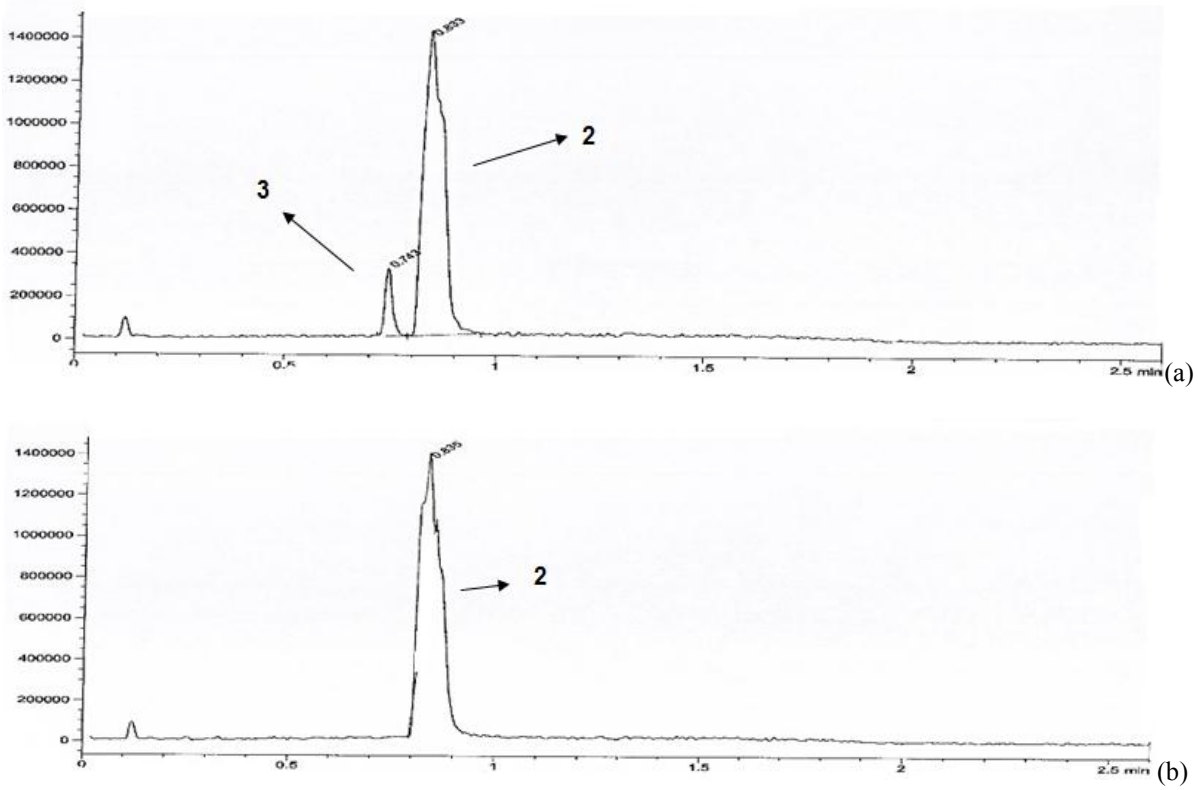


Figure S 3: Results for synthesis of monoester 2 (a) and monoester 3 (b) according to literature [1;2],

As shown, only monoester 2 could be synthesized successfully with both methods. Considering the by-product profile of both methods, conditions of (b) were chosen for the preparation of monoester 2.

Altering solvent composition, reaction time, temperature or pH-value of conditions (a), did not change the ratio between monoester 2 and 3. Therefore, basic hydrolysis (c) of AOT was conducted, as shown in Figure S 4. The reaction products were then analyzed via LC-MS as shown in Figure S 5.

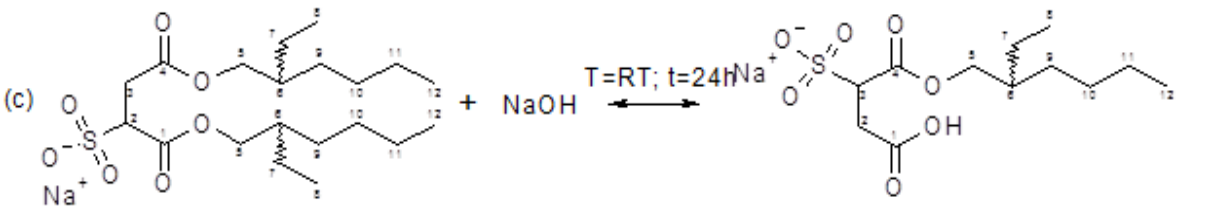


Figure S 4: Reaction condition for basic hydrolysis of AOT leading to monoester 3

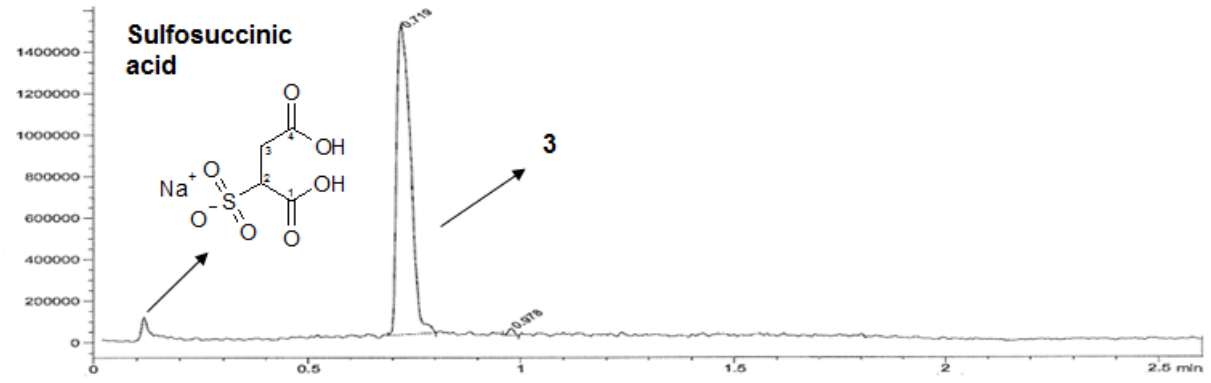


Figure S 5: Results for basic hydrolysis of AOT leading to sulfosuccinic acid and monoester 3

As shown, the hydrolysis of AOT led to monoester 3 and sulfosuccinic acid. Quantification via NMR showed a content of 25% (w/w) monoester 3 and 71% (w/w) of sulfosuccinic acid. As sulfosuccinic acid was not interfering with the analysis of either AOT or the monoesters 2 and 3, it could be tolerated, although it was the main reaction product. Under the given reaction condition complete hydrolysis of AOT was thermo-dynamically favored, with monoester 3 as intermediate. An explanation for monoester 3 as favored intermediate might be a slowed hydrolysis of the AOT at carbon atom 1, because of a keto-enol-tautomerism as shown in Figure S 6.

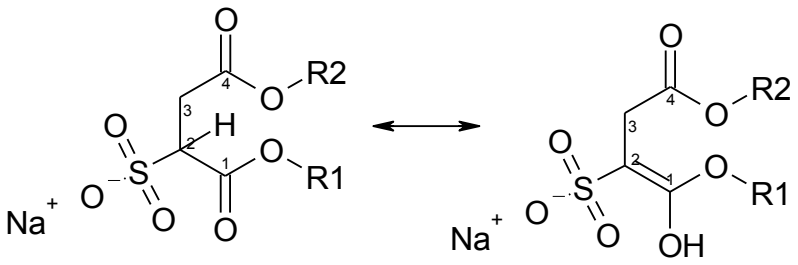


Figure S 6: Proposed keto-enol-tautomerism for AOT at position 2 and 1

In its enol-form, the higher electron density at position 1 compared with position 4 could be the reason for disfavoring the nucleophile addition of an OH^- group at position 1. Hydrolysis at position 4, hence, would be favored in the first step, leading to monoester 3 as intermediate, which was then in the next step further hydrolyzed at position 1, leading to sulfosuccinic acid as main product.

To check whether the keto- or the enol-form is favored under the reaction conditions of basic hydrolysis. ^{1}H-NMR-spectra were recorded at pH 1, 7 and 9. If the enol-form was favored, the proton signal of C*H* at the carbon atom indicated with 2 would diminish, due to deprotonation. Therefore, the ratio between the proton

signal at position 3 (C*HH*COOR$_2$) and position 2 (C*H*SO$_3$Na) of AOT should change in dependency of the pH-value. The results are shown in Table S 2.

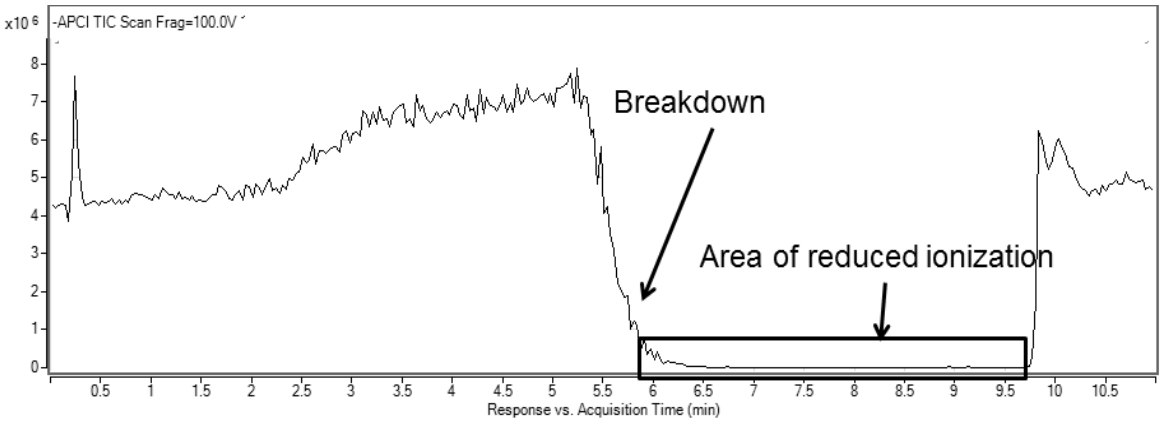
Table S 2: Ratio between ^{1}H-NMR integral C*HH*COOR$_2$ and integral C*H*SO$_3$Na at different pH-values for AOT

Spectra	Ratio integral C*HH*COOR$_2$/integral C*H*SO$_3$Na
AOT (pH 1)	4.08
AOT (pH 7)	3.96
AOT (pH 14)	43.6

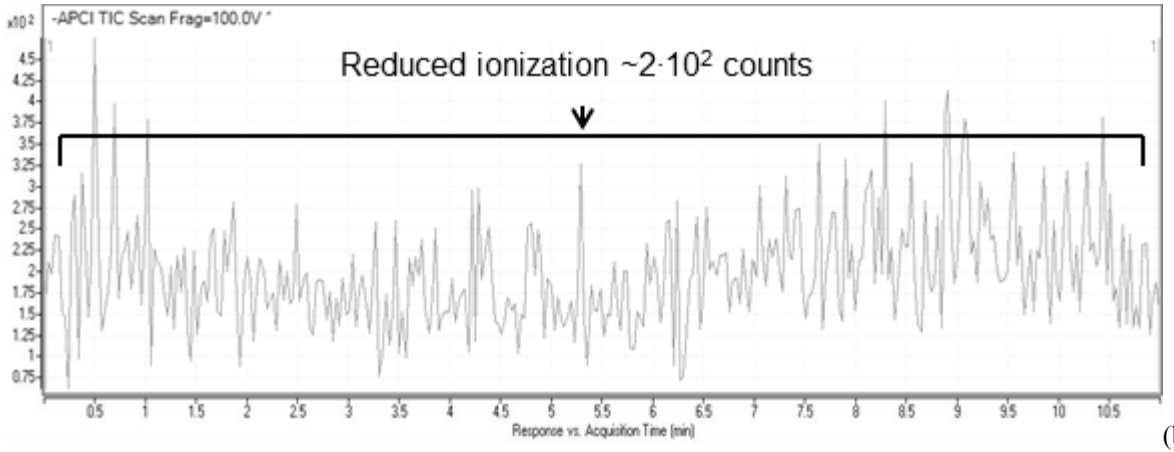
These results indicate that position 2 is significantly less protonated under basic pH conditions as chosen for the hydrolysis of AOT in this work, which supports the proposed reaction pathway. Although this could be an indicator for the proposed reaction pathway, other mechanism, however, have to be considered as well. As the acidity of the proton in counter position to the SO$_3$-group has been demonstrated also the formation of a partial salt may be considered, which would hindered a nucleophilic addition of the OH$^-$group and so promote the hydrolysis at position 4, as well.

APCI Performance in Dependency of the Composition of the Mobile Phase.

As shown in the manuscript ionization of AOT was not homogenous over the whole peak. This effect depended on the content of organic solvent in the mobile phase, as shown with injection of a blank sample containing acetonitrile/water (v/v) 1:1 in Figure S 7. For chromatogram (a) the developed gradient was used, for chromatogram (b) the gradient's starting point of B was set to 70%, which equaled the gradient's composition at the point of ionization breakdown. The other gradient parameters were left unchanged.



(a)



(b)

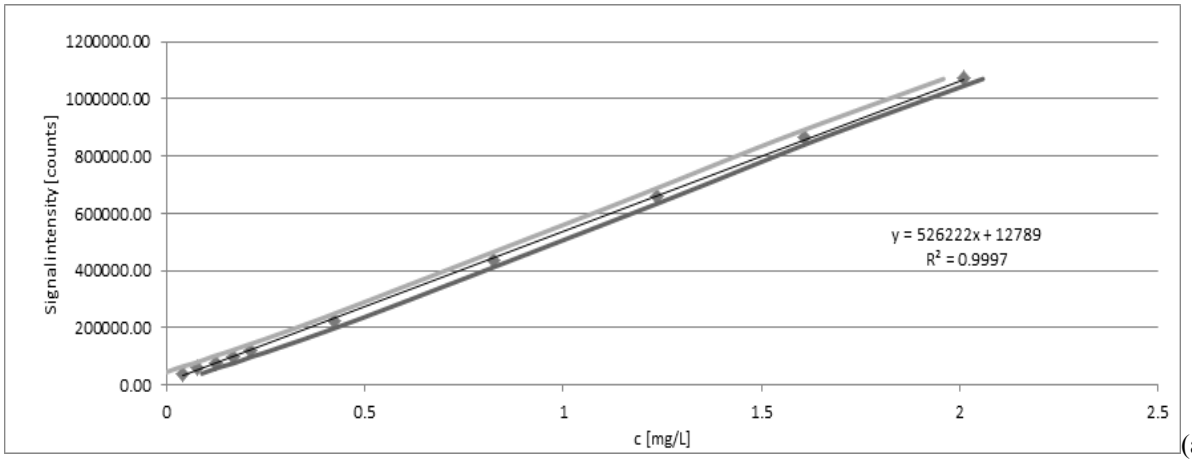
Figure S 7: Total ion chromatogram of a blank sample containing acetonitrile/water 1:1 (v/v) with an injection volume of 5µL, applying developed gradient with water and methanol as eluents (a) and applying developed gradient with changed starting point of 70% methanol (b), which equaled the composition of the gradient at the point of reduced ionization indicated in Figure S 7 (a)

As shown in chromatogram (b), the conditions after the breakdown in (a) could be simulated with raised organic content in the LC effluent over the whole run. Though ionization was then homogenous over the complete gradient, signal intensity was lower by a factor of 10 than for (a).

6.2.4 Validation

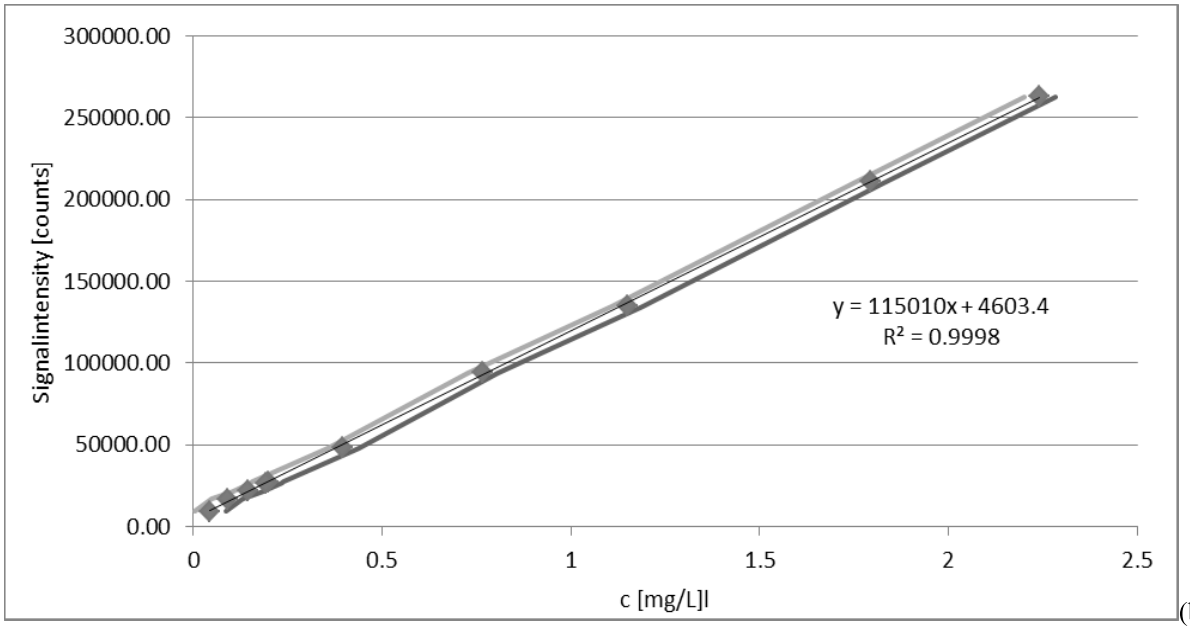
Linearity

Linearity and the band of prediction for AOT and monoesters 2 and 3, respectively, are shown in Figure S 8 as Supplementary for the validation.

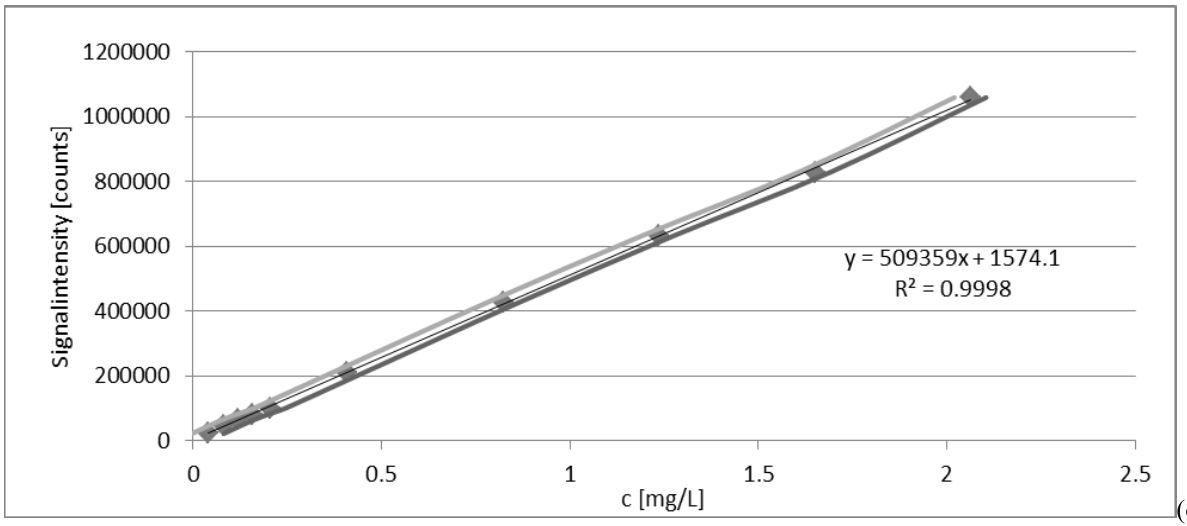


(a)

Supplementary



(b)



(c)

Figure S 8: Linear ranges for AOT (a) and monoesters 2 (b) and 3 (c) including the bands of prediction indicated with the upper and the lower line

6.2.5 Matrix Effects of a Model Agrochemical Formulation on the Analysis of Monoester 2 and 3

In Figure S 9 the total ion chromatogram (TIC) obtained in negative ESI mode is shown for the analysis of monoesters 2 and 3 in the matrix of an agrochemical formulation.

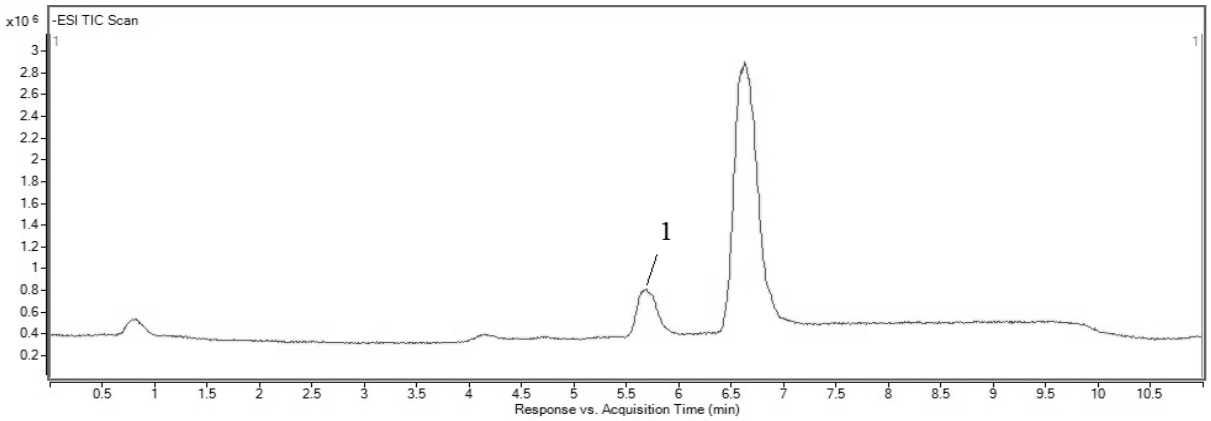


Figure S 9: Total ion chromatogram (TIC) obtained in negative ESI mode for the analysis of monoester 2 and 3 in the matrix of an agrochemical formulation.

Only the peak of AOT is visible in the TIC, the peaks of monoester 2 and 3 are not observed. In the mass range of 105 – 1700 m/z for the TIC there is too much interference from the matrix to detect the monoesters. In the following in Figure S 10 an extracted ion chromatogram (EIC) of the TIC in Figure S 9 for the masses of monoester 2 and 3 is shown, simulating the highest achievable mass resolution of a common quadrupole mass spectrometer by extracting the monoester [M-H]- molar mass and its A+1 and A+2 isotopic masses with a window of 0.1 amu around each mass.

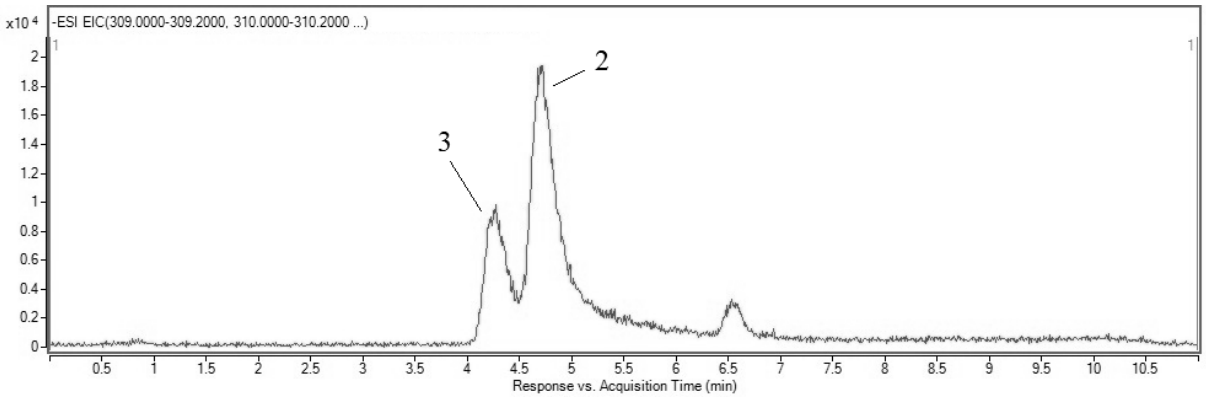


Figure S 10: Extracted ion chromatogram (EIC) of the TIC in Figure S 9 for the molar mass [M-H]- of monoester 2 (2) and monoester 3 (3) and it's A+1 and A+2 isotopic masses with a window of 0.1 amu, simulating the highest achievable mass resolution of a common quadrupole mass spectrometer.

Supplementary

As displayed, both monoesters are detectable as well, when only a mass spectrometer is available with lower mass resolution. Matrix, however, is interfering with the detection of monoester 2 at t_R = 6.5 min so that the method would have to be altered, in this case chromatographically, to ensure proper quantification of monoesters 2 and 3. Conversely, the interference were negligible observed with high resolution mass spectrometry with a detection window of 20 ppm around the molar mass of monoester 2 and 3 and it's A+1 and A+2 isotopic masses as shown in Figure 5 and 6 in the manuscript. To that end quantification via high resolution mass spectrometry is more robust as method adaption to changing matrix interferences is seldom necessary.

6.2.6 Results of the Measurement of AOT Product of Supplier A, B and C

Results of five independently weighed samples each supplier on the content of AOT and monoester 2 and 3 are given in Table S 3.

Table S 3: Results of the replicate measurements each sample on the content of AOT, monoester 2 and 3 in AOT product of supplier A, B, and C

	w(AOT) [%]	w(monoester 2) [%]	w(monoester 3) [%]
Supplier A-1	62.1%	1.30%	0.71%
Supplier A-2	63.5%	1.32%	0.75%
Supplier A-3	63.5%	1.28%	0.71%
Supplier A-4	61.7%	1.28%	0.74%
Supplier A-5	63.9%	1.27%	0.71%
Supplier B-1	65.1%	0.82%	0.2%
Supplier B-2	65.9%	0.82%	0.1%
Supplier B-3	65.4%	0.83%	0.1%
Supplier B-4	66.6%	0.82%	0.1%
Supplier B-5	66.2%	0.83%	0.2%
Supplier C-1	61.8%	3.2%	0.70%
Supplier C-2	62.5%	3.1%	0.66%
Supplier C-3	61.4%	3.2%	0.65%
Supplier C-4	60.6%	3.1%	0.65%
Supplier C-5	60.8%	3.2%	0.71%

6.2.7 Statistical Evaluation

Data of the measurement of AOT and monoester 2 and 3 in Aerosol OT of supplier A, B and C were statistically analyzed on significant differences between the suppliers. First an F-test on variance with a level of significance of p = 0.05 and a test F-value of 5.05. The results of the experimental determined F-values were shown in Table S 4 (a) for AOT, in (b) for monoester 2 and in (c) for monoester 3.

Supplementary

Table S 4: Results of the experimental determine F-value for the paired F-test on the results of the measurement of AOT (a) and monoester 2 (b) and 3 (c) in Aerosol OT of supplier A, B and C

AOT	**B**	**C**
A	2.6	1.6
B	-	1.6

(a)

Monoester 2	**B**	**C**
A	10.5	6.5
B	-	68.1

(b)

Monoester 3	**B**	**C**
A	28.5	2.2
B	-	62.5

(c)

As shown, the F-test on variance between the tests groups was passed for the values of AOT and monoester 3 for the groups A/C. Therefore an expanded t-test was conducted on them. As the F-test was not passed for the rest, the variances were varying significantly between test groups and so t-tests according to Welch had to be conducted. Both were conducted with a level of significance of $p = 0.05$. The corresponding test value of t for the paired expanded t-test was 2.8. For the t-tests according to Welch the test values of t had to be determined for each group. The values were displayed for the corresponding target analyte and test group in Table S 5.

Table S 5: Test values of t for the paired t-test according to Welch

	A/B	**A/C**	**B/C**
Monoester 2	2.8	2.6	2.8
Monoester 3	2.8	2.8	-

The results of the experimental determined t-value for each pair and target analyte were shown in Table S 6 (a) for AOT, in (b) for monoester 2 and in (c) for monoester 3.

Table S 6: Results of the determine t-value for the paired t-test on the results of the measurement of AOT and monoester 2 and 3 in Aerosol OT of supplier A, B and C

AOT	**B**	**C**
A	5.7	2.7
B	-	10.1

(a)

Monoester 2	**B**	**C**
A	53.0	81.0
B	-	108.0

(b)

Monoester 3	**B**	**C**
A	68.3	3.2
B	-	41.4

(c)

As the determined value of t was only lower for the t-test on the content of AOT between the supplier A and C, there was no significant difference in the content of AOT between these suppliers. In regard to the content of

Supplementary

AOT between supplier A/B and B/C there was significant difference between the investigated suppliers. This was also true for the content of monoester 2 between A/B, A/C and B/C. The content of monoester 3 differed significantly for A/B and B/C, but not for A/C.

To test whether the content of AOT was within its specified concentration range of 62.5-66.0 % (w/w) for supplier A, B and C, a one-side t-test with a level of significance of $p = 0.05$ and a corresponding test value of $t_{test} = 3.5$ was conducted. The results of the experimentally determined t-vales were $t_{exp.} = 0.9$ for supplier A, $t_{exp.} = 0.6$ for supplier B and $t_{exp.} = 2.8$ for supplier C. As none was higher than the test value of t the null hypothesis may be accepted and so the content of all suppliers was within the specified concentration range of 62.5-66.0 % (w/w).

6.2.8 Reference List

1. MacInnis JA, Boucher GD, Palepu R, Marangoni DG (1999) The properties of a family of two-headed surfactant systems: the 4-alkyl-3-sulfosuccinates 2.Surface properties of alkyl sulfosuccinate micelles. Can J Chem 77:340-347
2. Baczko K, Chasseray X, Larpent C (2001) Synthesis and surfactant properties of symmetric and unsymmetric sulfosuccinic diesters, Aerosol-OT homologues. J Chem Soc Perkin Trans 2:2179-2188

6.3 Composition of Commercial AOT Surfactant Products and its Effects on an Agrochemical Formulation

6.3.1 Sample for Testing on Mass Calibration of ToF-MS

The retention times and exact masses for the compounds in the test sample for checking on mass calibration of the used ToF-MS are given in Table S 7.

