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Characterisation of commercially available linear alkylbenzenesulfonates by LC-SPE-NMR/MS (liquid chromatography-solid phase extraction-nuclear magnetic resonance spectroscopy-mass spectroscopy)

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ABSTRACT

Commercially available linear alkylbenzenesulfonates (LASs) are a mixture of various homologues and isomers, leading to 20 major species. In this work we investigated the commercial product by liquid chromatography-solid phase extraction-nuclear magnetic resonance spectroscopy-mass spectrometry (LC-SPE-NMR/MS). The commercial product was separated into 17 fractions by liquid chromatography (LC). After chromatographic separation, 5% of the flow was split to a mass spectrometer (MS) while 95% was send to post-column solid phase extraction cartridges for enrichment of the analytes (LC-SPE). After elution from the SPE-cartridges a NMR-spectrometer equipped with a cryo-probe was used for the characterisation of the different LASs species. For the first time ^{1}H-1D and H-H-COSY spectra for 14 LASs species out of 20 major isomers are presented, whereas the 6 remaining species are detected as mixtures in 3 ^{1}H-1D and H-H-COSY spectra. These data were used to correlate the chromatographic retention of the LASs isomers to the substitution pattern of the alkyl chain.

1. Introduction

LASs are one of the most important anionic surfactants. The worldwide consumption in 2000 was about 3.2 MT, mostly used in detergents [1]. Due to its application and wide usage an acute contamination to sewage has been reported [2,3].

In activated sewage sludge treatment plants (as-STPs) about 99% of the LASs are removed from the influent. However, up to 20% remains in the sludge [4,5]. Under anaerobic conditions LASs will not be degenerated and accumulate in the sludge [6]. Due to the fast biodegradation under aerobic conditions [7–9], LASs concentrations in surface water treated by sewage treatment plants (STPs) are classified as low (concentrations downstream ranged up to 10 $\mu g\,L^{-1}$ [3,10]). However, in many regions and countries the sewage is not treated by STPs, due to its missing or the lack of connection to households. Eichhorn et al. determined LASs in Brazilian surface water which was burdened with untreated domestic sewage. The concentrations ranged between 14 and 155 $\mu g\,L^{-1}$ [11].

The commercial products of LASs are available as sodium salts and consist of up to 5 groups of LASs which differ in the number of CH_2-groups in the alkyl chain (C_{10}–C_{14}). Each group contains up to 6 positional isomers (Fig. 1). The relative distribution of these groups varies from 5 to 10% for C_{10}, 40 to 45% for C_{11}, 35 to 40% for C_{12}, 10 to 15% for C_{13} and 0 to 1% for C_{14} [12,13]. This leads to 20 major LASs isomers (26 species if C_{14} is included) (Fig. 1).

Monitoring studies determined LASs as a sum parameter or the distribution of the groups with a different numbers of CH_2-groups in the alkyl chain. Quantification is done by external calibration using the technical product itself [2,14,15] or by adding an internal standard [15–18] (mostly 4n-octylbenzenesulfonate (1-C_8-LAS)). Toxicity studies have shown, that the groups with different numbers of CH_2-groups in the alkyl chain offer a different degree of toxicity to aquatic organisms. Isomers carrying longer alkyl chains are found to be more toxic [19,20]. Moreover, the half-lives for different LASs species under aerobic conditions are different [7,21]. Biodegradation of the longer alkyl chain isomers is faster than for shorter ones. To achieve an explicit risk assessment, methods are needed which provide the potential to identify and quantify as many LASs species as possible.

Various methods for the determination of LASs are described in the literature. Gas chromatographic (GC) [15,22,23] and high performance liquid chromatographic (HPLC) [24–26] methods are

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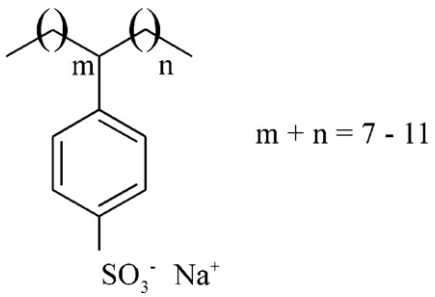

Fig. 1. General chemical structure of LASs. Variation of the marks m and n describe different amounts of CH_2 and all possible isomers in the commercial product.

widely used. Furthermore capillary electrophoresis (CE) [27,28] and immunoaffinity chromatography (IAC) methods are described [29]. The characterisation of LASs species can be arranged by GC–MS after derivatisation [15,23,30,31]. An adequate separation for most of the isomers is achieved with exception of some inner isomers (the phenyl group is substituted at the inner carbons of the alkyl chain). The fragmentation pattern of the different LASs species in the CI/EI-source is structure dependent, so that characterisation and structural assignment of each species is possible. The separation of LASs species which is achieved by LC is inferior to the GC separation and only the outer isomers are separated adequately [24,32,33]. The fragmentation pattern obtained from collision induced dissociation experiments (CID) of the (LC-) MS is not characteristic for positional isomers. This leads to uncertain conclusions in terms of an explicit LC–MS peak assignment. Therefore LC–MS is mostly used to differentiate between LASs groups with a different number of carbons in the alkyl chain [32,34]. Nevertheless, Lunar et al. described a LC–MS method which is capable to identify different LASs species in technical products [24].