Table S 7: Retention time and exact masses for compounds in the test sample for checking on mass calibration

Compound	t_N [min]	Exact mass [amu]
Imidacloprid	2.0	254.0450
Thiacloprid	2.5	252.0236
Tebuconazole (1.Isomer)	4.3	307.1451
Triadimenol	4.6	295.1088
Tebuconazole (2.Isomer)	4.9	307.1451
Distyrylethoxylate-5-EO	5.8	522,2981
Distyrylethoxylate-6-EO	5.8	566,3244
Distyrylethoxylate-7-EO	5.8	610,3506
Distyrylethoxylate-8-EO	5.8	654,3768
Distyrylethoxylate-9-EO	5.8	698,4030
Distyrylethoxylate-10-EO	5.8	742,4292
Distyrylethoxylate-11-EO	5.8	786,4554
Distyrylethoxylate-12-EO	5.8	830,4816
Distyrylethoxylate-13-EO	5.8	874,5079
Distyrylethoxylate-14-EO	5.8	918,5341
Distyrylethoxylate-15-EO	5.8	962,5603
Distyrylethoxylate-16-EO	5.8	1006,5865
Distyrylethoxylate-17-EO	5.9	1050,6127
Distyrylethoxylate-18-EO	5.9	1094,6389
Distyrylethoxylate-19-EO	5.9	1138,6651
Distyrylethoxylate-20-EO	5.9	1182,6914
Distyrylethoxylate-21-EO	5.9	1226,7176
Distyrylethoxylate-22-EO	5.9	1270,7438
Distyrylethoxylate-23-EO	5.9	1314,7700
Distyrylethoxylate-24-EO	5.9	1358,7962
Distyrylethoxylate-25-EO	5.9	1402,8224
Distyrylethoxylate-26-EO	5.9	1446,8486
Distyrylethoxylate-27-EO	5.9	1490,8749
Distyrylethoxylate-28-EO	5.9	1534,9011
Distyrylethoxylate-29-EO	5.9	1578,9273
Distyrylethoxylate-30-EO	5.9	1622,9535
Nonylphenolethoxylate-5-EO	6.6	440,3138
Nonylphenolethoxylate-6-EO	6.3	484,3400
Nonylphenolethoxylate-7-EO	6.2	528,3662
Nonylphenolethoxylate-8-EO	6.2	572,3924
Nonylphenolethoxylate-9-EO	6.2	616,4186
Nonylphenolethoxylate-10-EO	6.2	660,4449
Nonylphenolethoxylate-11-EO	6.2	704,4711
Nonylphenolethoxylate-12-EO	6.2	748,4973
Nonylphenolethoxylate-13-EO	6.2	792,5235
Nonylphenolethoxylate-14-EO	6.2	836,5497
Nonylphenolethoxylate-15-EO	6.2	880,5759

Supplementary

Compound	t_N [min]	Exact mass [amu]
Nonylphenolethoxylate-16-EO	6.2	924,6022
Nonylphenolethoxylate-17-EO	6.2	968,6284
Nonylphenolethoxylate-18-EO	6.2	1012,6546
Nonylphenolethoxylate-19-EO	6.2	1056,6808
Nonylphenolethoxylate-20-EO	6.2	1100,7070
Nonylphenolethoxylate-21-EO	6.2	1144,7332
Nonylphenolethoxylate-22-EO	6.2	1188,7594
Nonylphenolethoxylate-23-EO	6.2	1232,7857
Nonylphenolethoxylate-24-EO	6.2	1276,8119
Nonylphenolethoxylate-25-EO	6.2	1320,8381
Nonylphenolethoxylate-26-EO	5.9	1364,8643
Nonylphenolethoxylate-27-EO	5.9	1408,8905
Nonylphenolethoxylate-28-EO	5.9	1452,9167
Nonylphenolethoxylate-29-EO	5.9	1496,9429
Nonylphenolethoxylate-30-EO	5.9	1540,9692
Tristyrylethoxylate-5-EO	5.9	626,3607
Tristyrylethoxylate-6-EO	5.9	670,38695
Tristyrylethoxylate-7-EO	5.9	714,4132
Tristyrylethoxylate-8-EO	6.5	758,4394
Tristyrylethoxylate-9-EO	5.9	802,4656
Tristyrylethoxylate-10-EO	5.9	846,4918
Tristyrylethoxylate-11-EO	6.0	890,5180
Tristyrylethoxylate-12-EO	6.0	934,5442
Tristyrylethoxylate-13-EO	6.0	978,5705
Tristyrylethoxylate-14-EO	6.0	1022,5967
Tristyrylethoxylate-15-EO	6.0	1066,6229
Tristyrylethoxylate-16-EO	6.0	1110,6491
Tristyrylethoxylate-17-EO	6.0	1154,6753
Tristyrylethoxylate-18-EO	6.0	1198,7015
Tristyrylethoxylate-19-EO	6.0	1242,7278
Tristyrylethoxylate-20-EO	6.0	1286,7540
Tristyrylethoxylate-21-EO	5.9	1330,7802
Tristyrylethoxylate-22-EO	5.9	1374,8064
Tristyrylethoxylate-23-EO	5.9	1418,8326
Tristyrylethoxylate-24-EO	5.9	1462,8588
Tristyrylethoxylate-25-EO	5.9	1506,8850
Tristyrylethoxylate-26-EO	5.8	1550,9113
Tristyrylethoxylate-27-EO	5.8	1594,9375
Tristyrylethoxylate-28-EO	5.8	1638,9637
Tristyrylethoxylate-29-EO	5.8	1682,9899
Tristyrylethoxylate-30-EO	5.8	1727,0161

6.3.2 Content of AOT, Monoester 2 and Monoester 3 in different Production Batches of commercially available AOT Product of different Suppliers

In Table S 8 were given the content of AOT and the monoesters 2 and 3 in AOT product of at least eight production batches each investigated supplier A, B, C and D. The given data for each production batch are average values of five independently weighed repetition analyses after the removal of outliers with a Grubbs outlier test. The displayed data is given together with its interval of confidence of 95%.

Table S 8: Content of AOT and monoester 2 and 3 in AOT product together with their expanded measurement uncertainty. Analysis of five independently weight samples each batch number averaged. The expended measurement uncertainty is encompassing 95% of the distribution of values.

Sample [Supplier-Batch No.]	w(AOT) [%]	w(monoester 2) [%]	w(monoester 3) [%]
a-1	62.9 ± 1.2	1.3 ± 0.02	0.72 ± 0.02
a-2	58.6 ± 1.2	1.5 ± 0.04	0.58 ± 0.01
a-3	60.2 ± 0.6	1.7 ± 0.02	0.93 ± 0.01
a-4	61.3 ± 3.3	1.2 ± 0.05	0.48 ± 0.02
a-5	62.4 ± 2.1	2.0 ± 0.04	0.82 ± 0.03
a-6	61.2 ± 0.9	1.3 ± 0.01	0.72 ± 0.01
a-7	62.6 ± 1.2	1.5 ± 0.03	0.83 ± 0.01
a-8	62.2 ± 1.1	1.3 ± 0.03	0.69 ± 0.01
A-1	64.5 ± 1.0	2.8 ± 0.02	1.7 ± 0.03
A-2	57.8 ± 1.0	2.3 ± 0.05	2.1 ± 0.05
A-3	58.0 ± 1.6	2.6 ± 0.05	2.0 ± 0.04
A-4	56.3 ± 1.0	2.4 ± 0.04	1.9 ± 0.01
A-5	60.6 ± 0.6	2.5 ± 0.08	1.8 ± 0.05
B-1	65.8 ± 0.7	0.82 ± 0.01	0.15 ± 0.004
B-2	65.0 ± 3.5	0.58 ± 0.02	0.26 ± 0.01
B-3	65.3 ± 2.1	0.80 ± 0.02	0.15 ± 0.003
B-4	73.1 ± 1.3	1.2 ± 0.03	0.36 ± 0.01
B-5	61.3 ± 1.1	1.3 ± 0.04	0.28 ± 0.02
B-6	62.1 ± 0.7	1.0 ± 0.01	0.31 ± 0.01
B-7	63.0 ± 1.0	0.88 ± 0.01	0.21 ± 0.01
B-8	71.3 ± 1.0	1.2 ± 0.03	0.30 ± 0.01
C-1	61.4 ± 1.0	3.2 ± 0.06	0.67 ± 0.03
C-2	58.8 ± 0.7	2.5 ± 0.06	1.0 ± 0.02
C-3	55.7 ± 0.9	3.4 ± 0.02	1.0 ± 0.02
C-4	62.9 ± 0.6	2.5 ± 0.05	1.5 ± 0.03
C-5	60.1 ± 0.7	3.3 ± 0.05	0.73 ± 0.02
C-6	59.0 ± 0.8	2.3 ± 0.04	0.60 ± 0.01
C-7	57.1 ± 0.9	2.4 ± 0.04	0.53 ± 0.01
C-8	58.7 ± 0.9	2.4 ± 0.03	0.54 ± 0.01

Sample [Supplier-Batch No.]	w(AOT) [%]	w(monoester 2) [%]	w(monoester 3) [%]
D-1	63.9 ± 0.3	3.8 ± 0.09	2.7 ± 0.09
D-2	61.6 ± 1.1	3.4 ± 0.11	2.4 ± 0.03
D-3	64.8 ± 1.0	4.1 ± 0.06	2.7 ± 0.08
D-4	65.1 ± 0.9	4.0 ± 0.09	2.5 ± 0.04
D-5	64.1 ± 0.7	3.9 ± 0.08	2.3 ± 0.07
D-6	61.2 ± 1.3	4.1 ± 0.06	2.8 ± 0.04
D-7	64.6 ± 0.2	3.9 ± 0.05	2.0 ± 0.07
D-8	64.2 ± 1.0	3.8 ± 0.03	2.3 ± 0.03
D-9	65.0 ± 1.0	4.0 ± 0.03	2.0 ± 0.03
D-10	64.4 ± 0.5	3.1 ± 0.08	2.0 ± 0.05
D-11	65.3 ± 0.7	3.2 ± 0.07	2.2 ± 0.05
D-12	65.2 ± 0.4	3.0 ± 0.06	2.1 ± 0.04
D-13	65.2 ± 0.8	2.8 ± 0.09	1.9 ± 0.05
D-14	60.9 ± 0.7	2.9 ± 0.21	1.8 ± 0.09
D-15	63.3 ± 0.4	2.9 ± 0.05	2.0 ± 0.04
D-16	62.5 ± 0.8	3.3 ± 0.05	2.2 ± 0.06

6.3.3 Sedimentation in Trail Storage Formulation Samples

The observed sediment in the formulation samples after storage was photographed from above and shown in Figure S 11.

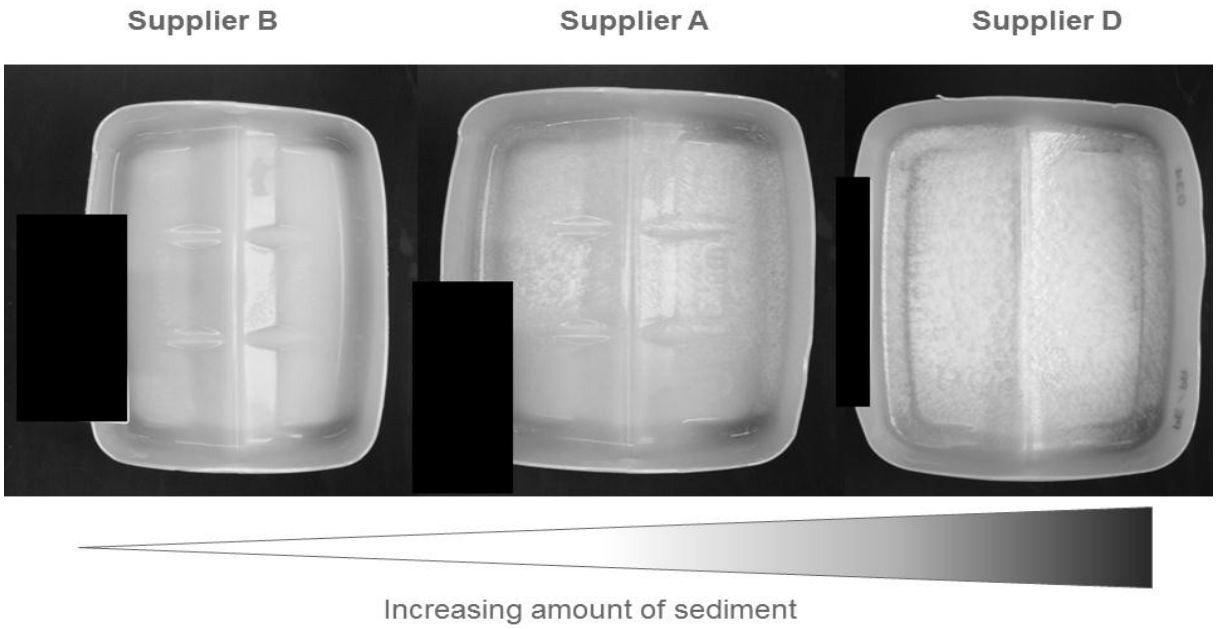


Figure S 11: Test on sedimentation after 0.5 a storage at room temperature of a model agrochemical formulation containing AOT product of supplier A1, B and D. Increasing amount of visible sediment from supplier A1 to supplier D

6.3.4 Centrifugation of a Model Agrochemical Formulation containing AOT Product of Supplier A1

A model agrochemical formulation containing AOT product of supplier A1 was centrifuged with a HEREAUS Labofuge 400 with 3000 rpm. The supernatant was removed and the sediment analyzed on AOT and monoester 2 and monoester 3. The results of the analyses given as percentage compositions of the AOT product used in the formulation are shown in Table S 9. Each value is the average of five replicate analyses given together with its interval of confidence of 95%.

Table S 9: Contents of AOT, monoester 2, and monoester 3 in supernatants and sediments, given as percentage compositions of commercial AOT product used in the formulation. The sediment was obtained after centrifugation of the model agrochemical formulation containing AOT product of supplier A1. Each value is the average of five replicates analyses, given together with its interval of confidence of 95%.

	w(AOT) [%]	**w(monoester 2) [%]**	**w(monoester 3) [%]**
Sediment sample	236.0 ± 36.2	1.8 ± 0.1	0.9 ± 0.08

6.3.5 Results of the Analysis of AOT Product of different Production Batches for inorganic Anions and Cations of different Suppliers

Selected production batches of AOT product of supplier A1, B, C and D were investigated on difference in their content of inorganic cations and anions, which are known to influence both ionic and non-ionic surfactants [1;2]. The samples were screened on the content of the cations Li^+ Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}, as well as, the anions of Br^-, Cl^-, F^-, NO_3^-, PO_4^{3-} and SO_4^{2-}. Variations in the content of inorganic ions between the suppliers of AOT product may explain the differences observed in sedimentation behavior after storage of a model agrochemical formulation containing AOT product of either supplier A1, B or D.

Analysis was conducted on an ICS 2000 ion chromatography instrument from Dionex. Chromatographic separation of the cations was performed with an IonPa CS12A column (250 x 2.0 mm). For mobile phase methanesulfonic acid (MSA) was taken. The sample was injected with a volume of 5.0 µL and gradient elution was applied for separation of the target analytes. Starting with a concentration of 30 mM MSA and raised to 40 mM in 10 min, lowered to 30 mM MSA in 1.0 min to 30mM MSA by column flushing and equilibration afterwards. Total run time was 15 min with a flow of 0.25 mL/min and a column temperature of 30°C.

Supplementary

For chromatographic separation of the anions an IonPac AS11 HC column (250 mm x 2.0 mm) was used. As mobile phase water plus 30mM KOH was taken. The sample was injected with 2.5 µL and the target analytes were eluted isocratically. Total run time was 15 min with a flow of 0.38 mL/min and column temperature of 30 °C. For detection an electrochemical detector connected upstream with a suppressor was used.

For analysis of the cations Dionex Six Cation-II Standard was used, containing lithium ($c(Li^+)$ = 50 mg/L), sodium ($c(Na^+)$ = 201 mg/L), ammonium ($c(NH_4^+)$ = 251 mg/L), potassium ($c(K^+)$ = 501 mg/L), magnesium ($c(Mg^{2+})$ = 250 mg/L) and calcium ($c(Ca^{2+})$ = 50 mg/L). This solution had to be further diluted by 1:10 (v/v) diluted to obtain the stock solution for the analysis of cations.

For the analysis of the anions a commercially available multi-element ion chromatography anion standard supplied by Fluka was used as standard solution containing, bromide ($c(Br^-)$ = 20 mg/L), chloride ($c(Cl^-)$ = 10 mg/L), fluoride ($c(F^-)$ = 3 mg/L), nitrate ($c(NO_3^-)$ = 20 mg/L), phosphate ($c(PO_4^{3-})$ = 20 mg/L) and sulfate ($c(SO_4^{2-})$ = 20 mg/L).

For preparation of the standard solutions the both stock solutions were diluted to fit the concentration range 20 mg/L to 1 mg/L.

For analysis the light aromatic solvent in AOT product was evaporated. An amount of 100 mg of the remainder was diluted with 50 mL of a mixture of 95/5 (v/v) water/methanol. The obtained solution could be directly injected without further dilution accepted for the analysis of Na^+, where the sample solution had to be diluted 1:10 (v/v) to be inside the linear range.

Of all investigated inorganic ions only the contents of Na^+, Ca^{2+}, Cl^-, NO_3^- and SO_4^{2-} were above the limit of quantification (LOQ) of 1 mg/L of the used analytical method. As this LOQ corresponds to a content of 0.05 % (w/w) in AOT product with the given sample preparation, no further attempts were made to detect the other inorganic ions screened for, as their content was considered negligible. In Figure S 12 is shown the chromatographic separation of the target cation (a) and anions (b) for the analysis of the production batch a-1.

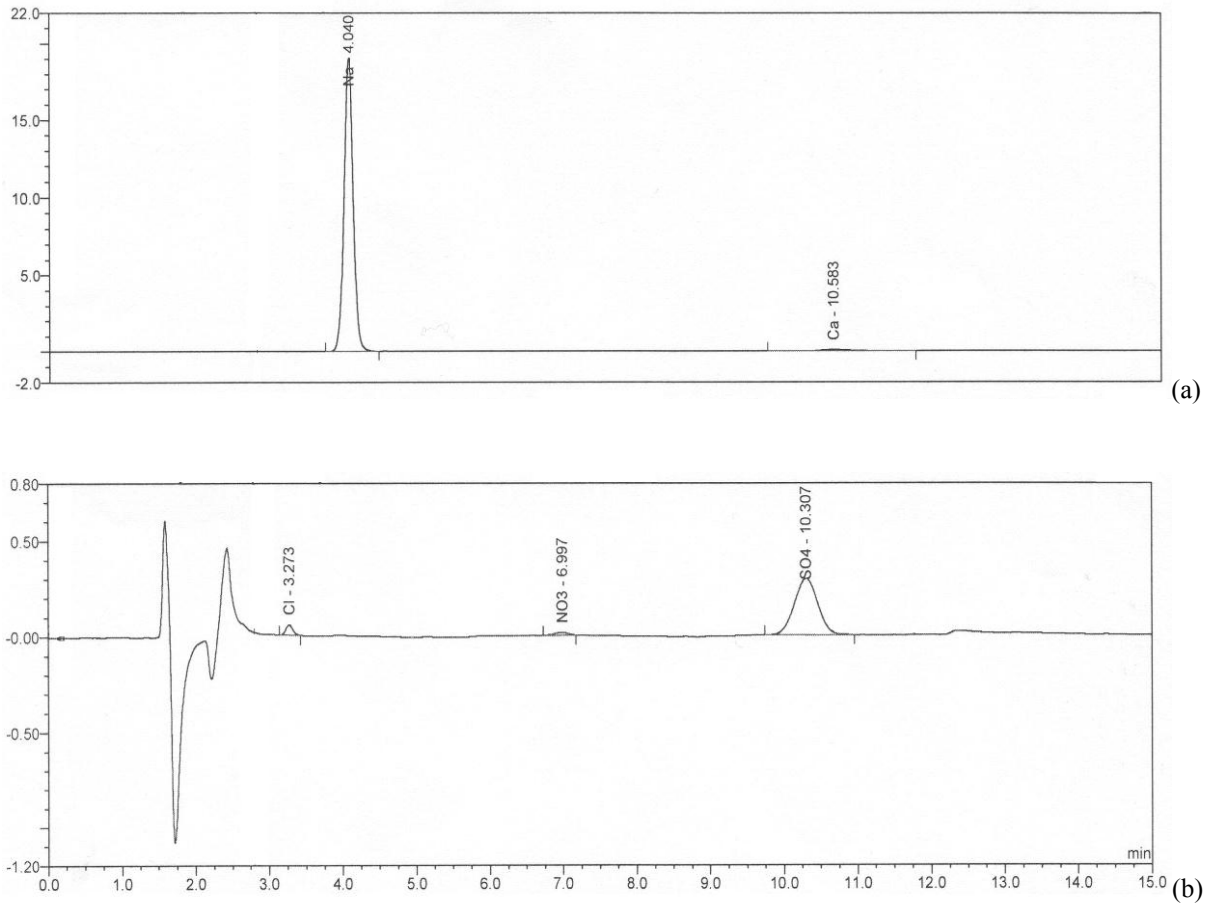


Figure S 12: Chromatographic separation of the cations Na^+ and Ca^{2+}(a) and the anions Cl^-, NO_3^- and SO_4^{2-} via ion chromatography.

The obtained results are shown in Table S 10 and are visualized as box-plots in Figure S 13 (a) for Na^+, in (b) for Ca^{2+}, in (c) for Cl^-, in (d) for NO_3^- and in (e) for SO_4^{2-}. Those ions, which contents were below the LOQ of the used method, were indicated with "<LOQ" and were not considered for the box-plot figures.

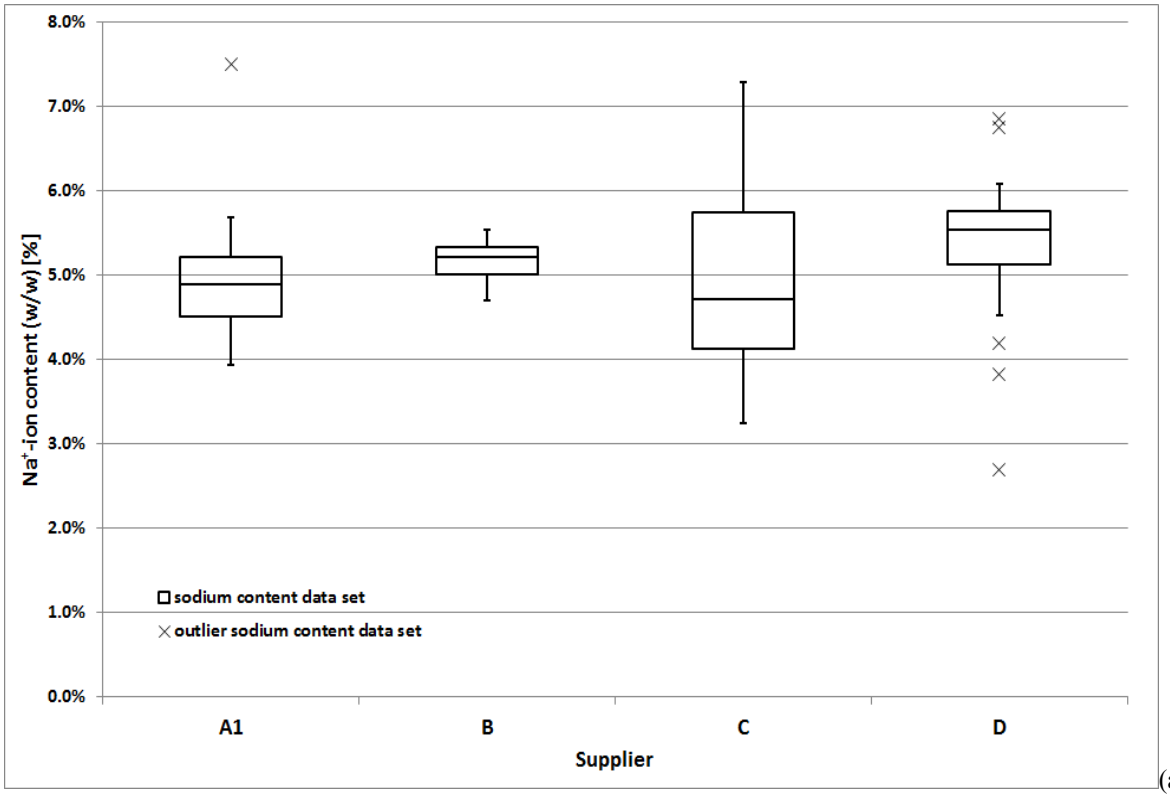
Table S 10: Content of Na^+, Ca^{2+}, Cl^-, NO_3^- and SO_4^{2-} in selected production batches of AOT product of supplier A1, supplier B, supplier C and supplier D. Those ions, which contents were below the LOQ of the used method were indicated with "<LOQ".

Sample [Supplier-Batch No.]	Na^+ (w/w) [%]	Ca^{2+} (w/w) [%]	Cl^- (w/w) [%]	NO_3^- (w/w) [%]	SO_4^{2-} (w/w) [%]
a-1	4.7	0.07	< LOQ	< LOQ	0.5
a-2	5.3	<LOQ	0.06	0.05	0.3
a-3	5.2	0.1	0.08	0.09	0.6
a-4	7.5	0.1	0.05	0.07	0.4
a-5	5.1	0.1	0.06	0.08	0.7
a-6	3.8	<LOQ	< LOQ	< LOQ	0.4
a-7	3.7	0.08	< LOQ	< LOQ	0.3
a-8	4.8	<LOQ	< LOQ	< LOQ	0.5

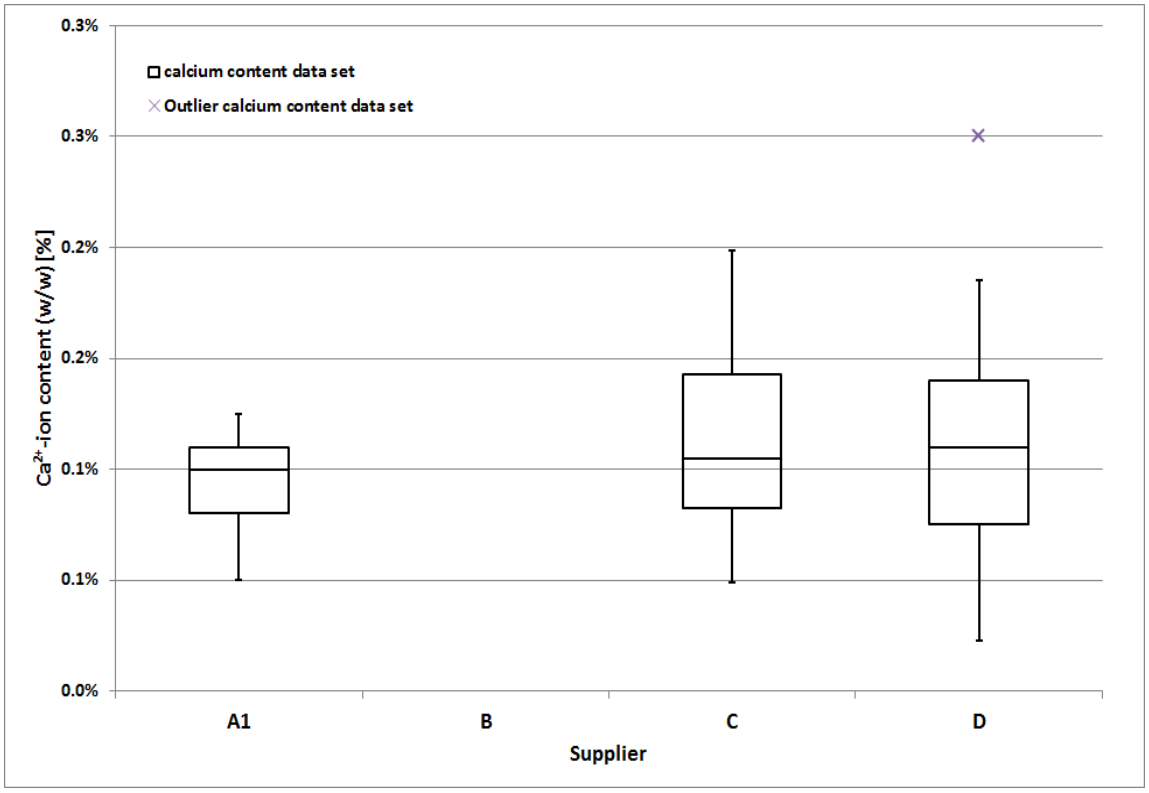
Supplementary

Sample [Supplier-Batch No.]	Na^+ (w/w) [%]	Ca^{2+} (w/w) [%]	Cl^- (w/w) [%]	NO_3^- (w/w) [%]	SO_4^{2-} (w/w) [%]
B-1	5.0	<LOQ	0.09	0.1	0.4
B-2	5.2	<LOQ	0.06	0.08	0.3
B-3	4.8	<LOQ	0.06	0.07	0.3
B-4	5.4	<LOQ	0.16	0.2	0.5
B-5	4.9	<LOQ	0.06	0.07	0.5
B-6	5.3	<LOQ	0.14	0.1	0.5
B-7	5.2	<LOQ	0.14	0.1	0.6
B-8	5.4	<LOQ	0.14	0.2	0.5
C-1	5.7	<LOQ	< LOQ	< LOQ	0.3
C-2	3.0	0.2	< LOQ	< LOQ	0.2
C-3	4.5	0.2	< LOQ	0.05	0.3
C-4	3.5	0.09	0.05	0.07	0.3
C-5	6.0	0.1	0.06	0.08	0.4
C-6	4.4	0.08	< LOQ	0.05	0.3
C-7	4.9	0.07	< LOQ	< LOQ	0.4
C-8	5.9	<LOQ	< LOQ	< LOQ	0.4
D-1	4.2	0.1	0.05	0.07	0.4
D-2	6.9	0.1	0.2	0.07	0.4
D-3	5.9	0.07	< LOQ	< LOQ	0.3
D-4	6.8	0.09	0.05	< LOQ	0.4
D-5	5.7	0.1	0.1	< LOQ	0.3
D-6	5.5	0.06	0.1	< LOQ	0.3
D-7	5.8	<LOQ	0.07	< LOQ	0.3
D-8	2.7	0.08	0.05	0.05	0.3
D-9	3.8	0.05	0.1	0.05	0.3
D-10	5.3	0.2	0.08	0.1	0.3
D-11	5.5	0.1	0.05	0.05	0.4
D-12	4.7	0.1	0.05	0.05	0.3
D-13	5.7	0.07	0.05	0.05	0.6
D-14	5.7	0.3	0.2	0.1	0.4
D-15	5.6	0.2	0.06	0.08	0.5
D-16	5.3	0.1	0.06	0.08	0.4

Supplementary



(a)



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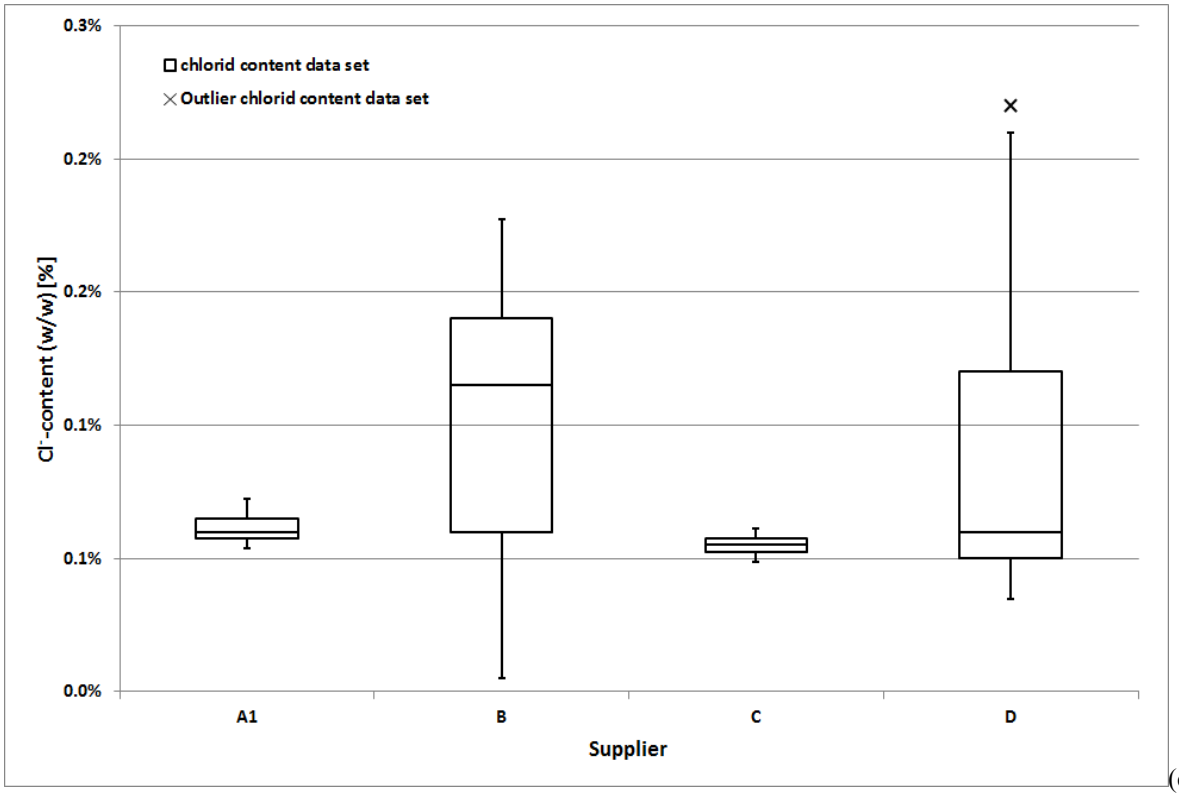

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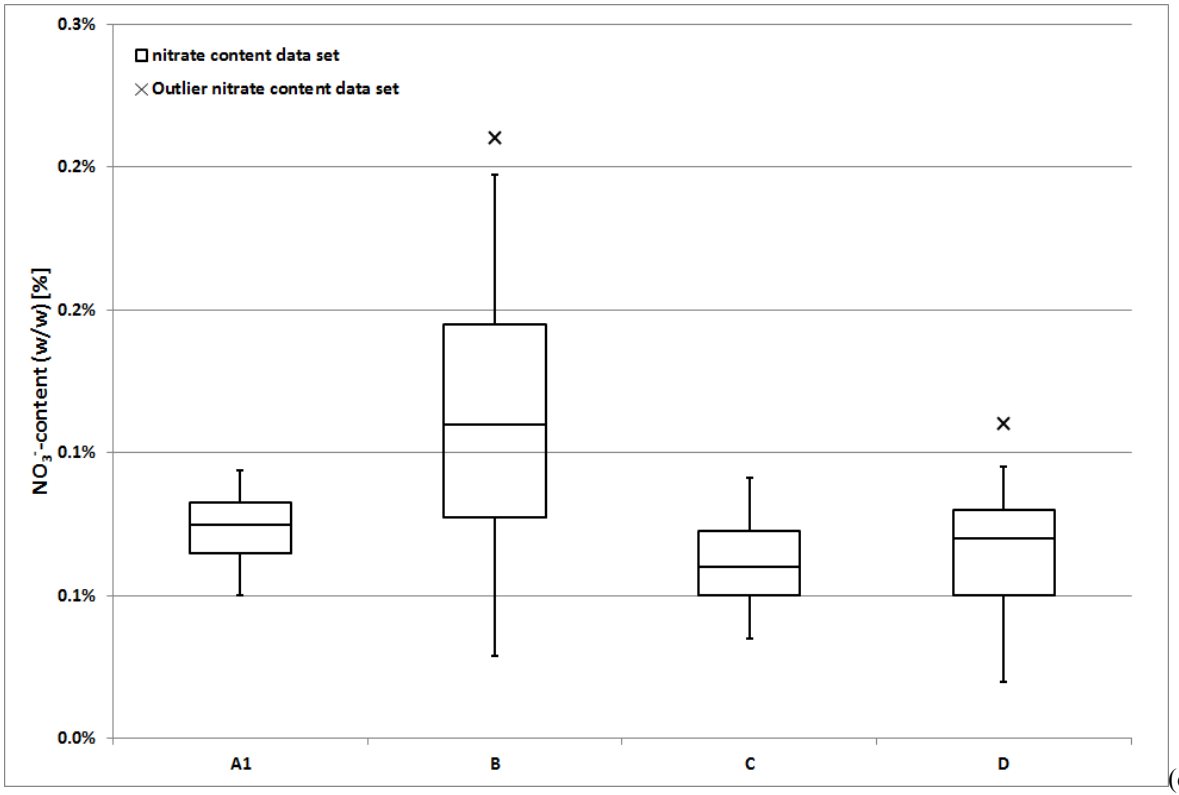

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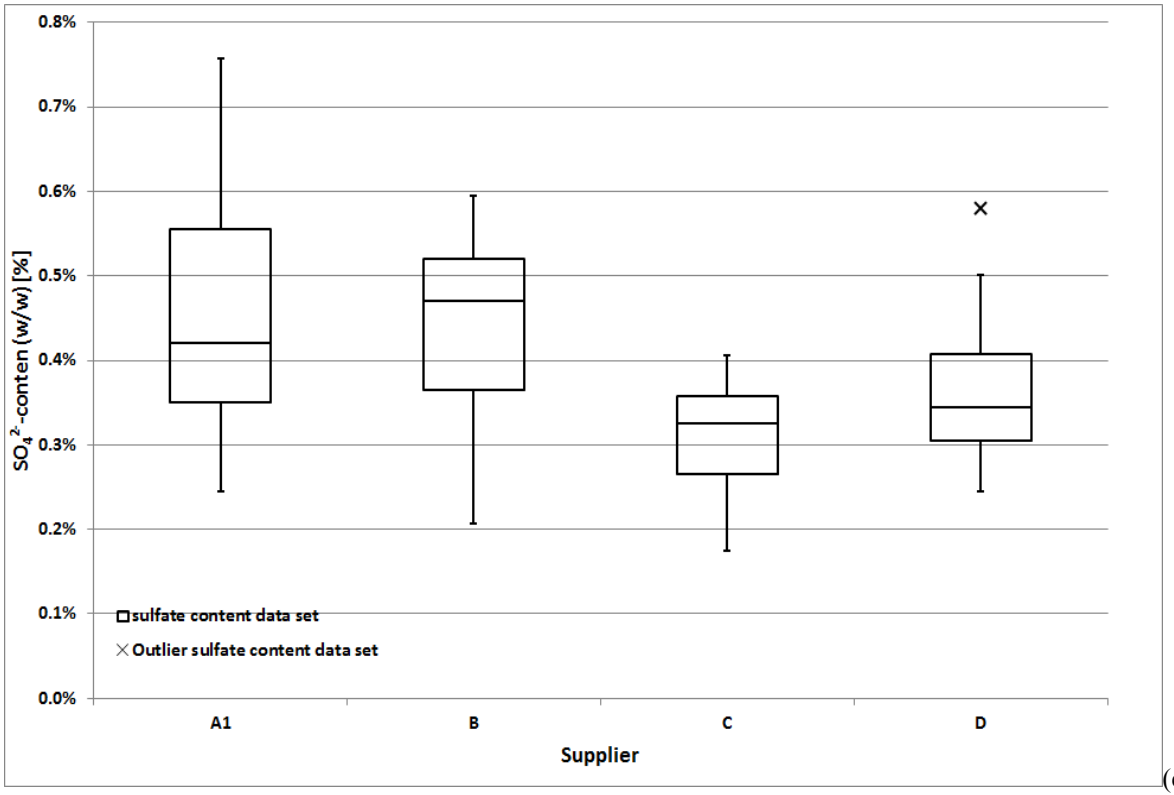


(e)

Figure S 13: Content of (a) Na^+, (b) NH_4^+, (c) Ca^{2+}, (d) Cl^-, (e) NO_3^- and (f) SO_4^{2-} in selected production batches of AOT product of supplier A1, B, C and D displayed as box-plots.

As shown the content of the investigated inorganic ions, Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, NO_3^- and SO_4^{2-}in AOT product was not different between the supplier A1, B, C and D. Therefore the observed differences in the physico-chemical properties of a model agrochemical formulation, containing AOT product of either supplier A1, B or D, could not be explained by differences in the content of inorganic ions.

6.3.6 Analysis of the Composition of the Solvent in AOT Product on Differences between the different Suppliers

Selected production batches of supplier A1, C and D were analyzed via GC-MS, to investigate, if there are differences in the composition of the light-aromatic naphtha solvent in which AOT is solved in, between the different suppliers of AOT product.

The analysis was performed via gas chromatography coupled to mass spectrometry with electron impact ionization on an Agilent 5973 GC/MS. The sample was injected with 0.2 µL, with a split of 1:60 (GC:waste) on a HP-5 capillary column of Agilent with an inner diameter of 0.18 mm, a length of 20 m and film thickness of 0.18 mm. Separation of the analytes was achieved with a temperature gradient, starting with 60 °C, raising

temperature to 200 °C in 28 min. For column cleaning the temperature was then raised to 280 °C in 4 min and held for 3 min at 280 °C. Total run time was 35 min with N_2-gas stream set at 150 kPa constant pressure. The Inlet temperature was set at 260 °C, the aux temperature at 280 °C, the temperature in the MS inlet at 250°C and in the MS quadrupole at 150 °C.

An amount of 20 mg each AOT product sample was solved in 50 mL of a mixture of 1:1 (v/v) ACN/H_2O. The obtained solution was then injected into the GC-MS, without further dilution or treatment.

The main components of the light-aromatic naphtha solvent was chromatographically separated and identified via a spectra library. The chromatographic separation is shown in Figure S 14 (a) for the early eluting and in Figure S 14 (b) for the late eluting compounds. The most likely hit regarding retention time and spectrum for the main components are displayed in Table S 11.

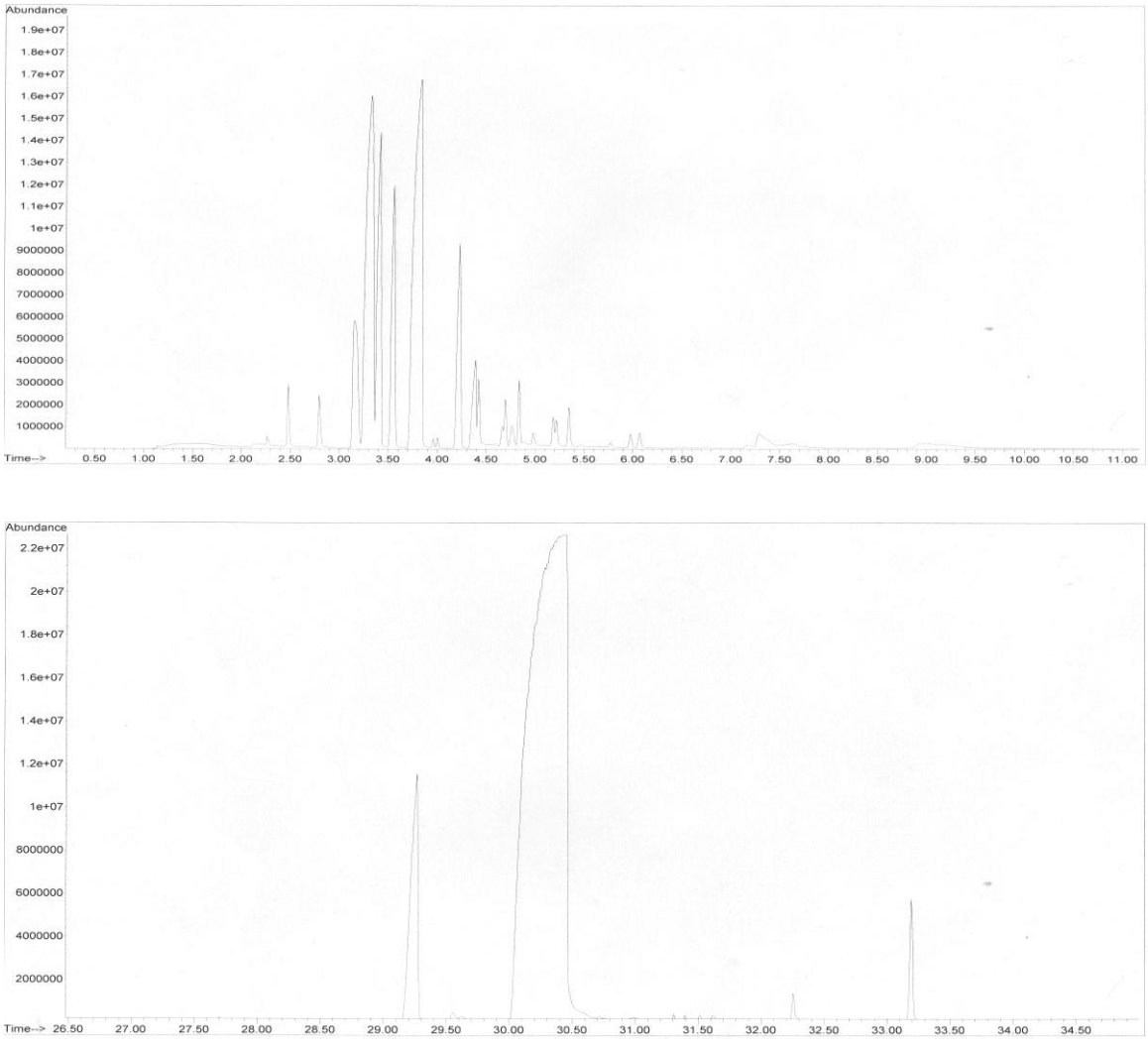


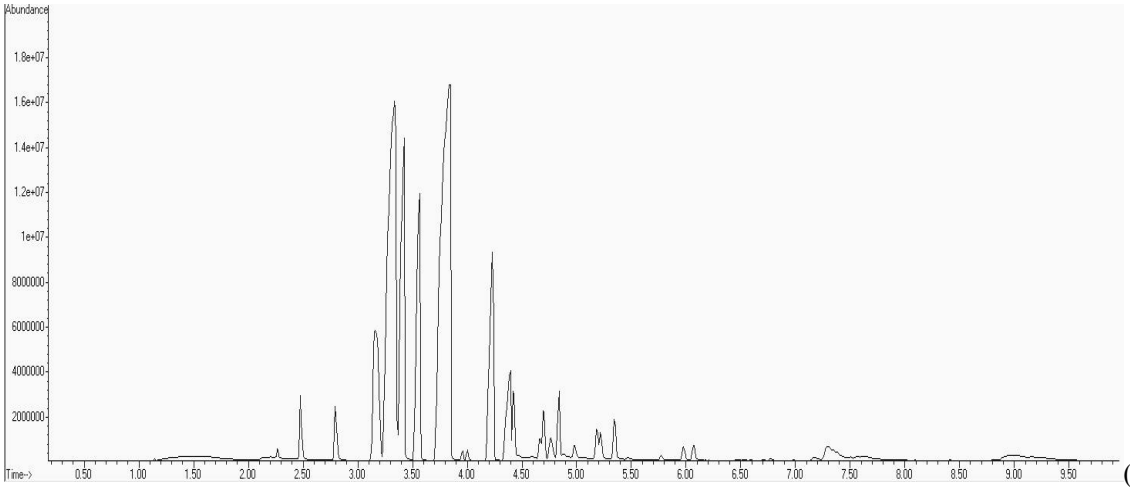
Figure S 14: Chromatographic separation of the light-aromatic naphtha solvent in AOT product, shown in (a) are the earlier eluting and in (b) the late eluting compounds.

Supplementary

Table S 11: Compounds in the light-aromatic naphtha solvent in AOT product, which were identified via spectra library. Shown are the most likely hits according to retention time and spectrum.

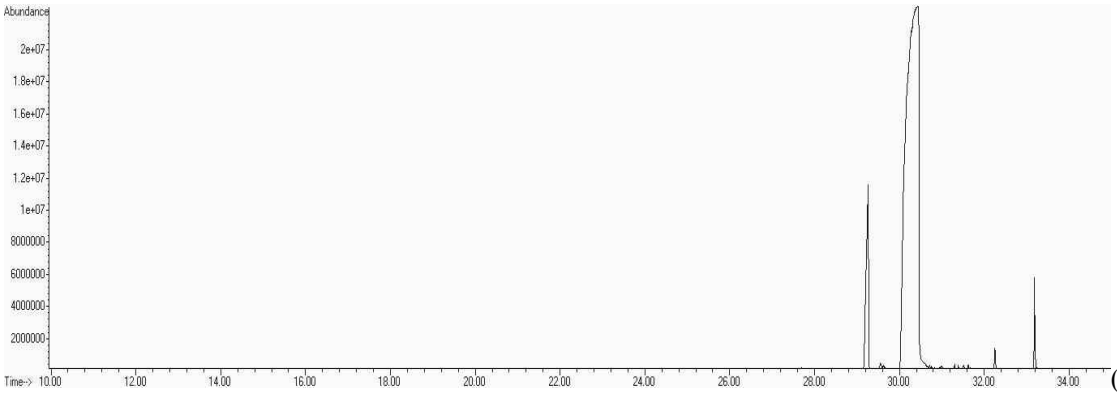
Retention time [min]	Compound
2.48	1,3-dimethyl-benzene
2.79	(1-methylethyl)-benzene
3.15	Propyl-benzene
3.30	1-ethyl-3-methyl-benzene
3.42	1-ethyl-2-methly-benzene
3.95	(2-methylpropyl)-benzene
3.99	(1-methylpropyl)-benzene
4.23	1, 2, 3-trimethylbenzene
4.43	Indane
4.66	1, 3-diethyl-benzene
4.69	1-methly-3-propyl-benzene
4.77	Diethyl-benzene
4.83	4-ethyl-1,2-dimethyl-benzene
4.88	1, 2-diethyl-benzene
4.98	1-methly-4-propyl-benzene
5.18	2-ethyl-1, 4-dimethyl-benzene
5.34	2-ethyl-1 ,3-dimethyl-benzene
5.97	1, 2, 4, 5-teramethly-benzene
6.06	1, 2, 3, 4-teramethly-benzene
7.19	alpha, 4-diemethyl-benzene-methanol
8.99	6-methylheptyl ester 2- propionic acid
29.24	Bis(2-ethylhexyl) maleate
30.24	1 ,2-Cyclohexanedione

As shown the main compounds identified are benzyl derivates of benzene, which confirms the characterization of the light-aromatic naphtha solvent by its supplier [3;4]. 8 different production batches each supplier A1, C and D were analyzed accordingly, on the composition of their light-aromatic solvent. Exemplary, are given in Figure S 15 the results for one production batch of AOT product each supplier, as variations between the analyzed production batches for suppliers were not detected. Shown are separately the range of time 0-10 min in A1-1, C-1 and D-1 and the time range 10-35min in A1-2, C-2 and D-2.

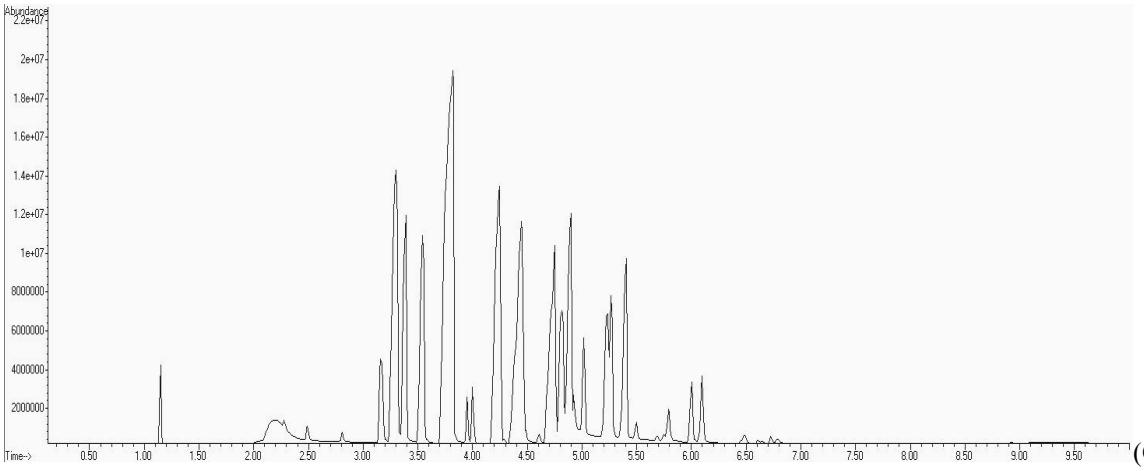


(A1-1)

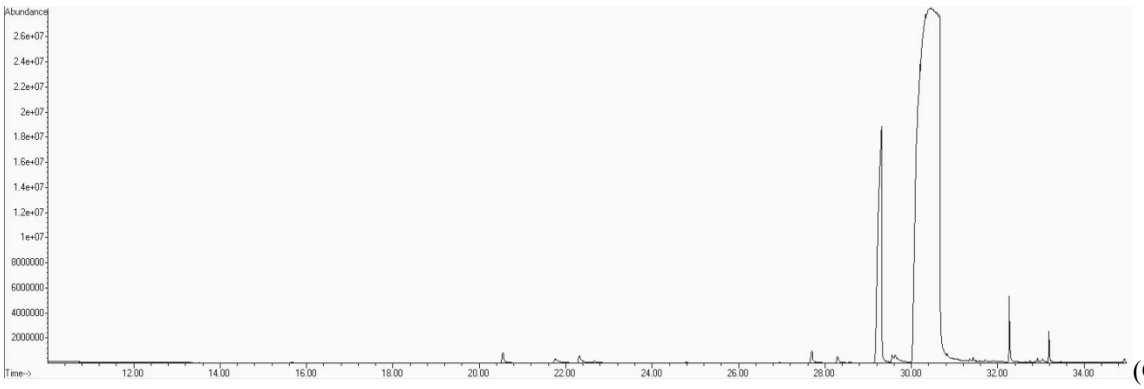
Supplementary



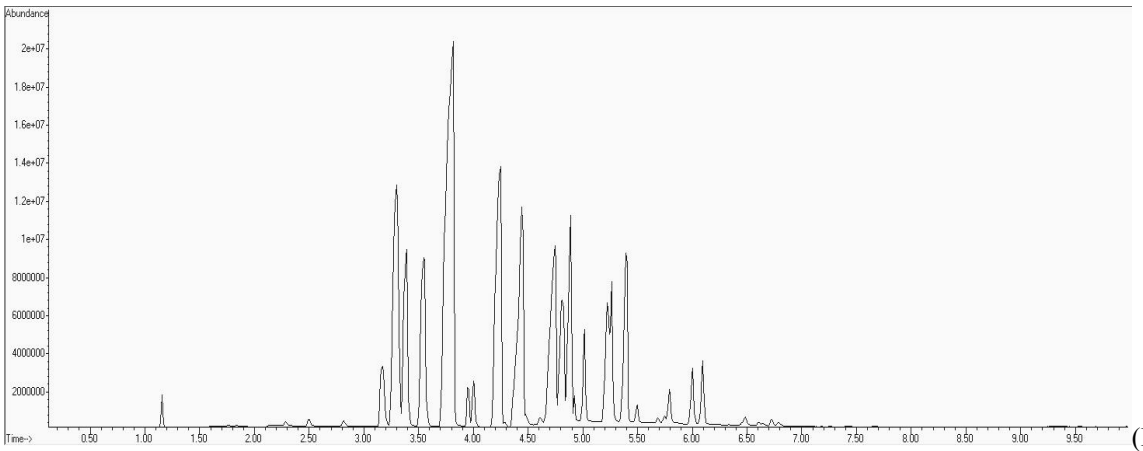
(A1-2)



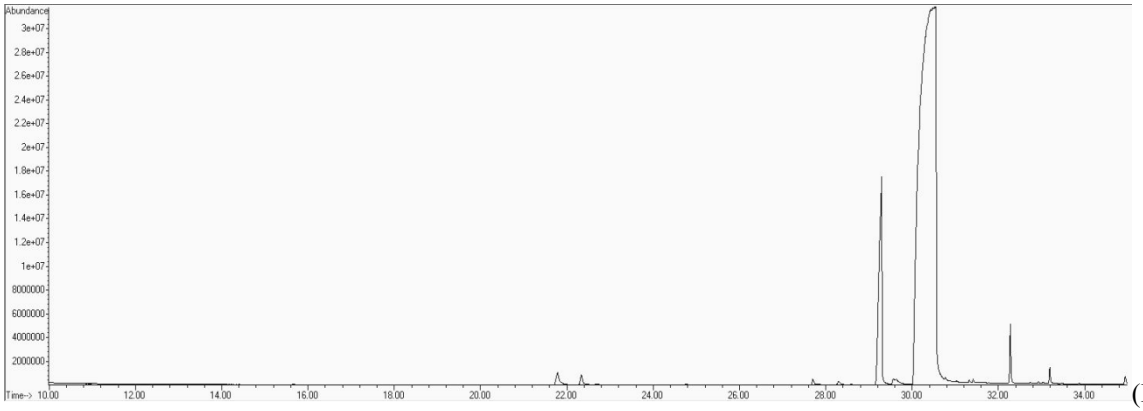
(C-1)



(C-2)



(D-1)



(D-2)

Figure S 15: Comparison of the chromatographic pattern of the light-aromatic naphtha solvent of selected production batches of AOT product of the suppliers A1, C and D. Shown are separately the retention time range 0-10 min (A1-1), C-1 and D-1) and 10-35 min (A1-2, C-2 and D-2). The analysis of the solvent was conducted on GC-MS

The compounds listed in Table S 11 were found for all three suppliers. Observed were, however, differences between the investigated suppliers of AOT product regarding the abundance of some compounds in the retention time window 2.0-7.0 min.

6.3.7 Statistical evaluation of the differences in the content of AOT, monoester 2 and 3 for product identification

After having analyzed the content of AOT, monoester 2 and 3 in AOT product samples from production batches of different suppliers the question arose if in the future such analytical data could be potentially helpful for identifying the supplier from which an unknown sample originates. The corresponding statistical analysis was provided by Molt K in personal communication and performed with R, a language and environment for statistical computing and graphics [5]. The data is prepared as displayed in Table S 12 (Samples from batches of various suppliers) and in Table S 13 (Trial storage formulation samples). The results of the data analysis in R are given in the following together with the corresponding code.

Table S 12: Data set samples from batches of various suppliers.

Content_AOT	Content_mono2	Content_mono3	Supplier	Plot Symbol	Sample Name	Identifier
1	62.9	1.3	0.72	A1	a	A1
2	58.6	1.5	0.58	A1	a	A2
3	60.2	1.7	0.93	A1	a	A3
4	61.3	1.2	0.48	A1	a	A4
5	62.4	2	0.82	A1	a	A5
6	61.2	1.3	0.72	A1	a	A6
7	62.6	1.5	0.83	A1	a	A7

Supplementary

Content_AOT	Content_mono2	Content_mono3	Supplier	Plot Symbol	Sample Name	Identifier
8	62.2	1.3	0.69	A1	a	A8
9	64.5	2.8	1.7	A2	A	A9
10	57.8	2.3	2.1	A2	A	A10
11	58	2.6	2	A2	A	A11
12	56.3	2.4	1.9	A2	A	A12
13	60.6	2.5	1.8	A2	A	A13
14	65.8	0.82	0.15	B	B	B1
15	65	0.58	0.26	B	B	B2
16	65.3	0.8	0.15	B	B	B3
17	73.1	1.2	0.36	B	B	B4
18	61.3	1.3	0.28	B	B	B5
19	62.1	1	0.31	B	B	B6
20	63	0.88	0.21	B	B	B7
21	71.3	1.2	0.3	B	B	B8
22	61.4	3.2	0.67	C	C	C1
23	58.8	2.5	1	C	C	C2
24	55.7	3.4	1	C	C	C3
25	62.9	2.5	1.5	C	C	C4
26	60.1	3.3	0.73	C	C	C5
27	59	2.3	0.6	C	C	C6
28	57.1	2.4	0.53	C	C	C7
29	58.7	2.4	0.54	C	C	C8
30	63.9	3.8	2.7	D	D	D1
31	61.6	3.4	2.4	D	D	D2
32	64.8	4.1	2.7	D	D	D3
33	65.1	4	2.5	D	D	D4
34	64.1	3.9	2.3	D	D	D5
35	61.2	4.1	2.8	D	D	D6
36	64.6	3.9	2	D	D	D7
37	64.2	3.8	2.3	D	D	D8
38	65	4	2	D	D	D9
39	64.4	3.1	2	D	D	D10
40	65.3	3.2	2.2	D	D	D11
41	65.2	3	2.1	D	D	D12
42	65.2	2.8	1.9	D	D	D13
43	60.9	2.9	1.8	D	D	D14
44	63.3	2.9	2	D	D	D15
45	62.5	3.3	2.2	D	D	D16

Table S 13: Data set trial storage formulation samples

Content_AOT	Content_mono 2	Content_mono3	Supplier	Plot Symbol	Sample Name
59.4	1.5	0.46	A1	a	formulation_1
63.8	1.3	0.24	B	B	formulation_2
59.7	3.7	1.9	D	D	formulation_3

Supplementary

Pairwise t-tests

First it is tested if the means of the contents of the individual components (AOT, monoester 2 and 3) in AOT product differ significantly between the individual suppliers.

> *D <- read.table("data_set_1.txt",header=TRUE)*

> *attach(D)*

In the following for each of the variables content of AOT (Content_AOT), monoester 2 (Content_mono2) and monoester 3 (Content_mono3) the following operations are performed:

- Bartlett's test of the null hypothesis that the variances in each of the groups (suppliers) are the same. These tests will show that the variances differ significantly between the individual groups.
- Paired t-test between the different groups of suppliers. Due to the results of Bartlett's test the paired t-tests will be performed with non-pooled variances. The differences between the means are regarded as significant between those pairs of suppliers where the observed significance level p of the paired t-test is less than 0.05 and as highly significant for p less than 0.01.
- Stripcharts including confidence intervals (95%) for the means are plotted.

AOT

> bartlett.test(Content_di ~ Supplier)

Bartlett test of homogeneity of variances data: Content_AOT by Supplier

Bartlett's K-squared = 14.4321, df = 4, p-value = 0.006036

> pairwise.t.test(Content_di,Supplier,pool.sd=FALSE)

Pairwise comparisons using t tests with non-pooled SD data: Content_AOT and Supplier

	A1	A2	B	C
A2	0.6782	-	-	-
B	0.1301	0.0772	-	-
C	0.1793	0.8946	0.0207	-
D	0.0166	0.1793	0.6782	0.0039

P value adjustment method: holm

Supplementary

The result shows that A1/D and B/C are significantly and C/D highly significantly different pairs with respect to their means.