Other investigations of LASs were carried out by using ^{1}H NMR. Goon et al. [35] used ^{1}H spectra to characterise a technical product. The signals in the ^{1}H spectra were not specific enough to differentiate individual LASs species. Furthermore ^{1}H spectra of some pure synthesised species were compared and discussed. Field et al. [36] and Thurman et al. [37] used ^{13}C spectra to determine LASs in groundwater. The ^{13}C spectra offer a higher spectral dispersion compared to the ^{1}H spectra and is thus more capable to discriminate between LASs species. The drawback of the ^{13}C NMR in comparison to the ^{1}H NMR is the lower sensitivity. Via ^{13}C NMR only the inner isomers can be differentiated from the outer isomers without providing a specific characterisation of homologues.

In this current work we investigated the technical LASs product with respect to LASs species characterisation via LC-SPE-NMR/MS. This hyphenated technique has become a valuable tool for the characterisation of drug metabolites [38] and phytochemical compounds [39–42]. In the current work we used HPLC to separate the LASs species into different fractions to address the problems reported by Goon et al. to characterise the technical LASs product by ^{1}H NMR. Subsequent fraction collection using post-column SPE allows an enrichment of LASs isomers resulting in a better signal to noise ratio for NMR spectra compared to traditional fractionation techniques. Mass spectra using electrospray ionization (ESI)-iontrap MS were recorded to ensure the elution order of LASs groups with a different number of CH_2-groups within the alkyl chain. To identify positional isomers, which is impossible with electrospray mass spectroscopy alone, NMR spectroscopy was used. Data are presented, which allow an explicit assignment of resonance signals to individual LASs species using ^{1}H-1D and H-H-COSY spectra. In addition, these data were used to assign individual LASs isomers to the chromatographic peak pattern.

2. Experimental

2.1. Chemicals

The commercial LASs product and ammonium acetate were purchased from Sigma-Aldrich. Acetonitrile and methanol-d_4 (purity 99.8%) were purchased from Deutero GmbH (Kastellaun, Germany). Water was obtained from a Milli-Q purification station from Millipore (Billerica, MA, USA).

2.2. Instrumentation

HPLC: An Agilent 1100 HPLC system equipped with a quaternary pump, an auto sampler and a DAD was used for liquid chromatographic separation prior to post-column solid phase extraction (Waldbronn, Germany). The separation was carried out on a Phenomenex Prodigy ODS3 column (250 mm × 4.6 mm, 5 μm particle size) (Aschaffenburg, Germany).

MS: MS spectra were recorded on an ESQUIRE-3000 ion trap mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with an electrospray ionization source.

SPE: The automatic post-column SPE was performed by a Prospekt 2 LC-SPE-NMR interface (Spark Holland, Emmen, The Netherlands) and individual LASs isomers where enriched on Hysphere GP (10 mm × 2 mm) SPE cartridges.

NMR: For NMR-experiments a Bruker 600 MHz AVII NMR spectrometer equipped with a 5 mm TCI-Cryoprobe and 30 μL Cryofit was used (Rheinstetten, Germany). Hystar 3.1 and Topspin 2.1 were used for LC-SPE-NMR data acquisition and processing (Bruker, Rheinstetten, Germany).

2.3. Conditions and procedure

Chromatography: Two solvent mixtures where prepared, solvent A (85% ACN and 15% H_2O) and solvent B (25% ACN and 75% H_2O). Both contained 10 mM Ammonium acetate as ion pair reagent for enhanced retention of LASs. A linear gradient (t = 0 min A = 22% to t = 40 min A = 68%) was used for LASs separation, followed by a washing- and equilibration step (5 min A = 100%, 10 min starting conditions). The flow rate was set to 0.6 mL min^{-1}. The injection volume was 30 μL and the LASs concentrations were 2.33 mg L^{-1}. LASs were dissolved in a solvent mixture which is equivalent to the mobile phase at starting conditions. The DAD detection wavelength was set to 225 nm. For LC-DAD-SPE-NMR/MS hyphenation a BNMI (Bruker NMR/MS-Interface, Rheinstetten, Germany) was used to split the eluate post-column into 2 fractions: 5% of the eluate was used for MS data acquisition and 95% of the eluate was used for post-column peak trapping via SPE.

SPE: A chromatographic test run was performed to determine the retention times of the different LASs species and the time settings for fraction collection (Fig. 2). To increase the retention of the separated LAS species on the SPE cartridges a post-column water addition was set to a flow rate of 1.8 mL min^{-1} (equivalent to 3:1 water/eluate ratio). The post-column water addition was applied with a Knauer K-120 HPLC pump (Knauer, Berlin, Germany). The separation was repeated 4 times and the eluate within each fraction collection time frame was automatically directed to the same SPE cartridge (multiple peak trapping). Subsequently the cartridges were dried with nitrogen for 30 min and each enriched LAS was eluted automatically with 295 μL of deuterated methanol into the NMR flow cell.