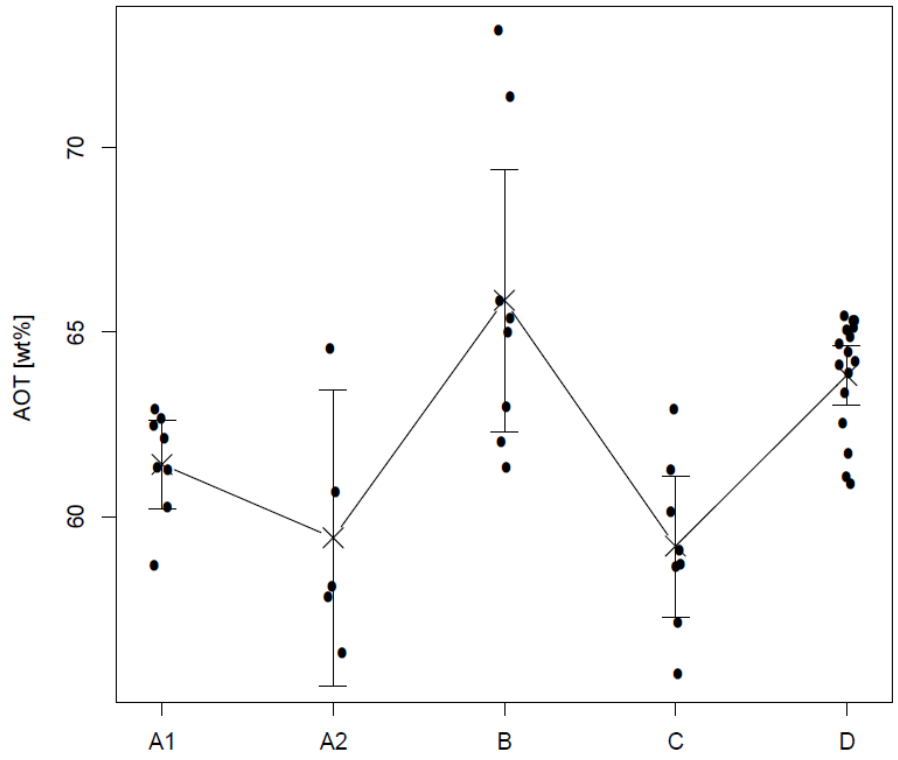


Figure S 16: Stripchart for AOT. As the pairwise t-tests show, none of the means of the individual suppliers differs significantly from the means of all the others.

Monoester 2

> bartlett.test(Content_mono2 ~ Supplier)

Bartlett test of homogeneity of variances data: Content_mono2 by Supplier

Bartlett's K-squared = 7.9693, df = 4, p-value = 0.09271

> pairwise.t.test(Content_mono2,Supplier,pool.sd=FALSE)

Supplementary

Pairwise comparisons using t tests with non-pooled SD data: Content_mono2 and Supplier

	A1	A2	B	C
A2	3.3e-05	-	-	-
B	0.00470	1.1e-06	-	-
C	0.00012	0.24172	9.9e-06	-
D	5.9e-11	2.0e-05	3.7e-13	0.00470

P value adjustment method: holm

The results show that besides the pair A2/C for which the means are not significantly different all other pairs have highly signficantly different means.

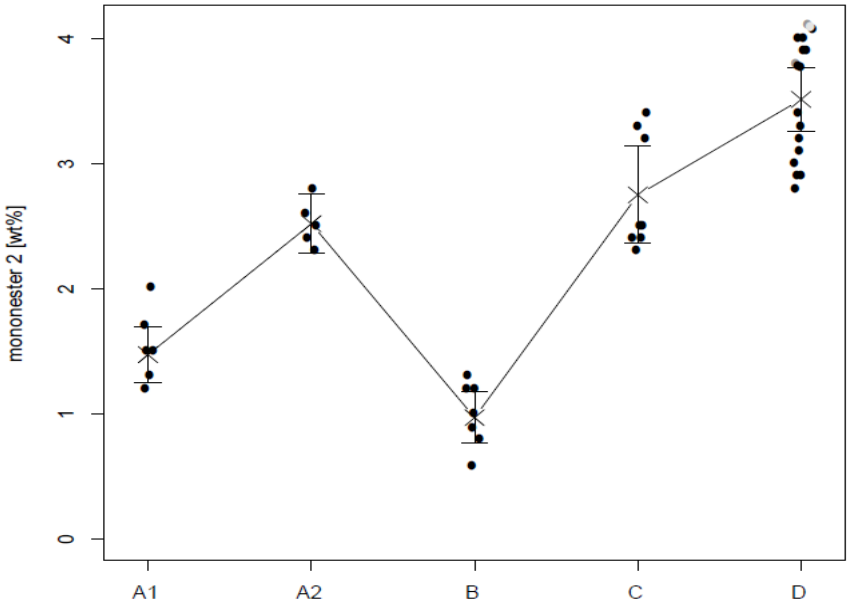


Figure S 17: Stripchart for monoester 2. As the pairwise t-tests show, the means from of each of the suppliers A1, B and D are highly significantly different all the others.

Supplementary

Monoester 3

> bartlett.test(Content_mono3 ~ Supplier)

Bartlett test of homogeneity of variances

data: Content_mono3 by Supplier

Bartlett's K-squared = 16.3527, df = 4, p-value = 0.002581

> pairwise.t.test(Content_mono3,Supplier,pool.sd=FALSE)

Pairwise comparisons using t tests with non-pooled SD data: Content_mono3 and Supplier

	A1	A2	B	C
A2	6.1e-06	-	-	-
B	3.3e-05	1.6e-05	-	-
C	0.4525	3.8e-05	0.0049	-
D	5.7e-13	0.0107	1.6e-14	1.1e-06

P value adjustment method: holm

The results show that besides the pair A1/C for which the means are not significantly different and the pair A2/D for which the means are significantly different all other pairs are have highly signficantly different means.

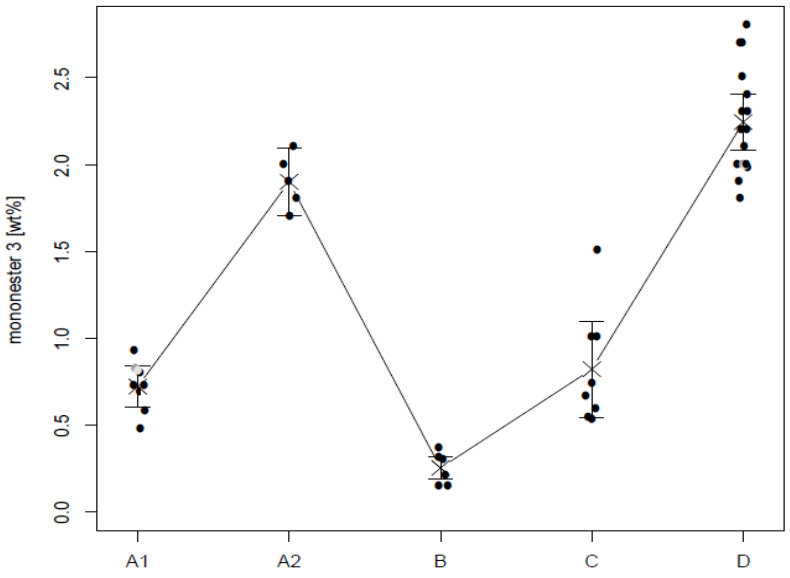


Figure S 18: Stripchart for monoester 3. As the pairwise t-tests show the mean of supplier B is highly significantly different form all the others.

Supplementary

The results of the pairwise t-tests show that none of the variables Content_di, Content_mono2 and Content_mono3 for itself will allow to discriminate between all of the suppliers. However the contents of monoester 2 and monoester 3 are definitely more characteristic for the kind of supplier than the content of AOT. A scatter plot together with Confidence ellipses (see Figure S 19) demonstrates that in the bivariate space spanned by these two variables clustering can be observed. There is however considerable overlap within the pairs A1/C and A2/D.

Discriminant analysis

The section above showed that the content of AOT and especially monoester 2 and 3 are variables potentially helpful for discriminating between different suppliers. In discriminant analysis the discrimination is optimized by calculating suitable discriminant co- ordinates, i.e. linear combinations of the original variables. Three different kinds of discriminant analyses were performed: [5] Linear discriminant analysis based on all three variables (Content_AOT, Content_mono2, Content_mono3), [6] linear discriminant analysis based only on the variables Content_mono2 and Content_mono3 [7] localized version of the latter.

Linear Discriminant Analysis based on three Variables

First a linear discriminant analysis was performed with all of the variables (Content_AOT, Content_mono2, Content_mono3) This requires the R-package MASS [8]. The confidence ellipses were generated with the command ellipsoidPoints within the R-package cluster [9].

```
>  require(MASS)

> z <- lda(Supplier~Content_AOT + Content_mono2 + Content_mono3, na.action="na.omit",

+          prior=c(1,1,1,1,1)/5,     CV=FALSE)

Call:

lda(Supplier ~ Content_AOT + Content_mono2 + Content_mono3, prior = c(1, 1, 1, 1, 1)/5, CV = FALSE,
na.action = "na.omit")

Prior probabilities of groups:        A1   A2   B     C     D

                                      0.2  0.2  0.2   0.2   0.2
```

Supplementary

Group means:

	Content_AOT	Content_mono2	Content_mono3
A1	61.4	1.48	0.72
A2	59.4	2.52	1.90
B	65.9	0.97	0.25
C	59.2	2.75	0.82
D	63.8	3.51	2.24

Coefficients of linear discriminants:

	LD1	LD2	LD3
Content_AOT	-0.069	0.18	-0.35
Content_mono2	0.82	-2.56	-1.10
Content_mono3	3.27	3.05	0.84

Proportion of trace:	LD1	LD2	LD3
	0.77	0.17	0.058

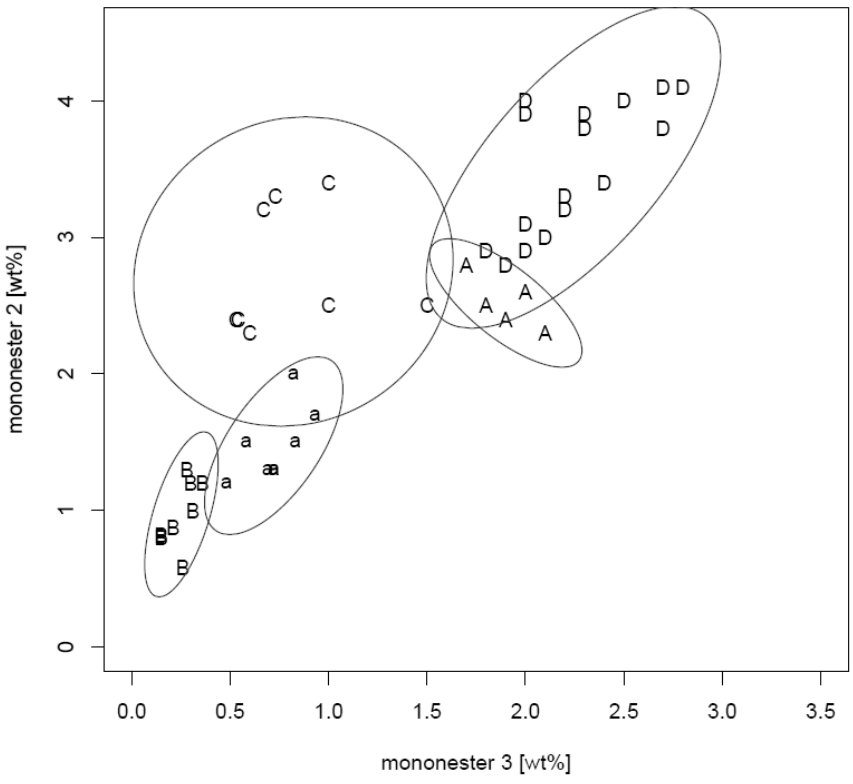


Figure S 19: Confidence ellipses for the variables content of monoester 2 and monoester 3. Supplier "A1" is designated as "a" and supplier "A2" as "A".

Supplementary

The following shows an internal validation, i.e. the classifications when predicting the data with the calculated discriminate model:

```
> p <- predict(z,D)

> Result <- (p$class == Supplier)

> internal.validation <- data.frame(Sample_Name, Real_Supplier=Supplier,

+ Predicted_Supplier=p$class, Result)

> internal.validation
```

Table S 14: Validation of the allocation to the correct supplier cluster of the single supplier samples achieved by linear discriminant analysis

	Sample_Name	Real_Supplier	Predicted_Supplier	Result
1	A1	A1	A1	TRUE
2	A2	A1	A1	TRUE
3	A3	A1	A1	TRUE
4	A4	A1	A1	TRUE
5	A5	A1	A1	TRUE
6	A6	A1	A1	TRUE
7	A7	A1	A1	TRUE
8	A8	A1	A1	TRUE
9	A9	A2	D	FALSE
10	A10	A2	A2	TRUE
11	A11	A2	A2	TRUE
12	A12	A2	A2	TRUE
13	A13	A2	A2	TRUE
14	B1	B	B	TRUE
15	B2	B	B	TRUE
16	B3	B	B	TRUE
17	B4	B	B	TRUE
18	B5	B	A1	FALSE
19	B6	B	B	TRUE
20	B7	B	B	TRUE
21	B8	B	B	TRUE
22	C1	C	C	TRUE
23	C2	C	C	TRUE
24	C3	C	C	TRUE
25	C4	C	A2	FALSE
26	C5	C	C	TRUE
27	C6	C	C	TRUE
28	C7	C	C	TRUE
29	C8	C	C	TRUE
30	D1	D	D	TRUE
31	D2	D	D	TRUE
32	D3	D	D	TRUE

Supplementary

	Sample_Name	Real_Supplier	Predicted_Supplier	Result
33	D4	D	D	TRUE
34	D5	D	D	TRUE
35	D6	D	D	TRUE
36	D7	D	D	TRUE
37	D8	D	D	TRUE
38	D9	D	D	TRUE
39	D10	D	D	TRUE
40	D11	D	D	TRUE
41	D12	D	D	TRUE
42	D13	D	D	TRUE
43	D14	D	A2	FALSE
44	D15	D	D	TRUE
45	D16	D	D	TRUE

> ct <- table(Supplier, p$class)

> ct

Table S 15: Allocation of the samples to the respective supplier achieved by linear discriminant analysis

Supplier	A1	A2	B	C	D
A1	8	0	0	0	0
A2	0	4	0	0	1
B	1	0	7	0	0
C	0	1	0	7	0
D	0	1	0	0	15

> (proportion_of_correct_classifications <- diag(prop.table(ct, 1)))

A1 A2 B C D

1.00 0.80 0.88 0.88 0.94

> (overall_proportion_of_incorrect_classifications <- 1-sum(diag(prop.table(ct))))

[1] 0.089

The overall proportion of incorrect classifications is about 9%.

Further an external validation is performed with the data form AOT product in stored agrochemical formulations. These data are read from the file "data set 2.txt".

> E <- read.table("data_set_2.txt",header=TRUE)

> E

Supplementary

	Content_AOT	Content_mono2	Content_mono3	Supplier	PlotSymbol	Sample_Name
1	59.4	1.5	0.46	A1	a	formulation_1
2	63.8	1.3	0.24	B	B	formulation_2
3	59.7	3.7	1.90	D	D	formulation_3

```
> E$Supplier <- factor(E$Supplier,levels=c("A1","A2","B","C","D"))
> p <- predict(z,newdata=E)
> Result <- (p$class == E$Supplier)
> external.validation <- data.frame(Sample_Name=E$Sample_Name, Real_Supplier=E$Supplier,
+ Predicted_Supplier=p$class, Result)
> external.validation
```

Sample_Name	Real_Supplier	Predicted_Supplier	Result
formulation_1	A1	A1	TRUE
formulation_2	B	B	TRUE
formulation_3	D	D	TRUE

The three classifications are correct.

Linear Discriminant Analysis based on two Variables

From the result of the discriminant analysis above it is seen that 77.4 + 16.8 = 94.2% of the between-group variance is covered by the first two discriminants and that the coefficients of these are mainly determined by the content of monoester 2 and monoester 3.

So in the following a new discriminant analysis is performed which is confined to these two variables.

Supplementary

```
> z <- lda(Supplier ~ Content_mono2 + Content_mono3, na.action="na.omit",
+          prior=c(1,1,1,1,1)/5,     CV=FALSE)
> z
Call:
lda(Supplier ~ Content_mono2 + Content_mono3, prior = c(1, 1, 1, 1, 1)/5, CV = FALSE, na.action = "na.omit")

Prior probabilities of groups:  A1   A2   B    C    D
                                0.2  0.2  0.2  0.2  0.2

Group means:
   Content_mono2 Content_mono3
A1          1.48          0.72
A2          2.52          1.90
B           0.97          0.25
C           2.75          0.82
D           3.51          2.24

Coefficients of linear discriminants:
                    LD1         LD2
Content_mono2  0.75          2.79
Content_mono3  3.36          -3.06

Proportion of trace:  LD1          LD2
                      0.84         0.16

>  p  <-  predict(z,D)
> Result <- (p$class == Supplier)
> internal.validation <- data.frame(Sample_Name, Real_Supplier=Supplier,
+   Predicted_Supplier=p$class,   Result)
>    internal.validation
```

Table S 16: Validation of the allocation to the correct supplier cluster of the single supplier samples achieved by linear discriminant analysis with two variables (Content_mono2 and Content_mono3)

	Sample_Name	Real_Supplier	Predicted_Supplier	Result
1	A1	A1	A1	TRUE
2	A2	A1	A1	TRUE
3	A3	A1	A1	TRUE
4	A4	A1	B	FALSE
5	A5	A1	A1	TRUE
6	A6	A1	A1	TRUE
7	A7	A1	A1	TRUE
8	A8	A1	A1	TRUE
9	A9	A2	A2	TRUE
10	A10	A2	A2	TRUE
11	A11	A2	A2	TRUE
12	A12	A2	A2	TRUE
13	A13	A2	A2	TRUE
14	B1	B	B	TRUE
15	B2	B	B	TRUE
16	B3	B	B	TRUE
17	B4	B	B	TRUE
18	B5	B	B	TRUE
19	B6	B	B	TRUE
20	B7	B	B	TRUE
21	B8	B	B	TRUE
22	C1	C	C	TRUE
23	C2	C	C	TRUE
24	C3	C	C	TRUE
25	C4	C	A2	FALSE
26	C5	C	C	TRUE
27	C6	C	C	TRUE
28	C7	C	C	TRUE
29	C8	C	C	TRUE
30	D1	D	D	TRUE
31	D2	D	D	TRUE
32	D3	D	D	TRUE
33	D4	D	D	TRUE
34	D5	D	D	TRUE
35	D6	D	D	TRUE
36	D7	D	D	TRUE
37	D8	D	D	TRUE
38	D9	D	D	TRUE
39	D10	D	D	TRUE
40	D11	D	D	TRUE
41	D12	D	A2	FALSE
42	D13	D	A2	FALSE
43	D14	D	A2	FALSE
44	D15	D	A2	FALSE
45	D16	D	D	TRUE

Supplementary

```
> ct <- table(Supplier, p$class)

> ct
```

Table S 17: Allocation of the samples to the respective supplier achieved by linear discriminant analysis with two variables (Content_mono2 and Content_mono3)

Supplier	A1	A2	B	C	D
A1	7	0	1	0	0
A2	0	5	0	0	0
B	0	0	8	0	0
C	0	1	0	7	0
D	0	4	0	0	12

```
> (proportion_of_correct_classifications <- diag(prop.table(ct, 1)))

A1   A2   B   C   D

0.88   1.00   1.00   0.88   0.75

> (overall_proportion_of_incorrect_classifications <- 1-sum(diag(prop.table(ct))))

[1] 0.1333333
```

The overall proportion of incorrect classifications has now increased to about 13%. The classification for the data from the stored formulations (Table S 13) is again correct:

```
> p <- predict(z,newdata=E)

> Result <- (p$class == E$Supplier)

> external.validation <- data.frame(Sample_Name=E$Sample_Name, Real_Supplier=E$Supplier,

+ Predicted_Supplier=p$class, Result)

> external.validation

Sample_Name   Real_Supplier   Predicted_Supplier   Result

formulation_1   A1   A1   TRUE

formulation_2   B   B   TRUE

formulation_3   D   D   TRUE
```

Supplementary

Figure S 20 shows the data in Table S 12 and Table S 13 on the two discriminant axes based on the variables Content_mono2 and Content_mono3.

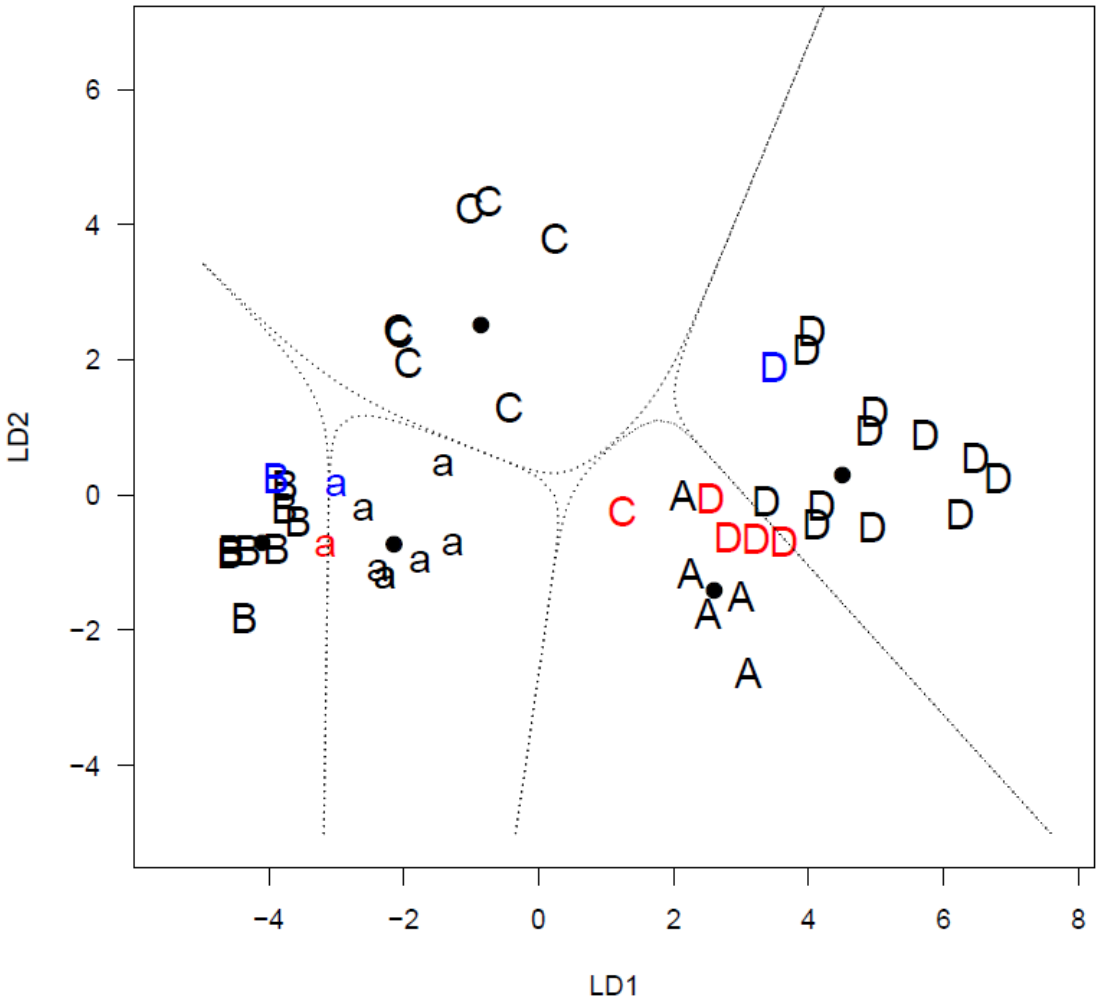


Figure S 20: Data from AOT product of different suppliers (Table S 12) on the two discriminant axes based on the variables Content_mono2 and Content_mono3. Supplier "A1" is designated as "a" and supplier "A2" as "A". Red character plot symbols show misclassifications within the data in Table S 12 and blue ones refer to the AOT product of the stored formulations (Table S 13).

Supplementary

Localized Linear Discriminant Analysis based on two Variables

An improvement of the discrimination with the two variables Content_mono2 and Con- tent_mono3 can be achieved by applying a localized version of linear discriminant analysis. For this the R-package klaR [7] is required.

```
> require(klaR)

> z <-loclda(Supplier ~ Content_mono2 + Content_mono3,method="lda")

> z

Call:

loclda(formula = Supplier ~ Content_mono2 + Content_mono3, method = "lda")

Weighting function: function (x) 1/exp(x)

<environment: 0x03155d5c>

Number of next neighbours that will be used for prediction: [1] 45

Usage of weighted a priori probabilities: [1] TRUE

> p <- predict(z,D)

> Result <- (p$class == Supplier)

> internal.validation <- data.frame(Sample_Name, Real_Supplier=Supplier,

+ Predicted_Supplier=p$class, Result)

> internal.validation
```

Table S 18: Validation of the allocation to the correct supplier cluster of the single supplier samples achieved by localized linear discriminant analysis with two variables (Content_mono2 and Content_mono3)

	Sample_Name	Real_Supplier	Predicted_Supplier	Result
1	A1	A1	A1	TRUE
2	A2	A1	A1	TRUE
3	A3	A1	A1	TRUE
4	A4	A1	B	FALSE
5	A5	A1	A1	TRUE
6	A6	A1	A1	TRUE

Supplementary

	Sample_Name	Real_Supplier	Predicted_Supplier	Result
7	A7	A1	A1	TRUE
8	A8	A1	A1	TRUE
9	A9	A2	A2	TRUE
10	A10	A2	A2	TRUE
11	A11	A2	A2	TRUE
12	A12	A2	A2	TRUE
13	A13	A2	A2	TRUE
14	B1	B	B	TRUE
15	B2	B	B	TRUE
16	B3	B	B	TRUE
17	B4	B	B	TRUE
18	B5	B	B	TRUE
19	B6	B	B	TRUE
20	B7	B	B	TRUE
21	B8	B	B	TRUE
22	C1	C	C	TRUE
23	C2	C	C	TRUE
24	C3	C	C	TRUE
25	C4	C	A2	FALSE
26	C5	C	C	TRUE
27	C6	C	C	TRUE
28	C7	C	C	TRUE
29	C8	C	C	TRUE
30	D1	D	D	TRUE
31	D2	D	D	TRUE
32	D3	D	D	TRUE
33	D4	D	D	TRUE
34	D5	D	D	TRUE
35	D6	D	D	TRUE
36	D7	D	D	TRUE
37	D8	D	D	TRUE
38	D9	D	D	TRUE
39	D10	D	D	TRUE
40	D11	D	D	TRUE
41	D12	D	D	TRUE
42	D13	D	A2	FALSE
43	D14	D	D	TRUE
44	D15	D	D	TRUE
45	D16	D	D	TRUE

```
> ct <- table(Supplier, p$class)

> ct
```

Table S 19: Allocation of the samples to the respective supplier achieved by localized linear discriminant analysis with two variables (Content_mono2 and Content_mono3)

Supplier	A1	A2	B	C	D
A1	7	0	1	0	0
A2	0	5	0	0	0
B	0	0	8	0	0
C	0	1	0	7	0
D	0	1	0	0	15

> (proportion_of_correct_classifications <- diag(prop.table(ct, 1)))

A1 A2 B C D

0.88 1.0 1.00 0.88 0.94

> (overall_proportion_of_incorrect_classifications <- 1-sum(diag(prop.table(ct))))

[1] 0.067

The overall proportion of incorrect classifications has now decreased to about 7%. The classification for the data from the stored formulations Table S 13) is again correct:

> p <- predict(z,newdata=E)

> Result <- (p$class == E$Supplier)

> external.validation <- data.frame(Sample_Name=E$Sample_Name, Real_Supplier=E$Supplier,

+ Predicted_Supplier=p$class, Result)

> external.validation

Sample_Name	Real_Supplier	Predicted_Supplier	Result
formulation_1	A1	A1	TRUE
formulation_2	B	B	TRUE
formulation_3	D	D	TRUE

Figure S 21 shows the partition plot based on the localized discriminant analysis. This plot was generated with the command partimat within the R-package klaR.

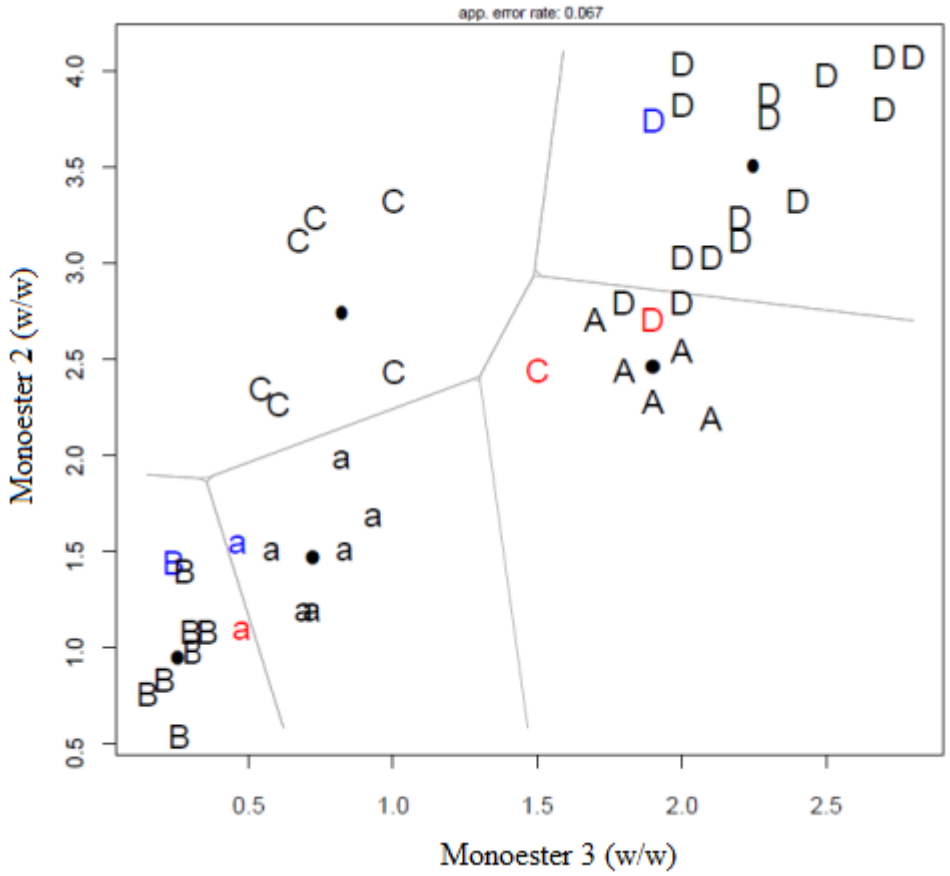


Figure S 21: Partition plot using the variables Content_mono2 and Content_mono3. Supplier "A1" is designated as "a" and supplier "A2" as "A". Red character plot symbols show misclassifications within the data in Table S 12 and blue ones refer to the AOT product in Table S 13.

6.3.8 Reference List

1. Porter MR (1994) Handbook of Surfactants. vol. 2 Chapman & Hall, Glasgow.
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8. Venables WN, Ripley BD (2002) Modern Applied Statistics with S. fourth edition, Springer, New York, ISBN 0-387-95457.
9. Maechler M, Rousseeuw P, Struyf A, Hubert M, Hornik K. (2012) Cluster: Cluster Analysis Basics and Extensions. R-Package.

6.4 Analytical Characterization and Comparison of Tristyrylphenol Ethoxylates used in Agrochemical Formulation

6.4.1 Sample for Testing on Mass Calibration of ToF-MS

The retention times and exact masses for the compounds in the test sample for checking mass calibration of the used ToF-MS are given in Table S 7.

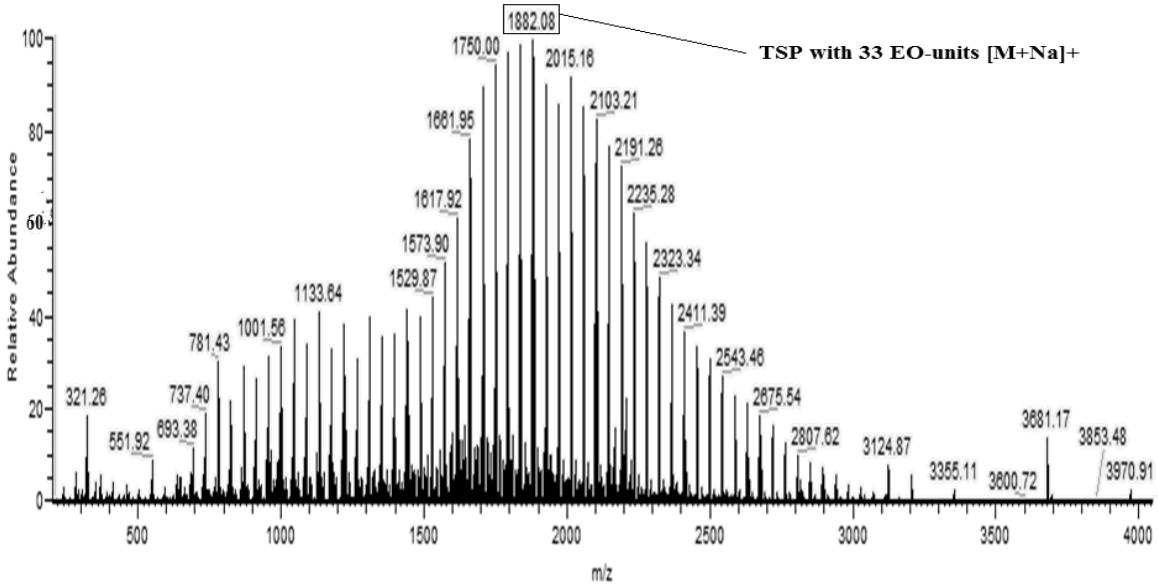
Table S 20: Retention time and exact masses for compounds in the test sample for checking on mass calibration

Compound	t_N [min]	Exact mass [amu]
Imidacloprid	2.0	254.0450
Thiacloprid	2.5	252.0236
Tebuconazole (1.Isomer)	4.3	307.1451
Triadimenol	4.6	295.1088
Tebuconazole (2.Isomer)	4.9	307.1451
Distyrylethoxylate-5-EO	5.8	522,2981
Distyrylethoxylate-6-EO	5.8	566,3244
Distyrylethoxylate-7-EO	5.8	610,3506
Distyrylethoxylate-8-EO	5.8	654,3768
Distyrylethoxylate-9-EO	5.8	698,4030
Distyrylethoxylate-10-EO	5.8	742,4292
Distyrylethoxylate-11-EO	5.8	786,4554
Distyrylethoxylate-12-EO	5.8	830,4816
Distyrylethoxylate-13-EO	5.8	874,5079
Distyrylethoxylate-14-EO	5.8	918,5341
Distyrylethoxylate-15-EO	5.8	962,5603
Distyrylethoxylate-16-EO	5.8	1006,5865
Distyrylethoxylate-17-EO	5.9	1050,6127
Distyrylethoxylate-18-EO	5.9	1094,6389
Distyrylethoxylate-19-EO	5.9	1138,6651
Distyrylethoxylate-20-EO	5.9	1182,6914
Distyrylethoxylate-21-EO	5.9	1226,7176
Distyrylethoxylate-22-EO	5.9	1270,7438
Distyrylethoxylate-23-EO	5.9	1314,7700
Distyrylethoxylate-24-EO	5.9	1358,7962
Distyrylethoxylate-25-EO	5.9	1402,8224
Distyrylethoxylate-26-EO	5.9	1446,8486
Distyrylethoxylate-27-EO	5.9	1490,8749
Distyrylethoxylate-28-EO	5.9	1534,9011
Distyrylethoxylate-29-EO	5.9	1578,9273
Distyrylethoxylate-30-EO	5.9	1622,9535
Nonylphenolethoxylate-5-EO	6.6	440,3138
Nonylphenolethoxylate-6-EO	6.3	484,3400
Nonylphenolethoxylate-7-EO	6.2	528,3662
Nonylphenolethoxylate-8-EO	6.2	572,3924
Nonylphenolethoxylate-9-EO	6.2	616,4186
Nonylphenolethoxylate-10-EO	6.2	660,4449
Nonylphenolethoxylate-11-EO	6.2	704,4711
Nonylphenolethoxylate-12-EO	6.2	748,4973
Nonylphenolethoxylate-13-EO	6.2	792,5235
Nonylphenolethoxylate-14-EO	6.2	836,5497
Nonylphenolethoxylate-15-EO	6.2	880,5759
Nonylphenolethoxylate-16-EO	6.2	924,6022

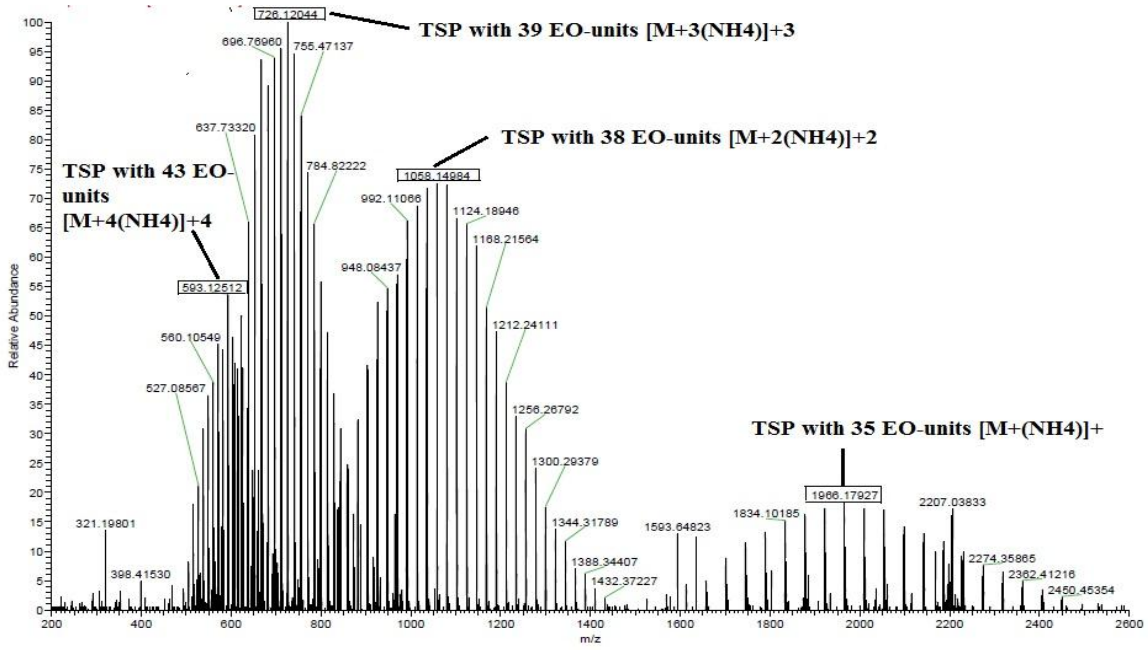
Compound	t_N [min]	Exact mass [amu]
Nonylphenolethoxylate-17-EO	6.2	968,6284
Nonylphenolethoxylate-18-EO	6.2	1012,6546
Nonylphenolethoxylate-19-EO	6.2	1056,6808
Nonylphenolethoxylate-20-EO	6.2	1100,7070
Nonylphenolethoxylate-21-EO	6.2	1144,7332
Nonylphenolethoxylate-22-EO	6.2	1188,7594
Nonylphenolethoxylate-23-EO	6.2	1232,7857
Nonylphenolethoxylate-24-EO	6.2	1276,8119
Nonylphenolethoxylate-25-EO	6.2	1320,8381
Nonylphenolethoxylate-26-EO	5.9	1364,8643
Nonylphenolethoxylate-27-EO	5.9	1408,8905
Nonylphenolethoxylate-28-EO	5.9	1452,9167
Nonylphenolethoxylate-29-EO	5.9	1496,9429
Nonylphenolethoxylate-30-EO	5.9	1540,9692
Tristyrylethoxylate-5-EO	5.9	626,3607
Tristyrylethoxylate-6-EO	5.9	670,38695
Tristyrylethoxylate-7-EO	5.9	714,4132
Tristyrylethoxylate-8-EO	6.5	758,4394
Tristyrylethoxylate-9-EO	5.9	802,4656
Tristyrylethoxylate-10-EO	5.9	846,4918
Tristyrylethoxylate-11-EO	6.0	890,5180
Tristyrylethoxylate-12-EO	6.0	934,5442
Tristyrylethoxylate-13-EO	6.0	978,5705
Tristyrylethoxylate-14-EO	6.0	1022,5967
Tristyrylethoxylate-15-EO	6.0	1066,6229
Tristyrylethoxylate-16-EO	6.0	1110,6491
Tristyrylethoxylate-17-EO	6.0	1154,6753
Tristyrylethoxylate-18-EO	6.0	1198,7015
Tristyrylethoxylate-19-EO	6.0	1242,7278
Tristyrylethoxylate-20-EO	6.0	1286,7540
Tristyrylethoxylate-21-EO	5.9	1330,7802
Tristyrylethoxylate-22-EO	5.9	1374,8064
Tristyrylethoxylate-23-EO	5.9	1418,8326
Tristyrylethoxylate-24-EO	5.9	1462,8588
Tristyrylethoxylate-25-EO	5.9	1506,8850
Tristyrylethoxylate-26-EO	5.8	1550,9113
Tristyrylethoxylate-27-EO	5.8	1594,9375
Tristyrylethoxylate-28-EO	5.8	1638,9637
Tristyrylethoxylate-29-EO	5.8	1682,9899
Tristyrylethoxylate-30-EO	5.8	1727,0161

6.4.2 Comparison of the Ionization Performance of APPI and ESI for the Analysis of TSP-40-ethoxylates

The ionization performance of APPI and ESI was compared for the analysis of TSP-40-ethoxylates. For comparison the mass spectra of TSP-ethoxylates were taken for each ionization technique. Results for APPI are displayed in Figure S 22 (a) and for ESI in Figure S 22 (b).



(a)



(b)

Figure S 22: Ionization behavior of TSP-40-ethoxylates ionized by APPI (a) and ESI (b). In each case the mass spectrum over the peak of TSP-ethoxylates is displayed. For each experiment the same elution conditions with water and methanol as mobile phase, plus 5 mM ammonium formiate, were chosen. The mass spectrometer used for this experiments was a Thermo Q-exactive.

For APPI a complex spectrum was obtained with a wide variety of signals, which can only partly be assigned to TSP-ethoxylates like the signal of TSP ethoxylate with 33 EO units. Given that the distribution of TSP-40-ethoxylates has its center on TSP with 33 EO units and not 40 EO units and taking into account the scatter of smaller peaks underlying the distribution it may be assumed that APPI is limited to ionization of entities with shorter EO chains. The ionization process, however, of entities with longer chain length leads to some sort of degradation shifting the center of distribution of ethoxylates and giving a wide variety of mass peaks, being

fragments of this process. As the spectrum is very hard to interpret APPI is less favorable for characterization of TSP ethoxylates with longer EO chain lengths.

By comparison, the spectrum obtained by ESI shows only single to fourfold-charged mol peaks of TSP ethoxylates without apparent degradation products or fragments. Analogous to the spectrum obtained for TSP-16-ethoxylates in the manuscript in Figure 3 (b) the higher charged entities are dominant for longer EO chain lengths. The spectrum obtained by ESI was easier to interpret and without apparent degradation products and so ESI was taken as coupling to the mass spectrometer in this work.

6.4.3 Determination of the Limit of Quantification

The limits of quantification (LOQ) for both analytes hexanophenone and TSP with 16 EO units have been defined as a signal-to-noise ratio of at least 20:1 to ensure acceptable quantification results. In the following the respective chromatograms at LOQ level are given for TSP with 16 EO units (a) and hexanophenone (b) in Figure S 23 and the linearity plots for TSP with 16 EO units (a) and hexanophenone (b) in Figure S 24.

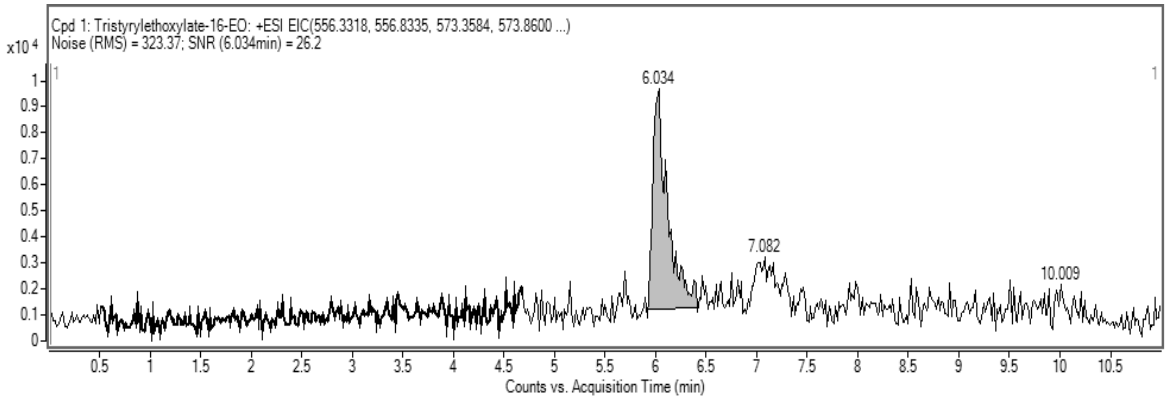
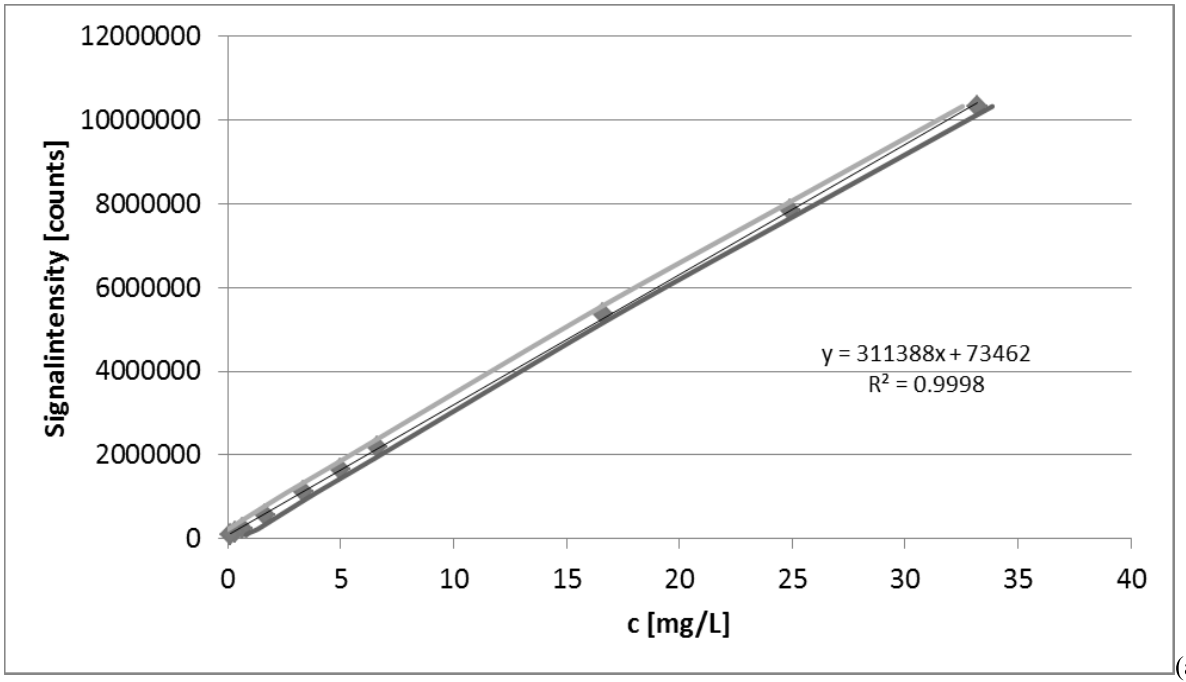
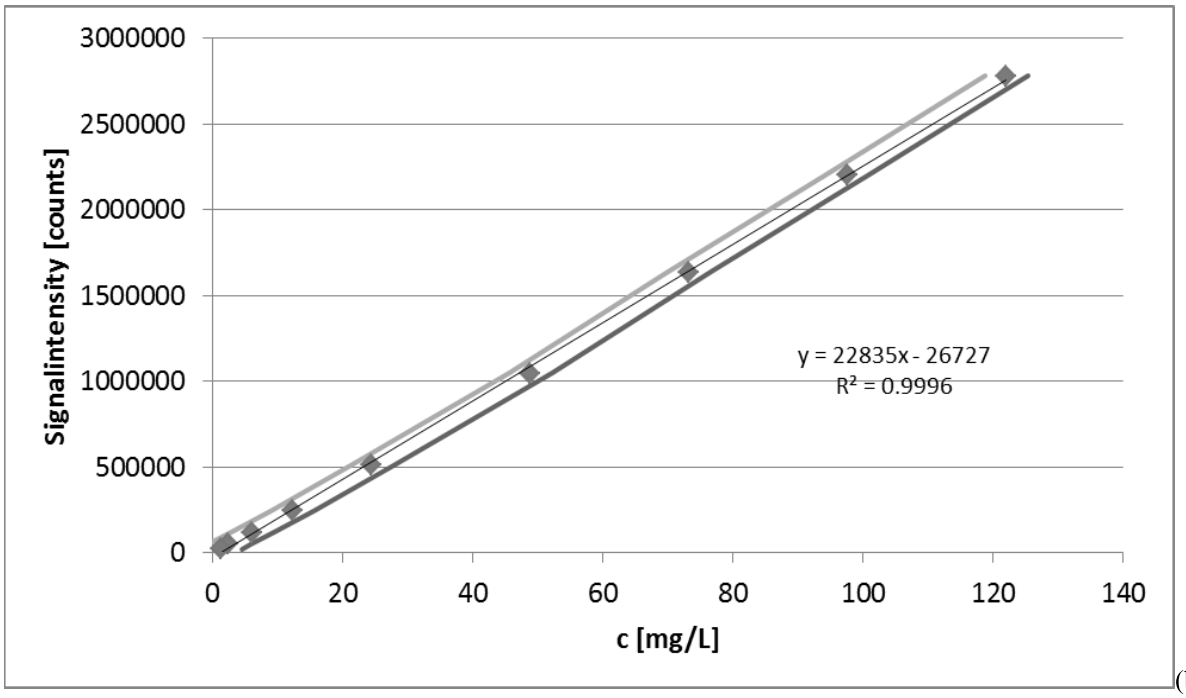


Figure S 23: Chromatograms for determination of the signal-to-noise ratio at the defined LOQ level for TSP with 16 EO units (a) and hexanophenone (b). The LOQ was defined as a signal-to-noise ratio of at least 20:1, which has been achieved for both analytes.



(a)



(b)

Figure S 24: Linear ranges for TSP with 16 EO units (a) and hexanophenone (b) including the bands of prediction indicated with the upper and the lower line

6.4.4 Comparison of TSP-16-ethoxylates of different Suppliers and Qualities

In Table S 21 the compounds in the different arrays determined by the hierarchical clustering (HCA) performed on the combined supplier data set are given.

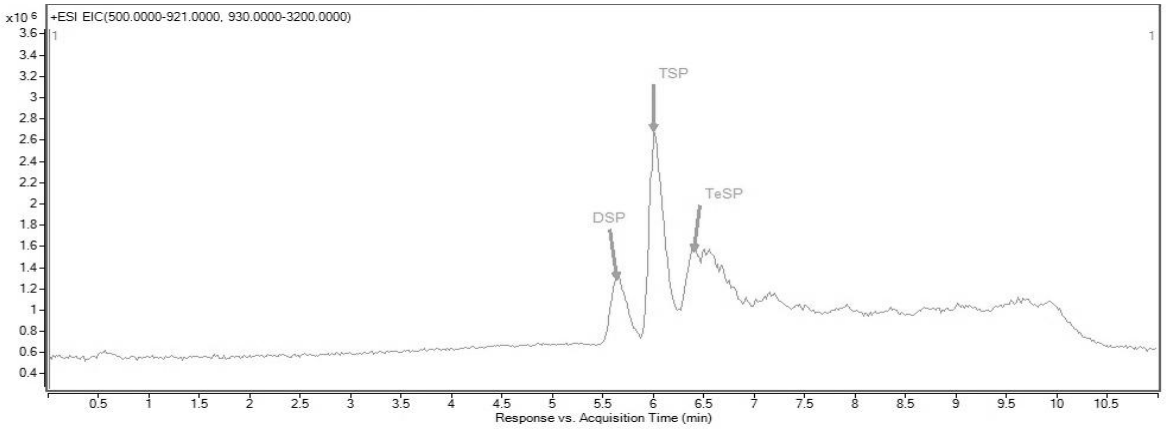
Table S 21: Compounds used for the combined hierarchical clustering listed together with the corresponding arrays as defined in Figure 26. The compounds are sorted according to the order obtained by the hierarchical clustering of the compounds.

Array	Compound	Array	Compound
1	Distyrylphenolprop-ethoxylate-16-EO 8-PO	5	Distyrylphenolethoxylate-15-EO
1	Distyrylphenolprop-ethoxylate-13-EO 8-PO	5	Distyrylphenolethoxylate-18-EO
1	Distyrylphenolprop-ethoxylate-11-EO 8-PO	5	Tristyrylphenolethoxylate-22-EO
1	Distyrylphenolprop-ethoxylate-12-EO 8-PO	5	Tristyrylphenolethoxylate-21-EO
1	Distyrylphenolprop-ethoxylate-14-EO 8-PO	5	Tristyrylphenolethoxylate-11-EO
1	Distyrylphenolprop-ethoxylate-8-EO 8-PO	5	Tristyrylphenolethoxylate-24-EO
1	Distyrylphenolprop-ethoxylate-7-EO 8-PO	5	Tristyrylphenolethoxylate-10-EO
1	Distyrylphenolprop-ethoxylate-9-EO 8-PO	5	Distyrylphenolethoxylate-21-EO
1	Distyrylphenolprop-ethoxylate-5-EO 8-PO	5	Distyrylphenolethoxylate-12-EO
1	Distyrylphenolprop-ethoxylate-6-EO 8-PO	5	Distyrylphenolethoxylate-20-EO
1	Distyrylphenolprop-ethoxylate-10-EO 8-PO	5	Tristyrylphenolethoxylate-9-EO
1	Monostyrylphenolprop-ethoxylate-11-EO 8-PO	5	Tristyrylphenolethoxylate-23-EO
1	Monostyrylphenolprop-ethoxylate-8-EO 8-PO	5	Tristyrylphenolethoxylate-8-EO
1	Monostyrylphenolprop-ethoxylate-9-EO 8-PO	5	Hexanophenone (Internal Standard)
1	Monostyrylphenolprop-ethoxylate-5-EO 8-PO	5	Distyrylphenolethoxylate-22-EO
1	Monostyrylphenolprop-ethoxylate-12-EO 8-PO	5	Distyrylphenolethoxylate-11-EO
1	Monostyrylphenolprop-ethoxylate-7-EO 8-PO	5	Distyrylphenolethoxylate-10-EO
1	Monostyrylphenolprop-ethoxylate-10-EO 8-PO	5	Tristyrylphenolethoxylate-26-EO
1	Monostyrylphenolprop-ethoxylate-6-EO 8-PO	5	Tristyrylphenolethoxylate-16-EO
1	Distyrylphenolprop-ethoxylate-15-EO 8-PO	5	Tristyrylphenolethoxylate-15-EO
1	Distyrylphenolprop-ethoxylate-18-EO 8-PO	5	Tristyrylphenolethoxylate-17-EO
2	Tristyrylphenolethoxylate-31-EO	5	Tristyrylphenolethoxylate-18-EO
2	Distyrylphenolprop-ethoxylate-19-EO 8-PO	5	Tristyrylphenolethoxylate-14-EO
2	Distyrylphenolprop-ethoxylate-12-EO 8-PO	5	Tristyrylphenolethoxylate-19-EO
2	Tetrastyrylphenolethoxylate-25-EO	5	Tristyrylphenolethoxylate-13-EO
2	Monostyrylphenolprop-ethoxylate-13-EO 8-PO	5	Tristyrylphenolethoxylate-20-EO
2	Tetratstyrylphenolethoxylate-5-EO	5	Tristyrylphenolethoxylate-12-EO
2	Distyrylphenolprop-ethoxylate-17-EO 8-PO	5	Tristyrylphenolethoxylate-28-EO
2	Monostyrylphenolethoxylate-24-EO	5	Tetrastyrylphenolethoxylate-14-EO
2	Tetrastyrylphenolethoxylate-6-EO	5	Tetrastyrylphenolethoxylate-15-EO
2	Tetrastyrylphenolethoxylate-24-EO	5	Distyrylphenolethoxylate-24-EO
2	Distyrylphenolethoxylate-29-EO	5	Tetrastyrylphenolethoxylate-16-EO
3	Monostyrylphenolethoxylate-18-EO	5	Tristyrylphenolethoxylate-6-EO
3	Monostyrylphenolethoxylate-19-EO	5	Distyrylphenolethoxylate-7-EO
3	Monostyrylphenolethoxylate-16-EO	5	Distyrylphenolethoxylate-8-EO

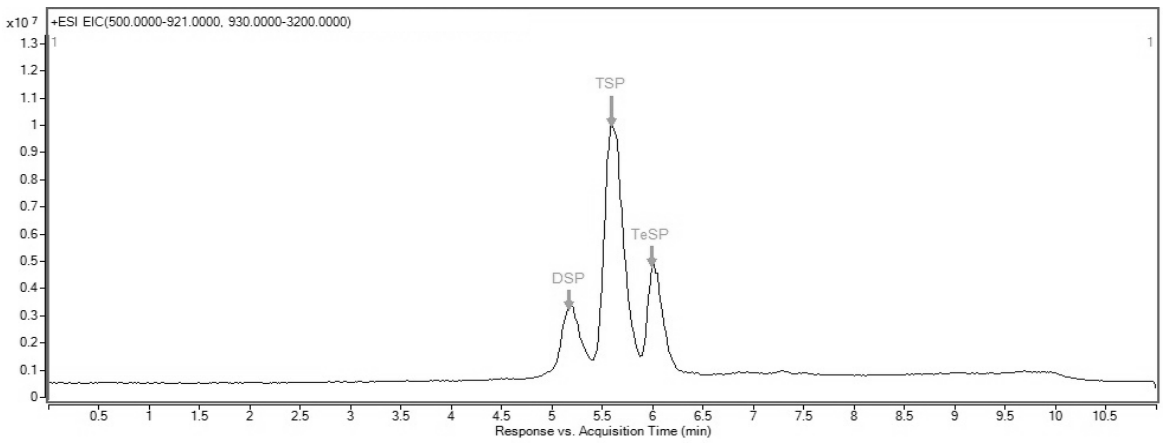
Array	Compound	Array	Compound
3	Monostyrylphenolethoxylate-17-EO	5	Tristyrylphenolethoxylate-7-EO
3	Monostyrylphenolethoxylate-15-EO	5	Distyrylphenolethoxylate-9-EO
3	Monostyrylphenolethoxylate-21-EO	5	Tristyrylphenolethoxylate-25-EO
3	Monostyrylphenolethoxylate-13-EO	5	Distyrylphenolethoxylate-23-EO
3	Monostyrylphenolethoxylate-14-EO	5	Tristyrylphenolethoxylate-5-EO
3	Monostyrylphenolethoxylate-12-EO	5	Tetrastyrylphenolethoxylate-18-EO
3	Monostyrylphenolethoxylate-20-EO	5	Tetrastyrylphenolethoxylate-13-EO
3	Monostyrylphenolethoxylate-11-EO	5	Tetrastyrylphenolethoxylate-12-EO
3	Monostyrylphenolethoxylate-23-EO	5	Tetrastyrylphenolethoxylate-17-EO
3	Monostyrylphenolethoxylate-22-EO	5	Distyrylphenolethoxylate-27-EO
4	Tristyrylphenolethoxylate-29-EO	5	Distyrylphenolethoxylate-5-EO
4	Tetrastyrylphenolethoxylate-21-EO	5	Distyrylphenolethoxylate-6-EO
4	Tetrastyrylphenolethoxylate-23-EO	5	Distyrylphenolethoxylate-26-EO
4	Tetrastyrylphenolethoxylate-8-EO	5	Tetrastyrylphenolethoxylate-22-EO
4	Tetrastyrylphenolethoxylate-7-EO	5	Tetrastyrylphenolethoxylate-20-EO
4	Tetrastyrylphenolethoxylate-9-EO	5	Tetrastyrylphenolethoxylate-11-EO
4	Tristyrylphenolethoxylate-30-EO	5	Tetrastyrylphenolethoxylate-19-EO
5	Distyrylphenolethoxylate-13-EO	5	Tetrastyrylphenolethoxylate-10-EO
5	Distyrylphenolethoxylate-19-EO	5	Tristyrylphenolethoxylate-27-EO
5	Distyrylphenolethoxylate-14-EO	5	Distyrylphenolethoxylate-28-EO
5	Distyrylphenolethoxylate-17-EO	5	Distyrylphenolethoxylate-25-EO
5	Distyrylphenolethoxylate-16-EO		

6.4.5 Example for Interference on Analysis of TSP-16-ethoxylates in Agrochemical Formulations

The identification of the different suppliers in an agrochemical formulation can be interfered by end group sulfated or phosphated TSP-ethoxylates, if they are contained in the agrochemical formulation. The chromatograms of commercially available TSP-16-ethoxylates terminal phosphated (a) and sulfated (b) are shown in Figure S 25 obtained in the positive ionization mode with the identified entities of DSP-, TSP- and TeSP-ethoxylates.



(a)



(b)

Figure S 25: Extracted ion chromatograms obtained in the positive ionization mode of terminal phosphated (a) and sulfated (b) commercially available TSP-16-ethoxylates. Indicated are the identified entities of DSP-, TSP and TeSP-ethoxylates.

As shown there are entities of DSP-, TSP- and TeSP-ethoxylates detectable in commercially available terminal sulfated and phosphated TSP-16-ethoxylates in the chosen ionization mode. These entities of DSP-, TSP- and TeSP-ethoxylates can be explained by incomplete phosphating or sulfating of the educt TSP-16-ethoxylates which was not removed after the reaction from the final commercially product. Possible interferences of these entities on the identification of the different suppliers of TSP-16-ethoxylates in the matrix of the model agrochemical formulations were investigated next. TSP-16-ethoxylates of supplier A, B2 and C and terminal sulfated TSP-16-ethoxylates were mixed in the model agrochemical as shown in Table S 22.

Table S 22: Table of composition of the model agrochemical formulation containing terminal sulfated TSP-16-ethoxylates alongside with TSP-16-ethoxylates

Raw material	**Content [%] (w/w)**
Active ingredient	23.0
TSP-16-ethoxylates	2.5
TSP-16-ethoxylates, sulfated	2.5
Dispersing agent (non-ionic)	10.0
Emulsifier 1 (non-ionic, functionalized PEG)	15.0
Emulsifier 2 (non-ionic, functionalized PPG-PEG-co-polymer)	9.0
Hydrophobically modified Clay	0.1
Acid	0.4
Solvent	37.5

Supplementary

These formulation samples were subjected to the analysis and multivariate data analysis techniques developed and used in this work, with the results of the principle component analysis (PCA) shown in Figure S 26.

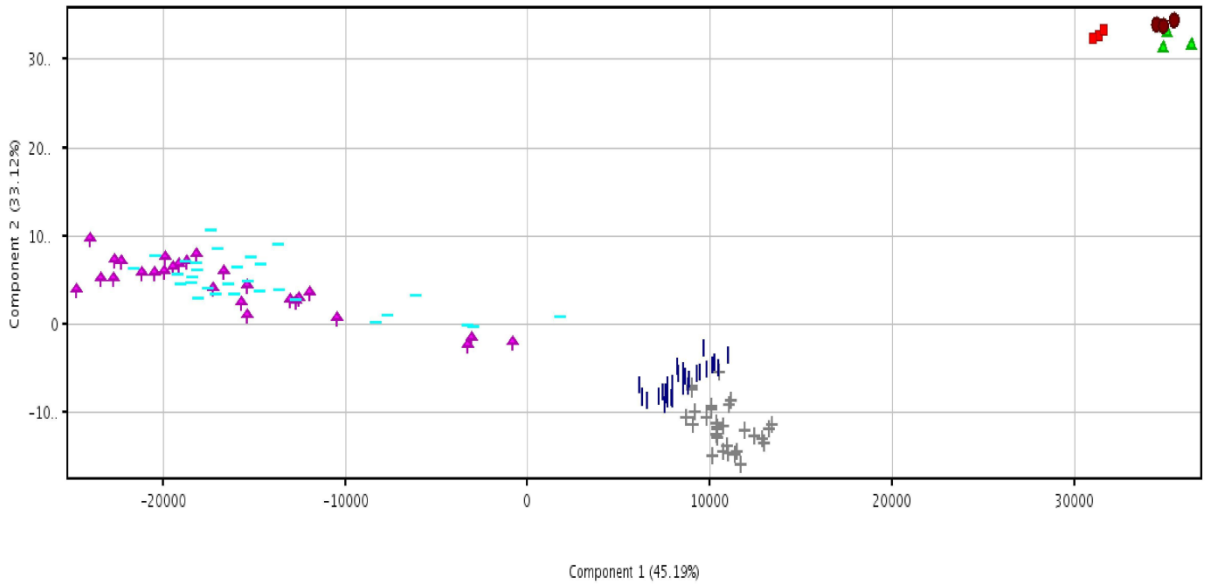


Figure S 26: Principle component analysis of the data sets from supplier A (Cross), B1 (Arrow), B2 (Horizontal Bar) and C (Vertical bar) together with the data of the formulation samples containing TSP-16-ethoxylates of supplier A (Square), B2 (Circle) and C (Triangle). For the PCA the whole data set was taken including the 3 repetition analysis each production batch and formulation sample.

As shown the entities of DSP-, TSP- and TeSP-ethoxylates contained in end group sulfated TSP-16-ethoxylates interfere with the developed method. As formulations using a combination of TSP-16-ethoxylates and another TSP-ethoxylates derivate are not widely spread this potential interference can be accepted. Nevertheless, further investigations should test the possibility for a correction of the observed interferences.

6.4.6 Exact Masses for Data Extraction in TSP-16-ethoxylate Samples

In the following table the exact masses used for compound finding the data extraction algorithms for the analysis of TSP-16-ethoxylate samples is displayed.

Table S 23: Exact masses used for data extraction in TSP-16-ethoxylate samples

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
Hexanophenon	177.1274	Distyrylphenol-prop-ethoxylate-7-EO-4-PO	842.5180284
Monostyrylphenolethoxylate-5-EO	418.2355	Distyrylphenol-prop-ethoxylate-8-EO-4-PO	886.5442432
Monostyrylphenolethoxylate-6-EO	462.2618	Distyrylphenol-prop-ethoxylate-9-EO-4-PO	930.5705
Monostyrylphenolethoxylate-7-EO	506.288	Distyrylphenol-prop-ethoxylate-10-EO-4-PO	974.5967
Monostyrylphenolethoxylate-8-EO	550.3142	Distyrylphenol-prop-ethoxylate-11-EO-4-PO	1018.623
Monostyrylphenolethoxylate-9-EO	594.3404	Distyrylphenol-prop-ethoxylate-12-EO-4-PO	1062.649
Monostyrylphenolethoxylate-10-EO	638.3666	Distyrylphenol-prop-ethoxylate-13-EO-4-PO	1106.675
Monostyrylphenolethoxylate-11-EO	682.3928	Distyrylphenol-prop-ethoxylate-14-EO-4-PO	1150.702
Monostyrylphenolethoxylate-12-EO	726.419	Distyrylphenol-prop-ethoxylate-15-EO-4-PO	1194.728
Monostyrylphenolethoxylate-13-EO	770.4453	Distyrylphenol-prop-ethoxylate-16-EO-4-PO	1238.754
Monostyrylphenolethoxylate-14-EO	814.4715	Distyrylphenol-prop-ethoxylate-17-EO-4-PO	1282.78
Monostyrylphenolethoxylate-15-EO	858.4977	Distyrylphenol-prop-ethoxylate-18-EO-4-PO	1326.806
Monostyrylphenolethoxylate-16-EO	902.5239	Distyrylphenol-prop-ethoxylate-19-EO-4-PO	1370.833
Monostyrylphenolethoxylate-17-EO	946.5501	Distyrylphenol-prop-ethoxylate-20-EO-4-PO	1414.859
Monostyrylphenolethoxylate-18-EO	990.5763	Distyrylphenol-prop-ethoxylate-21-EO-4-PO	1458.885
Monostyrylphenolethoxylate-19-EO	1034.6025	Distyrylphenol-prop-ethoxylate-22-EO-4-PO	1502.911
Monostyrylphenolethoxylate-20-EO	1078.6288	Distyrylphenol-prop-ethoxylate-23-EO-4-PO	1546.937
Monostyrylphenolethoxylate-21-EO	1122.655	Distyrylphenol-prop-ethoxylate-24-EO-4-PO	1590.964
Monostyrylphenolethoxylate-22-EO	1166.6812	Distyrylphenol-prop-ethoxylate-25-EO-4-PO	1634.99
Monostyrylphenolethoxylate-23-EO	1210.7074	Distyrylphenol-prop-ethoxylate-26-EO-4-PO	1679.016
Monostyrylphenolethoxylate-24-EO	1254.7336	Distyrylphenol-prop-ethoxylate-27-EO-4-PO	1723.042
Monostyrylphenolethoxylate-25-EO	1298.7598	Distyrylphenol-prop-ethoxylate-28-EO-4-PO	1767.069
Monostyrylphenolethoxylate-26-EO	1342.786	Distyrylphenol-prop-ethoxylate-29-EO-4-PO	1811.095
Monostyrylphenolethoxylate-27-EO	1386.8123	Distyrylphenol-prop-ethoxylate-30-EO-4-PO	1855.121
Monostyrylphenolethoxylate-28-EO	1430.8385	Distyrylphenol-prop-ethoxylate-31-EO-4-PO	1899.147
Monostyrylphenolethoxylate-29-EO	1474.8647	Distyrylphenol-prop-ethoxylate-32-EO-4-PO	1943.173
Monostyrylphenolethoxylate-30-EO	1518.8909	Distyrylphenol-prop-ethoxylate-33-EO-4-PO	1987.2
Monostyrylphenolethoxylate-31-EO	1562.9171	Distyrylphenol-prop-ethoxylate-34-EO-4-PO	2031.226

Supplementary

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
Monostyrylphenolethoxylate-32-EO	1606.9433	Distyrylphenol-prop-ethoxylate-35-EO-4-PO	2075.252
Monostyrylphenolethoxylate-33-EO	1650.9696	Distyrylphenol-prop-ethoxylate-36-EO-4-PO	2119.278
Monostyrylphenolethoxylate-34-EO	1694.9958	Distyrylphenol-prop-ethoxylate-37-EO-4-PO	2163.304
Monostyrylphenolethoxylate-35-EO	1739.022	Distyrylphenol-prop-ethoxylate-38-EO-4-PO	2207.331
Monostyrylphenolethoxylate-36-EO	1783.0482	Distyrylphenol-prop-ethoxylate-39-EO-4-PO	2251.357
Monostyrylphenolethoxylate-37-EO	1827.0744	Distyrylphenol-prop-ethoxylate-40-EO-4-PO	2295.383
Monostyrylphenolethoxylate-38-EO	1871.1006	Distyrylphenol-prop-ethoxylate-5-EO-5-PO	812.5075
Monostyrylphenolethoxylate-39-EO	1915.1268	Distyrylphenol-prop-ethoxylate-6-EO-5-PO	856.5337
Monostyrylphenolethoxylate-40-EO	1959.1531	Distyrylphenol-prop-ethoxylate-7-EO-5-PO	900.5599
Distyrylphenolethoxylate-5-EO	522.2981	Distyrylphenol-prop-ethoxylate-8-EO-5-PO	944.5861
Distyrylphenolethoxylate-6-EO	566.3244	Distyrylphenol-prop-ethoxylate-9-EO-5-PO	988.6123
Distyrylphenolethoxylate-7-EO	610.3506	Distyrylphenol-prop-ethoxylate-10-EO-5-PO	1032.639
Distyrylphenolethoxylate-8-EO	654.3768	Distyrylphenol-prop-ethoxylate-11-EO-5-PO	1076.665
Distyrylphenolethoxylate-9-EO	698.403	Distyrylphenol-prop-ethoxylate-12-EO-5-PO	1120.691
Distyrylphenolethoxylate-10-EO	742.4292	Distyrylphenol-prop-ethoxylate-13-EO-5-PO	1164.717
Distyrylphenolethoxylate-11-EO	786.4554	Distyrylphenol-prop-ethoxylate-14-EO-5-PO	1208.743
Distyrylphenolethoxylate-12-EO	830.4816	Distyrylphenol-prop-ethoxylate-15-EO-5-PO	1252.77
Distyrylphenolethoxylate-13-EO	874.5079	Distyrylphenol-prop-ethoxylate-16-EO-5-PO	1296.796
Distyrylphenolethoxylate-14-EO	918.5341	Distyrylphenol-prop-ethoxylate-17-EO-5-PO	1340.822
Distyrylphenolethoxylate-15-EO	962.5603	Distyrylphenol-prop-ethoxylate-18-EO-5-PO	1384.848
Distyrylphenolethoxylate-16-EO	1006.5865	Distyrylphenol-prop-ethoxylate-19-EO-5-PO	1428.874
Distyrylphenolethoxylate-17-EO	1050.6127	Distyrylphenol-prop-ethoxylate-20-EO-5-PO	1472.901
Distyrylphenolethoxylate-18-EO	1094.6389	Distyrylphenol-prop-ethoxylate-21-EO-5-PO	1516.927
Distyrylphenolethoxylate-19-EO	1138.6651	Distyrylphenol-prop-ethoxylate-22-EO-5-PO	1560.953
Distyrylphenolethoxylate-20-EO	1182.6914	Distyrylphenol-prop-ethoxylate-23-EO-5-PO	1604.979
Distyrylphenolethoxylate-21-EO	1226.7176	Distyrylphenol-prop-ethoxylate-24-EO-5-PO	1649.006
Distyrylphenolethoxylate-22-EO	1270.7438	Distyrylphenol-prop-ethoxylate-25-EO-5-PO	1693.032
Distyrylphenolethoxylate-23-EO	1314.77	Distyrylphenol-prop-ethoxylate-26-EO-5-PO	1737.058
Distyrylphenolethoxylate-24-EO	1358.7962	Distyrylphenol-prop-ethoxylate-27-EO-5-PO	1781.084
Distyrylphenolethoxylate-25-EO	1402.8224	Distyrylphenol-prop-ethoxylate-28-EO-5-PO	1825.11
Distyrylphenolethoxylate-26-EO	1446.8486	Distyrylphenol-prop-ethoxylate-29-EO-5-PO	1869.137
Distyrylphenolethoxylate-27-EO	1490.8749	Distyrylphenol-prop-ethoxylate-30-EO-5-PO	1913.163

Supplementary

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
Distyrylphenolethoxylate-28-EO	1534.9011	Distyrylphenol-prop-ethoxylate-31-EO-5-PO	1957.189
Distyrylphenolethoxylate-29-EO	1578.9273	Distyrylphenol-prop-ethoxylate-32-EO-5-PO	2001.215
Distyrylphenolethoxylate-30-EO	1622.9535	Distyrylphenol-prop-ethoxylate-33-EO-5-PO	2045.241
Distyrylphenolethoxylate-31-EO	1666.9797	Distyrylphenol-prop-ethoxylate-34-EO-5-PO	2089.268
Distyrylphenolethoxylate-32-EO	1711.0059	Distyrylphenol-prop-ethoxylate-35-EO-5-PO	2133.294
Distyrylphenolethoxylate-33-EO	1755.0322	Distyrylphenol-prop-ethoxylate-36-EO-5-PO	2177.32
Distyrylphenolethoxylate-34-EO	1799.0584	Distyrylphenol-prop-ethoxylate-37-EO-5-PO	2221.346
Distyrylphenolethoxylate-35-EO	1843.0846	Distyrylphenol-prop-ethoxylate-38-EO-5-PO	2265.373
Distyrylphenolethoxylate-36-EO	1887.1108	Distyrylphenol-prop-ethoxylate-39-EO-5-PO	2309.399
Distyrylphenolethoxylate-37-EO	1931.137	Distyrylphenol-prop-ethoxylate-40-EO-5-PO	2353.425
Distyrylphenolethoxylate-38-EO	1975.1632	Distyrylphenol-prop-ethoxylate-5-EO-6-PO	870.5493
Distyrylphenolethoxylate-39-EO	2019.1894	Distyrylphenol-prop-ethoxylate-6-EO-6-PO	914.5755
Distyrylphenolethoxylate-40-EO	2063.2157	Distyrylphenol-prop-ethoxylate-7-EO-6-PO	958.6018
Tristyrylphenolethoxylate-5-EO	626.3607	Distyrylphenol-prop-ethoxylate-8-EO-6-PO	1002.628
Tristyrylphenolethoxylate-6-EO	670.387	Distyrylphenol-prop-ethoxylate-9-EO-6-PO	1046.654
Tristyrylphenolethoxylate-7-EO	714.4132	Distyrylphenol-prop-ethoxylate-10-EO-6-PO	1090.68
Tristyrylphenolethoxylate-8-EO	758.4394	Distyrylphenol-prop-ethoxylate-11-EO-6-PO	1134.707
Tristyrylphenolethoxylate-9-EO	802.4656	Distyrylphenol-prop-ethoxylate-12-EO-6-PO	1178.733
Tristyrylphenolethoxylate-10-EO	846.4918	Distyrylphenol-prop-ethoxylate-13-EO-6-PO	1222.759
Tristyrylphenolethoxylate-11-EO	890.518	Distyrylphenol-prop-ethoxylate-14-EO-6-PO	1266.785
Tristyrylphenolethoxylate-12-EO	934.5442	Distyrylphenol-prop-ethoxylate-15-EO-6-PO	1310.811
Tristyrylphenolethoxylate-13-EO	978.5705	Distyrylphenol-prop-ethoxylate-16-EO-6-PO	1354.838
Tristyrylphenolethoxylate-14-EO	1022.5967	Distyrylphenol-prop-ethoxylate-17-EO-6-PO	1398.864
Tristyrylphenolethoxylate-15-EO	1066.6229	Distyrylphenol-prop-ethoxylate-18-EO-6-PO	1442.89
Tristyrylphenolethoxylate-16-EO	1110.6491	Distyrylphenol-prop-ethoxylate-19-EO-6-PO	1486.916
Tristyrylphenolethoxylate-17-EO	1154.6753	Distyrylphenol-prop-ethoxylate-20-EO-6-PO	1530.943
Tristyrylphenolethoxylate-18-EO	1198.7015	Distyrylphenol-prop-ethoxylate-21-EO-6-PO	1574.969
Tristyrylphenolethoxylate-19-EO	1242.7277	Distyrylphenol-prop-ethoxylate-22-EO-6-PO	1618.995
Tristyrylphenolethoxylate-20-EO	1286.754	Distyrylphenol-prop-ethoxylate-23-EO-6-PO	1663.021
Tristyrylphenolethoxylate-21-EO	1330.7802	Distyrylphenol-prop-ethoxylate-24-EO-6-PO	1707.047
Tristyrylphenolethoxylate-22-EO	1374.8064	Distyrylphenol-prop-ethoxylate-25-EO-6-PO	1751.074

Supplementary

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
Tristyrylphenolethoxylate-23-EO	1418.8326	Distyrylphenol-prop-ethoxylate-26-EO-6-PO	1795.1
Tristyrylphenolethoxylate-24-EO	1462.8588	Distyrylphenol-prop-ethoxylate-27-EO-6-PO	1839.126
Tristyrylphenolethoxylate-25-EO	1506.885	Distyrylphenol-prop-ethoxylate-28-EO-6-PO	1883.152
Tristyrylphenolethoxylate-26-EO	1550.9113	Distyrylphenol-prop-ethoxylate-29-EO-6-PO	1927.178
Tristyrylphenolethoxylate-27-EO	1594.9375	Distyrylphenol-prop-ethoxylate-30-EO-6-PO	1971.205
Tristyrylphenolethoxylate-28-EO	1638.9637	Distyrylphenol-prop-ethoxylate-31-EO-6-PO	2015.231
Tristyrylphenolethoxylate-29-EO	1682.9899	Distyrylphenol-prop-ethoxylate-32-EO-6-PO	2059.257
Tristyrylphenolethoxylate-30-EO	1727.0161	Distyrylphenol-prop-ethoxylate-33-EO-6-PO	2103.283
Tristyrylphenolethoxylate-31-EO	1771.0423	Distyrylphenol-prop-ethoxylate-34-EO-6-PO	2147.31
Tristyrylphenolethoxylate-32-EO	1815.0685	Distyrylphenol-prop-ethoxylate-35-EO-6-PO	2191.336
Tristyrylphenolethoxylate-33-EO	1859.0948	Distyrylphenol-prop-ethoxylate-36-EO-6-PO	2235.362
Tristyrylphenolethoxylate-34-EO	1903.121	Distyrylphenol-prop-ethoxylate-37-EO-6-PO	2279.388
Tristyrylphenolethoxylate-35-EO	1947.1472	Distyrylphenol-prop-ethoxylate-38-EO-6-PO	2323.414
Tristyrylphenolethoxylate-36-EO	1991.1734	Distyrylphenol-prop-ethoxylate-39-EO-6-PO	2367.441
Tristyrylphenolethoxylate-37-EO	2035.1996	Distyrylphenol-prop-ethoxylate-40-EO-6-PO	2411.467
Tristyrylphenolethoxylate-38-EO	2079.2258	Distyrylphenol-prop-ethoxylate-5-EO-7-PO	928.5912
Tristyrylphenolethoxylate-39-EO	2123.252	Distyrylphenol-prop-ethoxylate-6-EO-7-PO	972.6174
Tristyrylphenolethoxylate-40-EO	2167.2783	Distyrylphenol-prop-ethoxylate-7-EO-7-PO	1016.644
Tetrastyrylphenolethoxylate-5-EO	730.4233	Distyrylphenol-prop-ethoxylate-8-EO-7-PO	1060.67
Tetrastyrylphenolethoxylate-6-EO	774.4496	Distyrylphenol-prop-ethoxylate-9-EO-7-PO	1104.696
Tetrastyrylphenolethoxylate-7-EO	818.4758	Distyrylphenol-prop-ethoxylate-10-EO-7-PO	1148.722
Tetrastyrylphenolethoxylate-8-EO	862.502	Distyrylphenol-prop-ethoxylate-11-EO-7-PO	1192.748
Tetrastyrylphenolethoxylate-9-EO	906.5282	Distyrylphenol-prop-ethoxylate-12-EO-7-PO	1236.775
Tetrastyrylphenolethoxylate-10-EO	950.5544	Distyrylphenol-prop-ethoxylate-13-EO-7-PO	1280.801
Tetrastyrylphenolethoxylate-11-EO	994.5806	Distyrylphenol-prop-ethoxylate-14-EO-7-PO	1324.827
Tetrastyrylphenolethoxylate-12-EO	1038.6068	Distyrylphenol-prop-ethoxylate-15-EO-7-PO	1368.853
Tetrastyrylphenolethoxylate-13-EO	1082.6331	Distyrylphenol-prop-ethoxylate-16-EO-7-PO	1412.88
Tetrastyrylphenolethoxylate-14-EO	1126.6593	Distyrylphenol-prop-ethoxylate-17-EO-7-PO	1456.906
Tetrastyrylphenolethoxylate-15-EO	1170.6855	Distyrylphenol-prop-ethoxylate-18-EO-7-PO	1500.932
Tetrastyrylphenolethoxylate-16-EO	1214.7117	Distyrylphenol-prop-ethoxylate-19-EO-7-PO	1544.958
Tetrastyrylphenolethoxylate-17-EO	1258.7379	Distyrylphenol-prop-ethoxylate-20-EO-7-PO	1588.984

Supplementary

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
Tetrastyrylphenolethoxylate-18-EO	1302.7641	Distyrylphenol-prop-ethoxylate-21-EO-7-PO	1633.011
Tetrastyrylphenolethoxylate-19-EO	1346.7903	Distyrylphenol-prop-ethoxylate-22-EO-7-PO	1677.037
Tetrastyrylphenolethoxylate-20-EO	1390.8166	Distyrylphenol-prop-ethoxylate-23-EO-7-PO	1721.063
Tetrastyrylphenolethoxylate-21-EO	1434.8428	Distyrylphenol-prop-ethoxylate-24-EO-7-PO	1765.089
Tetrastyrylphenolethoxylate-22-EO	1478.869	Distyrylphenol-prop-ethoxylate-25-EO-7-PO	1809.115
Tetrastyrylphenolethoxylate-23-EO	1522.8952	Distyrylphenol-prop-ethoxylate-26-EO-7-PO	1853.142
Tetrastyrylphenolethoxylate-24-EO	1566.9214	Distyrylphenol-prop-ethoxylate-27-EO-7-PO	1897.168
Tetrastyrylphenolethoxylate-25-EO	1610.9476	Distyrylphenol-prop-ethoxylate-28-EO-7-PO	1941.194
Tetrastyrylphenolethoxylate-26-EO	1654.9739	Distyrylphenol-prop-ethoxylate-29-EO-7-PO	1985.22
Tetrastyrylphenolethoxylate-27-EO	1699.0001	Distyrylphenol-prop-ethoxylate-30-EO-7-PO	2029.247
Tetrastyrylphenolethoxylate-28-EO	1743.0263	Distyrylphenol-prop-ethoxylate-31-EO-7-PO	2073.273
Tetrastyrylphenolethoxylate-29-EO	1787.0525	Distyrylphenol-prop-ethoxylate-32-EO-7-PO	2117.299
Tetrastyrylphenolethoxylate-30-EO	1831.0787	Distyrylphenol-prop-ethoxylate-33-EO-7-PO	2161.325
Tetrastyrylphenolethoxylate-31-EO	1875.1049	Distyrylphenol-prop-ethoxylate-34-EO-7-PO	2205.351
Tetrastyrylphenolethoxylate-32-EO	1919.1311	Distyrylphenol-prop-ethoxylate-35-EO-7-PO	2249.378
Tetrastyrylphenolethoxylate-33-EO	1963.1574	Distyrylphenol-prop-ethoxylate-36-EO-7-PO	2293.404
Tetrastyrylphenolethoxylate-34-EO	2007.1836	Distyrylphenol-prop-ethoxylate-37-EO-7-PO	2337.43
Tetrastyrylphenolethoxylate-35-EO	2051.2098	Distyrylphenol-prop-ethoxylate-38-EO-7-PO	2381.456
Tetrastyrylphenolethoxylate-36-EO	2095.236	Distyrylphenol-prop-ethoxylate-39-EO-7-PO	2425.482
Tetrastyrylphenolethoxylate-37-EO	2139.2622	Distyrylphenol-prop-ethoxylate-40-EO-7-PO	2469.509
Tetrastyrylphenolethoxylate-38-EO	2183.2884	Distyrylphenol-prop-ethoxylate-5-EO-8-PO	986.6331
Tetrastyrylphenolethoxylate-39-EO	2227.3146	Distyrylphenol-prop-ethoxylate-6-EO-8-PO	1030.659
Tetrastyrylphenolethoxylate-40-EO	2271.3409	Distyrylphenol-prop-ethoxylate-7-EO-8-PO	1074.685
Monostyrylphenol-prop-ethoxylate--EO--PO	198.1044652	Distyrylphenol-prop-ethoxylate-8-EO-8-PO	1118.712
Monostyrylphenol-prop-ethoxylate-5-EO-1-PO	476.277404	Distyrylphenol-prop-ethoxylate-9-EO-8-PO	1162.738
Monostyrylphenol-prop-ethoxylate-6-EO-1-PO	520.3036187	Distyrylphenol-prop-ethoxylate-10-EO-8-PO	1206.764
Monostyrylphenol-prop-ethoxylate-7-EO-1-PO	564.3298335	Distyrylphenol-prop-ethoxylate-11-EO-8-PO	1250.79
Monostyrylphenol-prop-ethoxylate-8-EO-1-PO	608.3560483	Distyrylphenol-prop-ethoxylate-12-EO-8-PO	1294.817
Monostyrylphenol-prop-ethoxylate-9-EO-1-PO	652.3822631	Distyrylphenol-prop-ethoxylate-13-EO-8-PO	1338.843
Monostyrylphenol-prop-ethoxylate-10-EO-1-PO	696.4084779	Distyrylphenol-prop-ethoxylate-14-EO-8-PO	1382.869
Monostyrylphenol-prop-ethoxylate-11-EO-1-PO	740.4346927	Distyrylphenol-prop-ethoxylate-15-EO-8-PO	1426.895
Monostyrylphenol-prop-ethoxylate-12-EO-1-PO	784.4609075	Distyrylphenol-prop-ethoxylate-16-EO-8-PO	1470.921

Supplementary

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
Monostyrylphenol-prop-ethoxylate-13-EO-1-PO	828.4871223	Distyrylphenol-prop-ethoxylate-17-EO-8-PO	1514.948
Monostyrylphenol-prop-ethoxylate-14-EO-1-PO	872.5133371	Distyrylphenol-prop-ethoxylate-18-EO-8-PO	1558.974
Monostyrylphenol-prop-ethoxylate-15-EO-1-PO	916.5395518	Distyrylphenol-prop-ethoxylate-19-EO-8-PO	1603
Monostyrylphenol-prop-ethoxylate-16-EO-1-PO	960.5657666	Distyrylphenol-prop-ethoxylate-20-EO-8-PO	1647.026
Monostyrylphenol-prop-ethoxylate-17-EO-1-PO	1004.591981	Distyrylphenol-prop-ethoxylate-21-EO-8-PO	1691.052
Monostyrylphenol-prop-ethoxylate-18-EO-1-PO	1048.618196	Distyrylphenol-prop-ethoxylate-22-EO-8-PO	1735.079
Monostyrylphenol-prop-ethoxylate-19-EO-1-PO	1092.644411	Distyrylphenol-prop-ethoxylate-23-EO-8-PO	1779.105
Monostyrylphenol-prop-ethoxylate-20-EO-1-PO	1136.670626	Distyrylphenol-prop-ethoxylate-24-EO-8-PO	1823.131
Monostyrylphenol-prop-ethoxylate-21-EO-1-PO	1180.696841	Distyrylphenol-prop-ethoxylate-25-EO-8-PO	1867.157
Monostyrylphenol-prop-ethoxylate-22-EO-1-PO	1224.723055	Distyrylphenol-prop-ethoxylate-26-EO-8-PO	1911.184
Monostyrylphenol-prop-ethoxylate-23-EO-1-PO	1268.74927	Distyrylphenol-prop-ethoxylate-27-EO-8-PO	1955.21
Monostyrylphenol-prop-ethoxylate-24-EO-1-PO	1312.775485	Distyrylphenol-prop-ethoxylate-28-EO-8-PO	1999.236
Monostyrylphenol-prop-ethoxylate-25-EO-1-PO	1356.8017	Distyrylphenol-prop-ethoxylate-29-EO-8-PO	2043.262
Monostyrylphenol-prop-ethoxylate-26-EO-1-PO	1400.827915	Distyrylphenol-prop-ethoxylate-30-EO-8-PO	2087.288
Monostyrylphenol-prop-ethoxylate-27-EO-1-PO	1444.854129	Distyrylphenol-prop-ethoxylate-31-EO-8-PO	2131.315
Monostyrylphenol-prop-ethoxylate-28-EO-1-PO	1488.880344	Distyrylphenol-prop-ethoxylate-32-EO-8-PO	2175.341
Monostyrylphenol-prop-ethoxylate-29-EO-1-PO	1532.906559	Distyrylphenol-prop-ethoxylate-33-EO-8-PO	2219.367
Monostyrylphenol-prop-ethoxylate-30-EO-1-PO	1576.932774	Distyrylphenol-prop-ethoxylate-34-EO-8-PO	2263.393
Monostyrylphenol-prop-ethoxylate-31-EO-1-PO	1620.958988	Distyrylphenol-prop-ethoxylate-35-EO-8-PO	2307.42
Monostyrylphenol-prop-ethoxylate-32-EO-1-PO	1664.985203	Distyrylphenol-prop-ethoxylate-36-EO-8-PO	2351.446
Monostyrylphenol-prop-ethoxylate-33-EO-1-PO	1709.011418	Distyrylphenol-prop-ethoxylate-37-EO-8-PO	2395.472
Monostyrylphenol-prop-ethoxylate-34-EO-1-PO	1753.037633	Distyrylphenol-prop-ethoxylate-38-EO-8-PO	2439.498
Monostyrylphenol-prop-ethoxylate-35-EO-1-PO	1797.063848	Distyrylphenol-prop-ethoxylate-39-EO-8-PO	2483.524
Monostyrylphenol-prop-ethoxylate-36-EO-1-PO	1841.090062	Distyrylphenol-prop-ethoxylate-40-EO-8-PO	2527.551
Monostyrylphenol-prop-ethoxylate-37-EO-1-PO	1885.116277	Tristyrylphenol-prop-ethoxylate--EO--PO	406.2297
Monostyrylphenol-prop-ethoxylate-38-EO-1-PO	1929.142492	Tristyrylphenol-prop-ethoxylate-5-EO-1-PO	684.4026
Monostyrylphenol-prop-ethoxylate-39-EO-1-PO	1973.168707	Tristyrylphenol-prop-ethoxylate-6-EO-1-PO	728.4288
Monostyrylphenol-prop-ethoxylate-40-EO-1-PO	2017.194922	Tristyrylphenol-prop-ethoxylate-7-EO-1-PO	772.455
Monostyrylphenol-prop-ethoxylate-5-EO-2-PO	534.3192688	Tristyrylphenol-prop-ethoxylate-8-EO-1-PO	816.4812
Monostyrylphenol-prop-ethoxylate-6-EO-2-PO	578.3454836	Tristyrylphenol-prop-ethoxylate-9-EO-1-PO	860.5075
Monostyrylphenol-prop-ethoxylate-7-EO-2-PO	622.3716984	Tristyrylphenol-prop-ethoxylate-10-EO-1-PO	904.5337
Monostyrylphenol-prop-	666.3979132	Tristyrylphenol-prop-ethoxylate-11-	948.5599

Supplementary

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
ethoxylate-8-EO-2-PO		EO-1-PO	
Monostyrylphenol-prop-ethoxylate-9-EO-2-PO	710.424128	Tristyrylphenol-prop-ethoxylate-12-EO-1-PO	992.5861
Monostyrylphenol-prop-ethoxylate-10-EO-2-PO	754.4503428	Tristyrylphenol-prop-ethoxylate-13-EO-1-PO	1036.612
Monostyrylphenol-prop-ethoxylate-11-EO-2-PO	798.4765576	Tristyrylphenol-prop-ethoxylate-14-EO-1-PO	1080.639
Monostyrylphenol-prop-ethoxylate-12-EO-2-PO	842.5027723	Tristyrylphenol-prop-ethoxylate-15-EO-1-PO	1124.665
Monostyrylphenol-prop-ethoxylate-13-EO-2-PO	886.5289871	Tristyrylphenol-prop-ethoxylate-16-EO-1-PO	1168.691
Monostyrylphenol-prop-ethoxylate-14-EO-2-PO	930.5552019	Tristyrylphenol-prop-ethoxylate-17-EO-1-PO	1212.717
Monostyrylphenol-prop-ethoxylate-15-EO-2-PO	974.5814167	Tristyrylphenol-prop-ethoxylate-18-EO-1-PO	1256.743
Monostyrylphenol-prop-ethoxylate-16-EO-2-PO	1018.607631	Tristyrylphenol-prop-ethoxylate-19-EO-1-PO	1300.77
Monostyrylphenol-prop-ethoxylate-17-EO-2-PO	1062.633846	Tristyrylphenol-prop-ethoxylate-20-EO-1-PO	1344.796
Monostyrylphenol-prop-ethoxylate-18-EO-2-PO	1106.660061	Tristyrylphenol-prop-ethoxylate-21-EO-1-PO	1388.822
Monostyrylphenol-prop-ethoxylate-19-EO-2-PO	1150.686276	Tristyrylphenol-prop-ethoxylate-22-EO-1-PO	1432.848
Monostyrylphenol-prop-ethoxylate-20-EO-2-PO	1194.712491	Tristyrylphenol-prop-ethoxylate-23-EO-1-PO	1476.874
Monostyrylphenol-prop-ethoxylate-21-EO-2-PO	1238.738705	Tristyrylphenol-prop-ethoxylate-24-EO-1-PO	1520.901
Monostyrylphenol-prop-ethoxylate-22-EO-2-PO	1282.76492	Tristyrylphenol-prop-ethoxylate-25-EO-1-PO	1564.927
Monostyrylphenol-prop-ethoxylate-23-EO-2-PO	1326.791135	Tristyrylphenol-prop-ethoxylate-26-EO-1-PO	1608.953
Monostyrylphenol-prop-ethoxylate-24-EO-2-PO	1370.81735	Tristyrylphenol-prop-ethoxylate-27-EO-1-PO	1652.979
Monostyrylphenol-prop-ethoxylate-25-EO-2-PO	1414.843565	Tristyrylphenol-prop-ethoxylate-28-EO-1-PO	1697.006
Monostyrylphenol-prop-ethoxylate-26-EO-2-PO	1458.869779	Tristyrylphenol-prop-ethoxylate-29-EO-1-PO	1741.032
Monostyrylphenol-prop-ethoxylate-27-EO-2-PO	1502.895994	Tristyrylphenol-prop-ethoxylate-30-EO-1-PO	1785.058
Monostyrylphenol-prop-ethoxylate-28-EO-2-PO	1546.922209	Tristyrylphenol-prop-ethoxylate-31-EO-1-PO	1829.084
Monostyrylphenol-prop-ethoxylate-29-EO-2-PO	1590.948424	Tristyrylphenol-prop-ethoxylate-32-EO-1-PO	1873.11
Monostyrylphenol-prop-ethoxylate-30-EO-2-PO	1634.974639	Tristyrylphenol-prop-ethoxylate-33-EO-1-PO	1917.137
Monostyrylphenol-prop-ethoxylate-31-EO-2-PO	1679.000853	Tristyrylphenol-prop-ethoxylate-34-EO-1-PO	1961.163
Monostyrylphenol-prop-ethoxylate-32-EO-2-PO	1723.027068	Tristyrylphenol-prop-ethoxylate-35-EO-1-PO	2005.189
Monostyrylphenol-prop-ethoxylate-33-EO-2-PO	1767.053283	Tristyrylphenol-prop-ethoxylate-36-EO-1-PO	2049.215
Monostyrylphenol-prop-ethoxylate-34-EO-2-PO	1811.079498	Tristyrylphenol-prop-ethoxylate-37-EO-1-PO	2093.241
Monostyrylphenol-prop-ethoxylate-35-EO-2-PO	1855.105712	Tristyrylphenol-prop-ethoxylate-38-EO-1-PO	2137.268
Monostyrylphenol-prop-ethoxylate-36-EO-2-PO	1899.131927	Tristyrylphenol-prop-ethoxylate-39-EO-1-PO	2181.294
Monostyrylphenol-prop-ethoxylate-37-EO-2-PO	1943.158142	Tristyrylphenol-prop-ethoxylate-40-EO-1-PO	2225.32
Monostyrylphenol-prop-ethoxylate-38-EO-2-PO	1987.184357	Tristyrylphenol-prop-ethoxylate-5-EO-2-PO	742.4445
Monostyrylphenol-prop-ethoxylate-39-EO-2-PO	2031.210572	Tristyrylphenol-prop-ethoxylate-6-EO-2-PO	786.4707

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
Monostyrylphenol-prop-ethoxylate-40-EO-2-PO	2075.236786	Tristyrylphenol-prop-ethoxylate-7-EO-2-PO	830.4969
Monostyrylphenol-prop-ethoxylate-5-EO-3-PO	592.3611337	Tristyrylphenol-prop-ethoxylate-8-EO-2-PO	874.5231
Monostyrylphenol-prop-ethoxylate-6-EO-3-PO	636.3873485	Tristyrylphenol-prop-ethoxylate-9-EO-2-PO	918.5493
Monostyrylphenol-prop-ethoxylate-7-EO-3-PO	680.4135633	Tristyrylphenol-prop-ethoxylate-10-EO-2-PO	962.5755
Monostyrylphenol-prop-ethoxylate-8-EO-3-PO	724.439778	Tristyrylphenol-prop-ethoxylate-11-EO-2-PO	1006.602
Monostyrylphenol-prop-ethoxylate-9-EO-3-PO	768.4659928	Tristyrylphenol-prop-ethoxylate-12-EO-2-PO	1050.628
Monostyrylphenol-prop-ethoxylate-10-EO-3-PO	812.4922076	Tristyrylphenol-prop-ethoxylate-13-EO-2-PO	1094.654
Monostyrylphenol-prop-ethoxylate-11-EO-3-PO	856.5184224	Tristyrylphenol-prop-ethoxylate-14-EO-2-PO	1138.68
Monostyrylphenol-prop-ethoxylate-12-EO-3-PO	900.5446372	Tristyrylphenol-prop-ethoxylate-15-EO-2-PO	1182.707
Monostyrylphenol-prop-ethoxylate-13-EO-3-PO	944.570852	Tristyrylphenol-prop-ethoxylate-16-EO-2-PO	1226.733
Monostyrylphenol-prop-ethoxylate-14-EO-3-PO	988.5970668	Tristyrylphenol-prop-ethoxylate-17-EO-2-PO	1270.759
Monostyrylphenol-prop-ethoxylate-15-EO-3-PO	1032.623282	Tristyrylphenol-prop-ethoxylate-18-EO-2-PO	1314.785
Monostyrylphenol-prop-ethoxylate-16-EO-3-PO	1076.649496	Tristyrylphenol-prop-ethoxylate-19-EO-2-PO	1358.811
Monostyrylphenol-prop-ethoxylate-17-EO-3-PO	1120.675711	Tristyrylphenol-prop-ethoxylate-20-EO-2-PO	1402.838
Monostyrylphenol-prop-ethoxylate-18-EO-3-PO	1164.701926	Tristyrylphenol-prop-ethoxylate-21-EO-2-PO	1446.864
Monostyrylphenol-prop-ethoxylate-19-EO-3-PO	1208.728141	Tristyrylphenol-prop-ethoxylate-22-EO-2-PO	1490.89
Monostyrylphenol-prop-ethoxylate-20-EO-3-PO	1252.754356	Tristyrylphenol-prop-ethoxylate-23-EO-2-PO	1534.916
Monostyrylphenol-prop-ethoxylate-21-EO-3-PO	1296.78057	Tristyrylphenol-prop-ethoxylate-24-EO-2-PO	1578.943
Monostyrylphenol-prop-ethoxylate-22-EO-3-PO	1340.806785	Tristyrylphenol-prop-ethoxylate-25-EO-2-PO	1622.969
Monostyrylphenol-prop-ethoxylate-23-EO-3-PO	1384.833	Tristyrylphenol-prop-ethoxylate-26-EO-2-PO	1666.995
Monostyrylphenol-prop-ethoxylate-24-EO-3-PO	1428.859215	Tristyrylphenol-prop-ethoxylate-27-EO-2-PO	1711.021
Monostyrylphenol-prop-ethoxylate-25-EO-3-PO	1472.885429	Tristyrylphenol-prop-ethoxylate-28-EO-2-PO	1755.047
Monostyrylphenol-prop-ethoxylate-26-EO-3-PO	1516.911644	Tristyrylphenol-prop-ethoxylate-29-EO-2-PO	1799.074
Monostyrylphenol-prop-ethoxylate-27-EO-3-PO	1560.937859	Tristyrylphenol-prop-ethoxylate-30-EO-2-PO	1843.1
Monostyrylphenol-prop-ethoxylate-28-EO-3-PO	1604.964074	Tristyrylphenol-prop-ethoxylate-31-EO-2-PO	1887.126
Monostyrylphenol-prop-ethoxylate-29-EO-3-PO	1648.990289	Tristyrylphenol-prop-ethoxylate-32-EO-2-PO	1931.152
Monostyrylphenol-prop-ethoxylate-30-EO-3-PO	1693.016503	Tristyrylphenol-prop-ethoxylate-33-EO-2-PO	1975.178
Monostyrylphenol-prop-ethoxylate-31-EO-3-PO	1737.042718	Tristyrylphenol-prop-ethoxylate-34-EO-2-PO	2019.205
Monostyrylphenol-prop-ethoxylate-32-EO-3-PO	1781.068933	Tristyrylphenol-prop-ethoxylate-35-EO-2-PO	2063.231
Monostyrylphenol-prop-ethoxylate-33-EO-3-PO	1825.095148	Tristyrylphenol-prop-ethoxylate-36-EO-2-PO	2107.257
Monostyrylphenol-prop-ethoxylate-34-EO-3-PO	1869.121363	Tristyrylphenol-prop-ethoxylate-37-EO-2-PO	2151.283
Monostyrylphenol-prop-	1913.147577	Tristyrylphenol-prop-ethoxylate-38-	2195.31

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
ethoxylate-35-EO-3-PO		EO-2-PO	
Monostyrylphenol-prop-ethoxylate-36-EO-3-PO	1957.173792	Tristyrylphenol-prop-ethoxylate-39-EO-2-PO	2239.336
Monostyrylphenol-prop-ethoxylate-37-EO-3-PO	2001.200007	Tristyrylphenol-prop-ethoxylate-40-EO-2-PO	2283.362
Monostyrylphenol-prop-ethoxylate-38-EO-3-PO	2045.226222	Tristyrylphenol-prop-ethoxylate-5-EO-3-PO	800.4863
Monostyrylphenol-prop-ethoxylate-39-EO-3-PO	2089.252436	Tristyrylphenol-prop-ethoxylate-6-EO-3-PO	844.5125
Monostyrylphenol-prop-ethoxylate-40-EO-3-PO	2133.278651	Tristyrylphenol-prop-ethoxylate-7-EO-3-PO	888.5388
Monostyrylphenol-prop-ethoxylate-5-EO-4-PO	650.4029985	Tristyrylphenol-prop-ethoxylate-8-EO-3-PO	932.565
Monostyrylphenol-prop-ethoxylate-6-EO-4-PO	694.4292133	Tristyrylphenol-prop-ethoxylate-9-EO-3-PO	976.5912
Monostyrylphenol-prop-ethoxylate-7-EO-4-PO	738.4554281	Tristyrylphenol-prop-ethoxylate-10-EO-3-PO	1020.617
Monostyrylphenol-prop-ethoxylate-8-EO-4-PO	782.4816429	Tristyrylphenol-prop-ethoxylate-11-EO-3-PO	1064.644
Monostyrylphenol-prop-ethoxylate-9-EO-4-PO	826.5078577	Tristyrylphenol-prop-ethoxylate-12-EO-3-PO	1108.67
Monostyrylphenol-prop-ethoxylate-10-EO-4-PO	870.5340725	Tristyrylphenol-prop-ethoxylate-13-EO-3-PO	1152.696
Monostyrylphenol-prop-ethoxylate-11-EO-4-PO	914.5602873	Tristyrylphenol-prop-ethoxylate-14-EO-3-PO	1196.722
Monostyrylphenol-prop-ethoxylate-12-EO-4-PO	958.5865021	Tristyrylphenol-prop-ethoxylate-15-EO-3-PO	1240.748
Monostyrylphenol-prop-ethoxylate-13-EO-4-PO	1002.612717	Tristyrylphenol-prop-ethoxylate-16-EO-3-PO	1284.775
Monostyrylphenol-prop-ethoxylate-14-EO-4-PO	1046.638932	Tristyrylphenol-prop-ethoxylate-17-EO-3-PO	1328.801
Monostyrylphenol-prop-ethoxylate-15-EO-4-PO	1090.665146	Tristyrylphenol-prop-ethoxylate-18-EO-3-PO	1372.827
Monostyrylphenol-prop-ethoxylate-16-EO-4-PO	1134.691361	Tristyrylphenol-prop-ethoxylate-19-EO-3-PO	1416.853
Monostyrylphenol-prop-ethoxylate-17-EO-4-PO	1178.717576	Tristyrylphenol-prop-ethoxylate-20-EO-3-PO	1460.88
Monostyrylphenol-prop-ethoxylate-18-EO-4-PO	1222.743791	Tristyrylphenol-prop-ethoxylate-21-EO-3-PO	1504.906
Monostyrylphenol-prop-ethoxylate-19-EO-4-PO	1266.770006	Tristyrylphenol-prop-ethoxylate-22-EO-3-PO	1548.932
Monostyrylphenol-prop-ethoxylate-20-EO-4-PO	1310.79622	Tristyrylphenol-prop-ethoxylate-23-EO-3-PO	1592.958
Monostyrylphenol-prop-ethoxylate-21-EO-4-PO	1354.822435	Tristyrylphenol-prop-ethoxylate-24-EO-3-PO	1636.984
Monostyrylphenol-prop-ethoxylate-22-EO-4-PO	1398.84865	Tristyrylphenol-prop-ethoxylate-25-EO-3-PO	1681.011
Monostyrylphenol-prop-ethoxylate-23-EO-4-PO	1442.874865	Tristyrylphenol-prop-ethoxylate-26-EO-3-PO	1725.037
Monostyrylphenol-prop-ethoxylate-24-EO-4-PO	1486.90108	Tristyrylphenol-prop-ethoxylate-27-EO-3-PO	1769.063
Monostyrylphenol-prop-ethoxylate-25-EO-4-PO	1530.927294	Tristyrylphenol-prop-ethoxylate-28-EO-3-PO	1813.089
Monostyrylphenol-prop-ethoxylate-26-EO-4-PO	1574.953509	Tristyrylphenol-prop-ethoxylate-29-EO-3-PO	1857.115
Monostyrylphenol-prop-ethoxylate-27-EO-4-PO	1618.979724	Tristyrylphenol-prop-ethoxylate-30-EO-3-PO	1901.142
Monostyrylphenol-prop-ethoxylate-28-EO-4-PO	1663.005939	Tristyrylphenol-prop-ethoxylate-31-EO-3-PO	1945.168
Monostyrylphenol-prop-ethoxylate-29-EO-4-PO	1707.032153	Tristyrylphenol-prop-ethoxylate-32-EO-3-PO	1989.194
Monostyrylphenol-prop-ethoxylate-30-EO-4-PO	1751.058368	Tristyrylphenol-prop-ethoxylate-33-EO-3-PO	2033.22

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
Monostyrylphenol-prop-ethoxylate-31-EO-4-PO	1795.084583	Tristyrylphenol-prop-ethoxylate-34-EO-3-PO	2077.247
Monostyrylphenol-prop-ethoxylate-32-EO-4-PO	1839.110798	Tristyrylphenol-prop-ethoxylate-35-EO-3-PO	2121.273
Monostyrylphenol-prop-ethoxylate-33-EO-4-PO	1883.137013	Tristyrylphenol-prop-ethoxylate-36-EO-3-PO	2165.299
Monostyrylphenol-prop-ethoxylate-34-EO-4-PO	1927.163227	Tristyrylphenol-prop-ethoxylate-37-EO-3-PO	2209.325
Monostyrylphenol-prop-ethoxylate-35-EO-4-PO	1971.189442	Tristyrylphenol-prop-ethoxylate-38-EO-3-PO	2253.351
Monostyrylphenol-prop-ethoxylate-36-EO-4-PO	2015.215657	Tristyrylphenol-prop-ethoxylate-39-EO-3-PO	2297.378
Monostyrylphenol-prop-ethoxylate-37-EO-4-PO	2059.241872	Tristyrylphenol-prop-ethoxylate-40-EO-3-PO	2341.404
Monostyrylphenol-prop-ethoxylate-38-EO-4-PO	2103.268087	Tristyrylphenol-prop-ethoxylate-5-EO-4-PO	858.5282
Monostyrylphenol-prop-ethoxylate-39-EO-4-PO	2147.294301	Tristyrylphenol-prop-ethoxylate-6-EO-4-PO	902.5544
Monostyrylphenol-prop-ethoxylate-40-EO-4-PO	2191.320516	Tristyrylphenol-prop-ethoxylate-7-EO-4-PO	946.5806
Monostyrylphenol-prop-ethoxylate-5-EO-5-PO	708.4448634	Tristyrylphenol-prop-ethoxylate-8-EO-4-PO	990.6068
Monostyrylphenol-prop-ethoxylate-6-EO-5-PO	752.4710782	Tristyrylphenol-prop-ethoxylate-9-EO-4-PO	1034.633
Monostyrylphenol-prop-ethoxylate-7-EO-5-PO	796.497293	Tristyrylphenol-prop-ethoxylate-10-EO-4-PO	1078.659
Monostyrylphenol-prop-ethoxylate-8-EO-5-PO	840.5235078	Tristyrylphenol-prop-ethoxylate-11-EO-4-PO	1122.685
Monostyrylphenol-prop-ethoxylate-9-EO-5-PO	884.5497226	Tristyrylphenol-prop-ethoxylate-12-EO-4-PO	1166.712
Monostyrylphenol-prop-ethoxylate-10-EO-5-PO	928.5759373	Tristyrylphenol-prop-ethoxylate-13-EO-4-PO	1210.738
Monostyrylphenol-prop-ethoxylate-11-EO-5-PO	972.6021521	Tristyrylphenol-prop-ethoxylate-14-EO-4-PO	1254.764
Monostyrylphenol-prop-ethoxylate-12-EO-5-PO	1016.628367	Tristyrylphenol-prop-ethoxylate-15-EO-4-PO	1298.79
Monostyrylphenol-prop-ethoxylate-13-EO-5-PO	1060.654582	Tristyrylphenol-prop-ethoxylate-16-EO-4-PO	1342.817
Monostyrylphenol-prop-ethoxylate-14-EO-5-PO	1104.680797	Tristyrylphenol-prop-ethoxylate-17-EO-4-PO	1386.843
Monostyrylphenol-prop-ethoxylate-15-EO-5-PO	1148.707011	Tristyrylphenol-prop-ethoxylate-18-EO-4-PO	1430.869
Monostyrylphenol-prop-ethoxylate-16-EO-5-PO	1192.733226	Tristyrylphenol-prop-ethoxylate-19-EO-4-PO	1474.895
Monostyrylphenol-prop-ethoxylate-17-EO-5-PO	1236.759441	Tristyrylphenol-prop-ethoxylate-20-EO-4-PO	1518.921
Monostyrylphenol-prop-ethoxylate-18-EO-5-PO	1280.785656	Tristyrylphenol-prop-ethoxylate-21-EO-4-PO	1562.948
Monostyrylphenol-prop-ethoxylate-19-EO-5-PO	1324.81187	Tristyrylphenol-prop-ethoxylate-22-EO-4-PO	1606.974
Monostyrylphenol-prop-ethoxylate-20-EO-5-PO	1368.838085	Tristyrylphenol-prop-ethoxylate-23-EO-4-PO	1651
Monostyrylphenol-prop-ethoxylate-21-EO-5-PO	1412.8643	Tristyrylphenol-prop-ethoxylate-24-EO-4-PO	1695.026
Monostyrylphenol-prop-ethoxylate-22-EO-5-PO	1456.890515	Tristyrylphenol-prop-ethoxylate-25-EO-4-PO	1739.052
Monostyrylphenol-prop-ethoxylate-23-EO-5-PO	1500.91673	Tristyrylphenol-prop-ethoxylate-26-EO-4-PO	1783.079
Monostyrylphenol-prop-ethoxylate-24-EO-5-PO	1544.942944	Tristyrylphenol-prop-ethoxylate-27-EO-4-PO	1827.105
Monostyrylphenol-prop-ethoxylate-25-EO-5-PO	1588.969159	Tristyrylphenol-prop-ethoxylate-28-EO-4-PO	1871.131
Monostyrylphenol-prop-	1632.995374	Tristyrylphenol-prop-ethoxylate-29-	1915.157

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
ethoxylate-26-EO-5-PO		EO-4-PO	
Monostyrylphenol-prop-ethoxylate-27-EO-5-PO	1677.021589	Tristyrylphenol-prop-ethoxylate-30-EO-4-PO	1959.184
Monostyrylphenol-prop-ethoxylate-28-EO-5-PO	1721.047804	Tristyrylphenol-prop-ethoxylate-31-EO-4-PO	2003.21
Monostyrylphenol-prop-ethoxylate-29-EO-5-PO	1765.074018	Tristyrylphenol-prop-ethoxylate-32-EO-4-PO	2047.236
Monostyrylphenol-prop-ethoxylate-30-EO-5-PO	1809.100233	Tristyrylphenol-prop-ethoxylate-33-EO-4-PO	2091.262
Monostyrylphenol-prop-ethoxylate-31-EO-5-PO	1853.126448	Tristyrylphenol-prop-ethoxylate-34-EO-4-PO	2135.288
Monostyrylphenol-prop-ethoxylate-32-EO-5-PO	1897.152663	Tristyrylphenol-prop-ethoxylate-35-EO-4-PO	2179.315
Monostyrylphenol-prop-ethoxylate-33-EO-5-PO	1941.178877	Tristyrylphenol-prop-ethoxylate-36-EO-4-PO	2223.341
Monostyrylphenol-prop-ethoxylate-34-EO-5-PO	1985.205092	Tristyrylphenol-prop-ethoxylate-37-EO-4-PO	2267.367
Monostyrylphenol-prop-ethoxylate-35-EO-5-PO	2029.231307	Tristyrylphenol-prop-ethoxylate-38-EO-4-PO	2311.393
Monostyrylphenol-prop-ethoxylate-36-EO-5-PO	2073.257522	Tristyrylphenol-prop-ethoxylate-39-EO-4-PO	2355.42
Monostyrylphenol-prop-ethoxylate-37-EO-5-PO	2117.283737	Tristyrylphenol-prop-ethoxylate-40-EO-4-PO	2399.446
Monostyrylphenol-prop-ethoxylate-38-EO-5-PO	2161.309951	Tristyrylphenol-prop-ethoxylate-5-EO-5-PO	916.5701
Monostyrylphenol-prop-ethoxylate-39-EO-5-PO	2205.336166	Tristyrylphenol-prop-ethoxylate-6-EO-5-PO	960.5963
Monostyrylphenol-prop-ethoxylate-40-EO-5-PO	2249.362381	Tristyrylphenol-prop-ethoxylate-7-EO-5-PO	1004.622
Monostyrylphenol-prop-ethoxylate-5-EO-6-PO	766.4867283	Tristyrylphenol-prop-ethoxylate-8-EO-5-PO	1048.649
Monostyrylphenol-prop-ethoxylate-6-EO-6-PO	810.5129431	Tristyrylphenol-prop-ethoxylate-9-EO-5-PO	1092.675
Monostyrylphenol-prop-ethoxylate-7-EO-6-PO	854.5391578	Tristyrylphenol-prop-ethoxylate-10-EO-5-PO	1136.701
Monostyrylphenol-prop-ethoxylate-8-EO-6-PO	898.5653726	Tristyrylphenol-prop-ethoxylate-11-EO-5-PO	1180.727
Monostyrylphenol-prop-ethoxylate-9-EO-6-PO	942.5915874	Tristyrylphenol-prop-ethoxylate-12-EO-5-PO	1224.754
Monostyrylphenol-prop-ethoxylate-10-EO-6-PO	986.6178022	Tristyrylphenol-prop-ethoxylate-13-EO-5-PO	1268.78
Monostyrylphenol-prop-ethoxylate-11-EO-6-PO	1030.644017	Tristyrylphenol-prop-ethoxylate-14-EO-5-PO	1312.806
Monostyrylphenol-prop-ethoxylate-12-EO-6-PO	1074.670232	Tristyrylphenol-prop-ethoxylate-15-EO-5-PO	1356.832
Monostyrylphenol-prop-ethoxylate-13-EO-6-PO	1118.696447	Tristyrylphenol-prop-ethoxylate-16-EO-5-PO	1400.858
Monostyrylphenol-prop-ethoxylate-14-EO-6-PO	1162.722661	Tristyrylphenol-prop-ethoxylate-17-EO-5-PO	1444.885
Monostyrylphenol-prop-ethoxylate-15-EO-6-PO	1206.748876	Tristyrylphenol-prop-ethoxylate-18-EO-5-PO	1488.911
Monostyrylphenol-prop-ethoxylate-16-EO-6-PO	1250.775091	Tristyrylphenol-prop-ethoxylate-19-EO-5-PO	1532.937
Monostyrylphenol-prop-ethoxylate-17-EO-6-PO	1294.801306	Tristyrylphenol-prop-ethoxylate-20-EO-5-PO	1576.963
Monostyrylphenol-prop-ethoxylate-18-EO-6-PO	1338.827521	Tristyrylphenol-prop-ethoxylate-21-EO-5-PO	1620.99
Monostyrylphenol-prop-ethoxylate-19-EO-6-PO	1382.853735	Tristyrylphenol-prop-ethoxylate-22-EO-5-PO	1665.016
Monostyrylphenol-prop-ethoxylate-20-EO-6-PO	1426.87995	Tristyrylphenol-prop-ethoxylate-23-EO-5-PO	1709.042
Monostyrylphenol-prop-ethoxylate-21-EO-6-PO	1470.906165	Tristyrylphenol-prop-ethoxylate-24-EO-5-PO	1753.068

Supplementary

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
Monostyrylphenol-prop-ethoxylate-22-EO-6-PO	1514.93238	Tristyrylphenol-prop-ethoxylate-25-EO-5-PO	1797.094
Monostyrylphenol-prop-ethoxylate-23-EO-6-PO	1558.958594	Tristyrylphenol-prop-ethoxylate-26-EO-5-PO	1841.121
Monostyrylphenol-prop-ethoxylate-24-EO-6-PO	1602.984809	Tristyrylphenol-prop-ethoxylate-27-EO-5-PO	1885.147
Monostyrylphenol-prop-ethoxylate-25-EO-6-PO	1647.011024	Tristyrylphenol-prop-ethoxylate-28-EO-5-PO	1929.173
Monostyrylphenol-prop-ethoxylate-26-EO-6-PO	1691.037239	Tristyrylphenol-prop-ethoxylate-29-EO-5-PO	1973.199
Monostyrylphenol-prop-ethoxylate-27-EO-6-PO	1735.063454	Tristyrylphenol-prop-ethoxylate-30-EO-5-PO	2017.225
Monostyrylphenol-prop-ethoxylate-28-EO-6-PO	1779.089668	Tristyrylphenol-prop-ethoxylate-31-EO-5-PO	2061.252
Monostyrylphenol-prop-ethoxylate-29-EO-6-PO	1823.115883	Tristyrylphenol-prop-ethoxylate-32-EO-5-PO	2105.278
Monostyrylphenol-prop-ethoxylate-30-EO-6-PO	1867.142098	Tristyrylphenol-prop-ethoxylate-33-EO-5-PO	2149.304
Monostyrylphenol-prop-ethoxylate-31-EO-6-PO	1911.168313	Tristyrylphenol-prop-ethoxylate-34-EO-5-PO	2193.33
Monostyrylphenol-prop-ethoxylate-32-EO-6-PO	1955.194528	Tristyrylphenol-prop-ethoxylate-35-EO-5-PO	2237.357
Monostyrylphenol-prop-ethoxylate-33-EO-6-PO	1999.220742	Tristyrylphenol-prop-ethoxylate-36-EO-5-PO	2281.383
Monostyrylphenol-prop-ethoxylate-34-EO-6-PO	2043.246957	Tristyrylphenol-prop-ethoxylate-37-EO-5-PO	2325.409
Monostyrylphenol-prop-ethoxylate-35-EO-6-PO	2087.273172	Tristyrylphenol-prop-ethoxylate-38-EO-5-PO	2369.435
Monostyrylphenol-prop-ethoxylate-36-EO-6-PO	2131.299387	Tristyrylphenol-prop-ethoxylate-39-EO-5-PO	2413.461
Monostyrylphenol-prop-ethoxylate-37-EO-6-PO	2175.325601	Tristyrylphenol-prop-ethoxylate-40-EO-5-PO	2457.488
Monostyrylphenol-prop-ethoxylate-38-EO-6-PO	2219.351816	Tristyrylphenol-prop-ethoxylate-5-EO-6-PO	974.6119
Monostyrylphenol-prop-ethoxylate-39-EO-6-PO	2263.378031	Tristyrylphenol-prop-ethoxylate-6-EO-6-PO	1018.638
Monostyrylphenol-prop-ethoxylate-40-EO-6-PO	2307.404246	Tristyrylphenol-prop-ethoxylate-7-EO-6-PO	1062.664
Monostyrylphenol-prop-ethoxylate-5-EO-7-PO	824.5285931	Tristyrylphenol-prop-ethoxylate-8-EO-6-PO	1106.691
Monostyrylphenol-prop-ethoxylate-6-EO-7-PO	868.5548079	Tristyrylphenol-prop-ethoxylate-9-EO-6-PO	1150.717
Monostyrylphenol-prop-ethoxylate-7-EO-7-PO	912.5810227	Tristyrylphenol-prop-ethoxylate-10-EO-6-PO	1194.743
Monostyrylphenol-prop-ethoxylate-8-EO-7-PO	956.6072375	Tristyrylphenol-prop-ethoxylate-11-EO-6-PO	1238.769
Monostyrylphenol-prop-ethoxylate-9-EO-7-PO	1000.633452	Tristyrylphenol-prop-ethoxylate-12-EO-6-PO	1282.795
Monostyrylphenol-prop-ethoxylate-10-EO-7-PO	1044.659667	Tristyrylphenol-prop-ethoxylate-13-EO-6-PO	1326.822
Monostyrylphenol-prop-ethoxylate-11-EO-7-PO	1088.685882	Tristyrylphenol-prop-ethoxylate-14-EO-6-PO	1370.848
Monostyrylphenol-prop-ethoxylate-12-EO-7-PO	1132.712097	Tristyrylphenol-prop-ethoxylate-15-EO-6-PO	1414.874
Monostyrylphenol-prop-ethoxylate-13-EO-7-PO	1176.738311	Tristyrylphenol-prop-ethoxylate-16-EO-6-PO	1458.9
Monostyrylphenol-prop-ethoxylate-14-EO-7-PO	1220.764526	Tristyrylphenol-prop-ethoxylate-17-EO-6-PO	1502.927
Monostyrylphenol-prop-ethoxylate-15-EO-7-PO	1264.790741	Tristyrylphenol-prop-ethoxylate-18-EO-6-PO	1546.953
Monostyrylphenol-prop-ethoxylate-16-EO-7-PO	1308.816956	Tristyrylphenol-prop-ethoxylate-19-EO-6-PO	1590.979
Monostyrylphenol-prop-	1352.843171	Tristyrylphenol-prop-ethoxylate-20-	1635.005

Supplementary

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
ethoxylate-17-EO-7-PO		EO-6-PO	
Monostyrylphenol-prop-ethoxylate-18-EO-7-PO	1396.869385	Tristyrylphenol-prop-ethoxylate-21-EO-6-PO	1679.031
Monostyrylphenol-prop-ethoxylate-19-EO-7-PO	1440.8956	Tristyrylphenol-prop-ethoxylate-22-EO-6-PO	1723.058
Monostyrylphenol-prop-ethoxylate-20-EO-7-PO	1484.921815	Tristyrylphenol-prop-ethoxylate-23-EO-6-PO	1767.084
Monostyrylphenol-prop-ethoxylate-21-EO-7-PO	1528.94803	Tristyrylphenol-prop-ethoxylate-24-EO-6-PO	1811.11
Monostyrylphenol-prop-ethoxylate-22-EO-7-PO	1572.974245	Tristyrylphenol-prop-ethoxylate-25-EO-6-PO	1855.136
Monostyrylphenol-prop-ethoxylate-23-EO-7-PO	1617.000459	Tristyrylphenol-prop-ethoxylate-26-EO-6-PO	1899.162
Monostyrylphenol-prop-ethoxylate-24-EO-7-PO	1661.026674	Tristyrylphenol-prop-ethoxylate-27-EO-6-PO	1943.189
Monostyrylphenol-prop-ethoxylate-25-EO-7-PO	1705.052889	Tristyrylphenol-prop-ethoxylate-28-EO-6-PO	1987.215
Monostyrylphenol-prop-ethoxylate-26-EO-7-PO	1749.079104	Tristyrylphenol-prop-ethoxylate-29-EO-6-PO	2031.241
Monostyrylphenol-prop-ethoxylate-27-EO-7-PO	1793.105318	Tristyrylphenol-prop-ethoxylate-30-EO-6-PO	2075.267
Monostyrylphenol-prop-ethoxylate-28-EO-7-PO	1837.131533	Tristyrylphenol-prop-ethoxylate-31-EO-6-PO	2119.294
Monostyrylphenol-prop-ethoxylate-29-EO-7-PO	1881.157748	Tristyrylphenol-prop-ethoxylate-32-EO-6-PO	2163.32
Monostyrylphenol-prop-ethoxylate-30-EO-7-PO	1925.183963	Tristyrylphenol-prop-ethoxylate-33-EO-6-PO	2207.346
Monostyrylphenol-prop-ethoxylate-31-EO-7-PO	1969.210178	Tristyrylphenol-prop-ethoxylate-34-EO-6-PO	2251.372
Monostyrylphenol-prop-ethoxylate-32-EO-7-PO	2013.236392	Tristyrylphenol-prop-ethoxylate-35-EO-6-PO	2295.398
Monostyrylphenol-prop-ethoxylate-33-EO-7-PO	2057.262607	Tristyrylphenol-prop-ethoxylate-36-EO-6-PO	2339.425
Monostyrylphenol-prop-ethoxylate-34-EO-7-PO	2101.288822	Tristyrylphenol-prop-ethoxylate-37-EO-6-PO	2383.451
Monostyrylphenol-prop-ethoxylate-35-EO-7-PO	2145.315037	Tristyrylphenol-prop-ethoxylate-38-EO-6-PO	2427.477
Monostyrylphenol-prop-ethoxylate-36-EO-7-PO	2189.341252	Tristyrylphenol-prop-ethoxylate-39-EO-6-PO	2471.503
Monostyrylphenol-prop-ethoxylate-37-EO-7-PO	2233.367466	Tristyrylphenol-prop-ethoxylate-40-EO-6-PO	2515.529
Monostyrylphenol-prop-ethoxylate-38-EO-7-PO	2277.393681	Tristyrylphenol-prop-ethoxylate-5-EO-7-PO	1032.654
Monostyrylphenol-prop-ethoxylate-39-EO-7-PO	2321.419896	Tristyrylphenol-prop-ethoxylate-6-EO-7-PO	1076.68
Monostyrylphenol-prop-ethoxylate-40-EO-7-PO	2365.446111	Tristyrylphenol-prop-ethoxylate-7-EO-7-PO	1120.706
Monostyrylphenol-prop-ethoxylate-5-EO-8-PO	882.570458	Tristyrylphenol-prop-ethoxylate-8-EO-7-PO	1164.732
Monostyrylphenol-prop-ethoxylate-6-EO-8-PO	926.5966728	Tristyrylphenol-prop-ethoxylate-9-EO-7-PO	1208.759
Monostyrylphenol-prop-ethoxylate-7-EO-8-PO	970.6228876	Tristyrylphenol-prop-ethoxylate-10-EO-7-PO	1252.785
Monostyrylphenol-prop-ethoxylate-8-EO-8-PO	1014.649102	Tristyrylphenol-prop-ethoxylate-11-EO-7-PO	1296.811
Monostyrylphenol-prop-ethoxylate-9-EO-8-PO	1058.675317	Tristyrylphenol-prop-ethoxylate-12-EO-7-PO	1340.837
Monostyrylphenol-prop-ethoxylate-10-EO-8-PO	1102.701532	Tristyrylphenol-prop-ethoxylate-13-EO-7-PO	1384.864
Monostyrylphenol-prop-ethoxylate-11-EO-8-PO	1146.727747	Tristyrylphenol-prop-ethoxylate-14-EO-7-PO	1428.89
Monostyrylphenol-prop-ethoxylate-12-EO-8-PO	1190.753962	Tristyrylphenol-prop-ethoxylate-15-EO-7-PO	1472.916

Supplementary

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
Monostyrylphenol-prop-ethoxylate-13-EO-8-PO	1234.780176	Tristyrylphenol-prop-ethoxylate-16-EO-7-PO	1516.942
Monostyrylphenol-prop-ethoxylate-14-EO-8-PO	1278.806391	Tristyrylphenol-prop-ethoxylate-17-EO-7-PO	1560.968
Monostyrylphenol-prop-ethoxylate-15-EO-8-PO	1322.832606	Tristyrylphenol-prop-ethoxylate-18-EO-7-PO	1604.995
Monostyrylphenol-prop-ethoxylate-16-EO-8-PO	1366.858821	Tristyrylphenol-prop-ethoxylate-19-EO-7-PO	1649.021
Monostyrylphenol-prop-ethoxylate-17-EO-8-PO	1410.885035	Tristyrylphenol-prop-ethoxylate-20-EO-7-PO	1693.047
Monostyrylphenol-prop-ethoxylate-18-EO-8-PO	1454.91125	Tristyrylphenol-prop-ethoxylate-21-EO-7-PO	1737.073
Monostyrylphenol-prop-ethoxylate-19-EO-8-PO	1498.937465	Tristyrylphenol-prop-ethoxylate-22-EO-7-PO	1781.099
Monostyrylphenol-prop-ethoxylate-20-EO-8-PO	1542.96368	Tristyrylphenol-prop-ethoxylate-23-EO-7-PO	1825.126
Monostyrylphenol-prop-ethoxylate-21-EO-8-PO	1586.989895	Tristyrylphenol-prop-ethoxylate-24-EO-7-PO	1869.152
Monostyrylphenol-prop-ethoxylate-22-EO-8-PO	1631.016109	Tristyrylphenol-prop-ethoxylate-25-EO-7-PO	1913.178
Monostyrylphenol-prop-ethoxylate-23-EO-8-PO	1675.042324	Tristyrylphenol-prop-ethoxylate-26-EO-7-PO	1957.204
Monostyrylphenol-prop-ethoxylate-24-EO-8-PO	1719.068539	Tristyrylphenol-prop-ethoxylate-27-EO-7-PO	2001.231
Monostyrylphenol-prop-ethoxylate-25-EO-8-PO	1763.094754	Tristyrylphenol-prop-ethoxylate-28-EO-7-PO	2045.257
Monostyrylphenol-prop-ethoxylate-26-EO-8-PO	1807.120969	Tristyrylphenol-prop-ethoxylate-29-EO-7-PO	2089.283
Monostyrylphenol-prop-ethoxylate-27-EO-8-PO	1851.147183	Tristyrylphenol-prop-ethoxylate-30-EO-7-PO	2133.309
Monostyrylphenol-prop-ethoxylate-28-EO-8-PO	1895.173398	Tristyrylphenol-prop-ethoxylate-31-EO-7-PO	2177.335
Monostyrylphenol-prop-ethoxylate-29-EO-8-PO	1939.199613	Tristyrylphenol-prop-ethoxylate-32-EO-7-PO	2221.362
Monostyrylphenol-prop-ethoxylate-30-EO-8-PO	1983.225828	Tristyrylphenol-prop-ethoxylate-33-EO-7-PO	2265.388
Monostyrylphenol-prop-ethoxylate-31-EO-8-PO	2027.252042	Tristyrylphenol-prop-ethoxylate-34-EO-7-PO	2309.414
Monostyrylphenol-prop-ethoxylate-32-EO-8-PO	2071.278257	Tristyrylphenol-prop-ethoxylate-35-EO-7-PO	2353.44
Monostyrylphenol-prop-ethoxylate-33-EO-8-PO	2115.304472	Tristyrylphenol-prop-ethoxylate-36-EO-7-PO	2397.466
Monostyrylphenol-prop-ethoxylate-34-EO-8-PO	2159.330687	Tristyrylphenol-prop-ethoxylate-37-EO-7-PO	2441.493
Monostyrylphenol-prop-ethoxylate-35-EO-8-PO	2203.356902	Tristyrylphenol-prop-ethoxylate-38-EO-7-PO	2485.519
Monostyrylphenol-prop-ethoxylate-36-EO-8-PO	2247.383116	Tristyrylphenol-prop-ethoxylate-39-EO-7-PO	2529.545
Monostyrylphenol-prop-ethoxylate-37-EO-8-PO	2291.409331	Tristyrylphenol-prop-ethoxylate-40-EO-7-PO	2573.571
Monostyrylphenol-prop-ethoxylate-38-EO-8-PO	2335.435546	Tristyrylphenol-prop-ethoxylate-5-EO-8-PO	1090.696
Monostyrylphenol-prop-ethoxylate-39-EO-8-PO	2379.461761	Tristyrylphenol-prop-ethoxylate-6-EO-8-PO	1134.722
Monostyrylphenol-prop-ethoxylate-40-EO-8-PO	2423.487976	Tristyrylphenol-prop-ethoxylate-7-EO-8-PO	1178.748
Distyrylphenol-prop-ethoxylate--EO--PO	302.1670655	Tristyrylphenol-prop-ethoxylate-8-EO-8-PO	1222.774
Distyrylphenol-prop-ethoxylate-5-EO-1-PO	580.3400043	Tristyrylphenol-prop-ethoxylate-9-EO-8-PO	1266.801
Distyrylphenol-prop-ethoxylate-6-EO-1-PO	624.366219	Tristyrylphenol-prop-ethoxylate-10-EO-8-PO	1310.827
Distyrylphenol-prop-ethoxylate-7-	668.3924338	Tristyrylphenol-prop-ethoxylate-11-	1354.853

Supplementary

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
EO-1-PO		EO-8-PO	
Distyrylphenol-prop-ethoxylate-8-EO-1-PO	712.4186486	Tristyrylphenol-prop-ethoxylate-12-EO-8-PO	1398.879
Distyrylphenol-prop-ethoxylate-9-EO-1-PO	756.4448634	Tristyrylphenol-prop-ethoxylate-13-EO-8-PO	1442.905
Distyrylphenol-prop-ethoxylate-10-EO-1-PO	800.4710782	Tristyrylphenol-prop-ethoxylate-14-EO-8-PO	1486.932
Distyrylphenol-prop-ethoxylate-11-EO-1-PO	844.497293	Tristyrylphenol-prop-ethoxylate-15-EO-8-PO	1530.958
Distyrylphenol-prop-ethoxylate-12-EO-1-PO	888.5235078	Tristyrylphenol-prop-ethoxylate-16-EO-8-PO	1574.984
Distyrylphenol-prop-ethoxylate-13-EO-1-PO	932.5497226	Tristyrylphenol-prop-ethoxylate-17-EO-8-PO	1619.01
Distyrylphenol-prop-ethoxylate-14-EO-1-PO	976.5759373	Tristyrylphenol-prop-ethoxylate-18-EO-8-PO	1663.036
Distyrylphenol-prop-ethoxylate-15-EO-1-PO	1020.602152	Tristyrylphenol-prop-ethoxylate-19-EO-8-PO	1707.063
Distyrylphenol-prop-ethoxylate-16-EO-1-PO	1064.628367	Tristyrylphenol-prop-ethoxylate-20-EO-8-PO	1751.089
Distyrylphenol-prop-ethoxylate-17-EO-1-PO	1108.654582	Tristyrylphenol-prop-ethoxylate-21-EO-8-PO	1795.115
Distyrylphenol-prop-ethoxylate-18-EO-1-PO	1152.680797	Tristyrylphenol-prop-ethoxylate-22-EO-8-PO	1839.141
Distyrylphenol-prop-ethoxylate-19-EO-1-PO	1196.707011	Tristyrylphenol-prop-ethoxylate-23-EO-8-PO	1883.168
Distyrylphenol-prop-ethoxylate-20-EO-1-PO	1240.733226	Tristyrylphenol-prop-ethoxylate-24-EO-8-PO	1927.194
Distyrylphenol-prop-ethoxylate-21-EO-1-PO	1284.759441	Tristyrylphenol-prop-ethoxylate-25-EO-8-PO	1971.22
Distyrylphenol-prop-ethoxylate-22-EO-1-PO	1328.785656	Tristyrylphenol-prop-ethoxylate-26-EO-8-PO	2015.246
Distyrylphenol-prop-ethoxylate-23-EO-1-PO	1372.81187	Tristyrylphenol-prop-ethoxylate-27-EO-8-PO	2059.272
Distyrylphenol-prop-ethoxylate-24-EO-1-PO	1416.838085	Tristyrylphenol-prop-ethoxylate-28-EO-8-PO	2103.299
Distyrylphenol-prop-ethoxylate-25-EO-1-PO	1460.8643	Tristyrylphenol-prop-ethoxylate-29-EO-8-PO	2147.325
Distyrylphenol-prop-ethoxylate-26-EO-1-PO	1504.890515	Tristyrylphenol-prop-ethoxylate-30-EO-8-PO	2191.351
Distyrylphenol-prop-ethoxylate-27-EO-1-PO	1548.91673	Tristyrylphenol-prop-ethoxylate-31-EO-8-PO	2235.377
Distyrylphenol-prop-ethoxylate-28-EO-1-PO	1592.942944	Tristyrylphenol-prop-ethoxylate-32-EO-8-PO	2279.403
Distyrylphenol-prop-ethoxylate-29-EO-1-PO	1636.969159	Tristyrylphenol-prop-ethoxylate-33-EO-8-PO	2323.43
Distyrylphenol-prop-ethoxylate-30-EO-1-PO	1680.995374	Tristyrylphenol-prop-ethoxylate-34-EO-8-PO	2367.456
Distyrylphenol-prop-ethoxylate-31-EO-1-PO	1725.021589	Tristyrylphenol-prop-ethoxylate-35-EO-8-PO	2411.482
Distyrylphenol-prop-ethoxylate-32-EO-1-PO	1769.047804	Tristyrylphenol-prop-ethoxylate-36-EO-8-PO	2455.508
Distyrylphenol-prop-ethoxylate-33-EO-1-PO	1813.074018	Tristyrylphenol-prop-ethoxylate-37-EO-8-PO	2499.535
Distyrylphenol-prop-ethoxylate-34-EO-1-PO	1857.100233	Tristyrylphenol-prop-ethoxylate-38-EO-8-PO	2543.561
Distyrylphenol-prop-ethoxylate-35-EO-1-PO	1901.126448	Tristyrylphenol-prop-ethoxylate-39-EO-8-PO	2587.587
Distyrylphenol-prop-ethoxylate-36-EO-1-PO	1945.152663	Tristyrylphenol-prop-ethoxylate-40-EO-8-PO	2631.613
Distyrylphenol-prop-ethoxylate-37-EO-1-PO	1989.178877	Polyethanglykol-2-EO	106.063
Distyrylphenol-prop-ethoxylate-38-EO-1-PO	2033.205092	Polyethanglykol-3-EO	150.0892

Supplementary

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
Distyrylphenol-prop-ethoxylate-39-EO-1-PO	2077.231307	Polyethanglykol-4-EO	194.1154
Distyrylphenol-prop-ethoxylate-40-EO-1-PO	2121.257522	Polyethanglykol-5-EO	238.1416
Distyrylphenol-prop-ethoxylate-5-EO-2-PO	638.3818691	Polyethanglykol-6-EO	282.1679
Distyrylphenol-prop-ethoxylate-6-EO-2-PO	682.4080839	Polyethanglykol-7-EO	326.1941
Distyrylphenol-prop-ethoxylate-7-EO-2-PO	726.4342987	Polyethanglykol-8-EO	370.2203
Distyrylphenol-prop-ethoxylate-8-EO-2-PO	770.4605135	Polyethanglykol-9-EO	414.2465
Distyrylphenol-prop-ethoxylate-9-EO-2-PO	814.4867283	Polyethanglykol-10-EO	458.2727
Distyrylphenol-prop-ethoxylate-10-EO-2-PO	858.5129431	Polyethanglykol-11-EO	502.2989
Distyrylphenol-prop-ethoxylate-11-EO-2-PO	902.5391578	Polyethanglykol-12-EO	546.3251
Distyrylphenol-prop-ethoxylate-12-EO-2-PO	946.5653726	Polyethanglykol-13-EO	590.3514
Distyrylphenol-prop-ethoxylate-13-EO-2-PO	990.5915874	Polyethanglykol-14-EO	634.3776
Distyrylphenol-prop-ethoxylate-14-EO-2-PO	1034.617802	Polyethanglykol-15-EO	678.4038
Distyrylphenol-prop-ethoxylate-15-EO-2-PO	1078.644017	Polyethanglykol-16-EO	722.43
Distyrylphenol-prop-ethoxylate-16-EO-2-PO	1122.670232	Polyethanglykol-17-EO	766.4562
Distyrylphenol-prop-ethoxylate-17-EO-2-PO	1166.696447	Polyethanglykol-18-EO	810.4824
Distyrylphenol-prop-ethoxylate-18-EO-2-PO	1210.722661	Polyethanglykol-19-EO	854.5086
Distyrylphenol-prop-ethoxylate-19-EO-2-PO	1254.748876	Polyethanglykol-20-EO	898.5349
Distyrylphenol-prop-ethoxylate-20-EO-2-PO	1298.775091	Polyethanglykol-21-EO	942.5611
Distyrylphenol-prop-ethoxylate-21-EO-2-PO	1342.801306	Polyethanglykol-22-EO	986.5873
Distyrylphenol-prop-ethoxylate-22-EO-2-PO	1386.827521	Polyethanglykol-23-EO	1030.614
Distyrylphenol-prop-ethoxylate-23-EO-2-PO	1430.853735	Polyethanglykol-24-EO	1074.64
Distyrylphenol-prop-ethoxylate-24-EO-2-PO	1474.87995	Polyethanglykol-25-EO	1118.666
Distyrylphenol-prop-ethoxylate-25-EO-2-PO	1518.906165	Polyethanglykol-26-EO	1162.692
Distyrylphenol-prop-ethoxylate-26-EO-2-PO	1562.93238	Polyethanglykol-27-EO	1206.718
Distyrylphenol-prop-ethoxylate-27-EO-2-PO	1606.958594	Polyethanglykol-28-EO	1250.745
Distyrylphenol-prop-ethoxylate-28-EO-2-PO	1650.984809	Polyethanglykol-29-EO	1294.771
Distyrylphenol-prop-ethoxylate-29-EO-2-PO	1695.011024	Polyethanglykol-30-EO	1338.797
Distyrylphenol-prop-ethoxylate-30-EO-2-PO	1739.037239	Polyethanglykol-31-EO	1382.823
Distyrylphenol-prop-ethoxylate-31-EO-2-PO	1783.063454	Polyethanglykol-32-EO	1426.849
Distyrylphenol-prop-ethoxylate-32-EO-2-PO	1827.089668	Polyethanglykol-33-EO	1470.876
Distyrylphenol-prop-ethoxylate-33-EO-2-PO	1871.115883	Polyethanglykol-34-EO	1514.902
Distyrylphenol-prop-ethoxylate-34-EO-2-PO	1915.142098	Polyethanglykol-35-EO	1558.928

Supplementary

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
Distyrylphenol-prop-ethoxylate-35-EO-2-PO	1959.168313	Polyethanglykol-36-EO	1602.954
Distyrylphenol-prop-ethoxylate-36-EO-2-PO	2003.194528	Polyethanglykol-37-EO	1646.981
Distyrylphenol-prop-ethoxylate-37-EO-2-PO	2047.220742	Polyethanglykol-38-EO	1691.007
Distyrylphenol-prop-ethoxylate-38-EO-2-PO	2091.246957	Polyethanglykol-39-EO	1735.033
Distyrylphenol-prop-ethoxylate-39-EO-2-PO	2135.273172	Polyethanglykol-40-EO	1779.059
Distyrylphenol-prop-ethoxylate-40-EO-2-PO	2179.299387	Methyl-Ethandiol-2-EO	134.0943
Distyrylphenol-prop-ethoxylate-5-EO-3-PO	696.423734	Methyl-Ethandiol-3-EO	192.1362
Distyrylphenol-prop-ethoxylate-6-EO-3-PO	740.4499488	Methyl-Ethandiol-4-EO	250.178
Distyrylphenol-prop-ethoxylate-7-EO-3-PO	784.4761636	Methyl-Ethandiol-5-EO	308.2199
Distyrylphenol-prop-ethoxylate-8-EO-3-PO	828.5023783	Methyl-Ethandiol-6-EO	366.2618
Distyrylphenol-prop-ethoxylate-9-EO-3-PO	872.5285931	Methyl-Ethandiol-7-EO	424.3036
Distyrylphenol-prop-ethoxylate-10-EO-3-PO	916.5548079	Methyl-Ethandiol-8-EO	482.3455
Distyrylphenol-prop-ethoxylate-11-EO-3-PO	960.5810227	Methyl-Ethandiol-9-EO	540.3873
Distyrylphenol-prop-ethoxylate-12-EO-3-PO	1004.607237	Methyl-Ethandiol-10-EO	598.4292
Distyrylphenol-prop-ethoxylate-13-EO-3-PO	1048.633452	Methyl-Ethandiol-11-EO	656.4711
Distyrylphenol-prop-ethoxylate-14-EO-3-PO	1092.659667	Methyl-Ethandiol-12-EO	714.5129
Distyrylphenol-prop-ethoxylate-15-EO-3-PO	1136.685882	Methyl-Ethandiol-13-EO	772.5548
Distyrylphenol-prop-ethoxylate-16-EO-3-PO	1180.712097	Methyl-Ethandiol-14-EO	830.5967
Distyrylphenol-prop-ethoxylate-17-EO-3-PO	1224.738311	Methyl-Ethandiol-15-EO	888.6385
Distyrylphenol-prop-ethoxylate-18-EO-3-PO	1268.764526	Methyl-Ethandiol-16-EO	946.6804
Distyrylphenol-prop-ethoxylate-19-EO-3-PO	1312.790741	Methyl-Ethandiol-17-EO	1004.722
Distyrylphenol-prop-ethoxylate-20-EO-3-PO	1356.816956	Methyl-Ethandiol-18-EO	1062.764
Distyrylphenol-prop-ethoxylate-21-EO-3-PO	1400.843171	Methyl-Ethandiol-19-EO	1120.806
Distyrylphenol-prop-ethoxylate-22-EO-3-PO	1444.869385	Methyl-Ethandiol-20-EO	1178.848
Distyrylphenol-prop-ethoxylate-23-EO-3-PO	1488.8956	Methyl-Ethandiol-21-EO	1236.89
Distyrylphenol-prop-ethoxylate-24-EO-3-PO	1532.921815	Methyl-Ethandiol-22-EO	1294.932
Distyrylphenol-prop-ethoxylate-25-EO-3-PO	1576.94803	Methyl-Ethandiol-23-EO	1352.973
Distyrylphenol-prop-ethoxylate-26-EO-3-PO	1620.974245	Methyl-Ethandiol-24-EO	1411.015
Distyrylphenol-prop-ethoxylate-27-EO-3-PO	1665.000459	Methyl-Ethandiol-25-EO	1469.057
Distyrylphenol-prop-ethoxylate-28-EO-3-PO	1709.026674	Methyl-Ethandiol-26-EO	1527.099
Distyrylphenol-prop-ethoxylate-29-EO-3-PO	1753.052889	Methyl-Ethandiol-27-EO	1585.141
Distyrylphenol-prop-ethoxylate-30-EO-3-PO	1797.079104	Methyl-Ethandiol-28-EO	1643.183

Supplementary

Compound	Exact Mass [m/z]	Compound	Exact Mass [m/z]
Distyrylphenol-prop-ethoxylate-31-EO-3-PO	1841.105318	Methyl-Ethandiol-29-EO	1701.225
Distyrylphenol-prop-ethoxylate-32-EO-3-PO	1885.131533	Methyl-Ethandiol-30-EO	1759.267
Distyrylphenol-prop-ethoxylate-33-EO-3-PO	1929.157748	Methyl-Ethandiol-31-EO	1817.308
Distyrylphenol-prop-ethoxylate-34-EO-3-PO	1973.183963	Methyl-Ethandiol-32-EO	1875.35
Distyrylphenol-prop-ethoxylate-35-EO-3-PO	2017.210178	Methyl-Ethandiol-33-EO	1933.392
Distyrylphenol-prop-ethoxylate-36-EO-3-PO	2061.236392	Methyl-Ethandiol-34-EO	1991.434
Distyrylphenol-prop-ethoxylate-37-EO-3-PO	2105.262607	Methyl-Ethandiol-35-EO	2049.476
Distyrylphenol-prop-ethoxylate-38-EO-3-PO	2149.288822	Methyl-Ethandiol-36-EO	2107.518
Distyrylphenol-prop-ethoxylate-39-EO-3-PO	2193.315037	Methyl-Ethandiol-37-EO	2165.56
Distyrylphenol-prop-ethoxylate-40-EO-3-PO	2237.341252	Methyl-Ethandiol-38-EO	2223.601
Distyrylphenol-prop-ethoxylate-5-EO-4-PO	754.4655988	Methyl-Ethandiol-39-EO	2281.643
Distyrylphenol-prop-ethoxylate-6-EO-4-PO	798.4918136	Methyl-Ethandiol-40-EO	2339.685

6.5 General Conclusion and Outlook

No supplemental

6.6 List of Publications

Publications in peer-reviewed journals

Glaubitz J, Schmidt TC (2013)

LC-MS Quantification of a Sulfosuccinate Surfactant in Agrochemical Formulations. Chromatographia 76:1729-1737.

Oral presentations

Glaubitz J, Schmidt TC

Characterization of formulation additives in agrochemical products via liquid chromatography-mass spectrometry

Essen (Germany) Anakon 2013, March 4 – March 7, 2013

Glaubitz J, Schmidt TC

Characterisation of formulation additives in agrochemical products via liquid chromatography-mass spectrometry

Hohenroda (Germany) 23. Doktorandenseminar des Arbeitskreises Separation Science der GDCh-Fachgruppe Analytische Chemie, January 6 – January 8, 2013

6.7 Curriculum Vitae

The curriculum vitae is not included in this online version, due to protection of data privacy.

Supplementary

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All the love to Haoyun my wife, without her everlasting support, patience and love this work would have never been possible or even a success.

6.8 Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit mit dem Titel

„Chromatographic and Spectroscopic Characterization of Surfactants used for Agrochemical Products"

selbst verfasst und keine außer den angegebenen Hilfsmitteln und Quellen benutzt habe, und dass die Arbeit in dieser oder ähnlicher Form noch bei keiner anderen Universität eingereicht wurde.

Essen, im März 2014