MS: MS data were recorded in a scan range between 50 and 400 Da using an electrospray interface in negative ionization mode. The $[M-Na]^-$ ions were selected for MS/MS experiments. The ESI-MS parameters are summarised in Table 1.

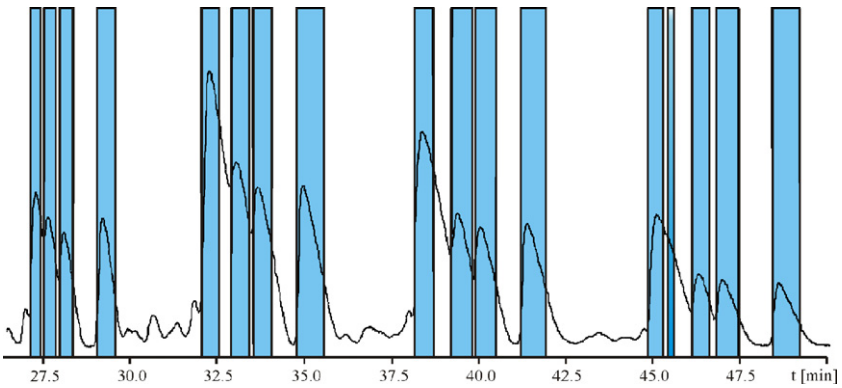

Fig. 2. HPLC-UV chromatogram of a commercial LASs product. The time frames for fraction collection are blue coloured.

Table 1
ESI-MS acquisition parameters.

Capi1lary voltage [V]	3500
Capillary exit voltage [V]	−93.4
Dry temperature [°C]	300
Dry gas flow [L min^{-1}]	11
Nebuliser gas pressure [psi]	69

NMR: Prior to the elution process the flow cell was dried with nitrogen to avoid diffusion effects inside the transfer capillary or the NMR probe. For ^{1}H measurements a 1D version of the NOESY pulse sequence (lc1pncwps) was used. This pulse programme offers double solvent suppression via presaturation with shaped pulses to obtain the suppression of the residual HDO water signal and the CD_2HOD methanol signal. Additionally this programme uses CW-decoupling for the elimination of the ^{13}C satellites. H-H-COSY spectra were recorded using a gradient-selected magnitude mode COSY (cosygpqf). The NMR acquisition and processing parameter for ^{1}H-1D and H-H-COSY experiments are listed in Table 2.

3. Results and discussion

3.1. HPLC-UV-SPE

For the characterisation of LASs species an HPLC method was developed and optimised. A polar-embedded stationary phase with a water/acetonitrile-gradient and ammonium acetate as ion pair reagent was used. The UV-chromatogram of the commercial LASs, which was obtained under these conditions, is shown in Fig. 2. The peak tailing can be explained by the high concentrations of LASs in the sample analysed (2.33 mg mL^{-1}) in combination with the high injection volume (30 μL).

The resolution of the LC separation is not sufficient for a chromatographic separation of all 20 major LASs species. Only 16 peaks appear in the chromatogram, as some isomers are co-eluting. 17 time windows for fraction collection have been set for enrichment of the LASs species by SPE. The fraction collection and accumulation by post-column SPE are described in Section 2.

3.2. MS

Investigations concerning the identification of LASs species were mostly done by LC–MS [24,32–34]. The determination of the isomers is not straightforward. However, the distinction between the groups with different molecular masses (C_{10}, C_{11}, C_{12}, C_{13}) is easy to achieve by mass spectrometry. These LASs groups elute according to the length of their alkyl chain, whereas C_{10} elutes first. In the present work for LC-SPE-NMR/MS investigations different HPLC conditions were used compared to those which are described in literature. For this reason mass spectrometry was used to ensure the elution order of LASs groups via their $[M-Na]^-$ mass peaks. Additionally MS/MS spectra were recorded to proof the existence of isomer specific mass fragments [24]. MS and MS/MS spectra of 4 selected peaks (retention time: 29.15 min, 34.85 min, 41.25 min,

Table 2
NMR acquisition and processing parameters for ^{1}H and H-H-COSY experiments.

	^{1}H	H-H-COSY
Acquisition		
Pulse programme	1c1pncwps	cosygpqf
Number of complex data points in F2	16,384	2048
Number of complex data points in Fl	–	256
Numbers of scans	128	16 or 32
Number of dummy scans	16	8
Spectral width F2 [Hz]	6009.6	1539.4
Spectral width Fl [Hz]		1539.4
Transmitter frequency offset (O1) in F2 [Hz]	2922.6	1079.8
Transmitter frequency offset (O1) in F1 [Hz]		1079.8
Transmitter frequency offset (O2) in F2 [Hz] (not observed)	7391.0	–
Relaxation delay (D1) [s]	3.00	2.00
Processing		
Size of real spectrum in F2	32,768	1024
Size of real spectrum in Fl	–	1024
Window function for F2	em	sine
Window function for F1	–	sine
Line broadening for cm in F2 [Hz]	1.0	–
Number of FID data points used by ft in F2	–	512
Number of FID data points used by ft in F1	–	0

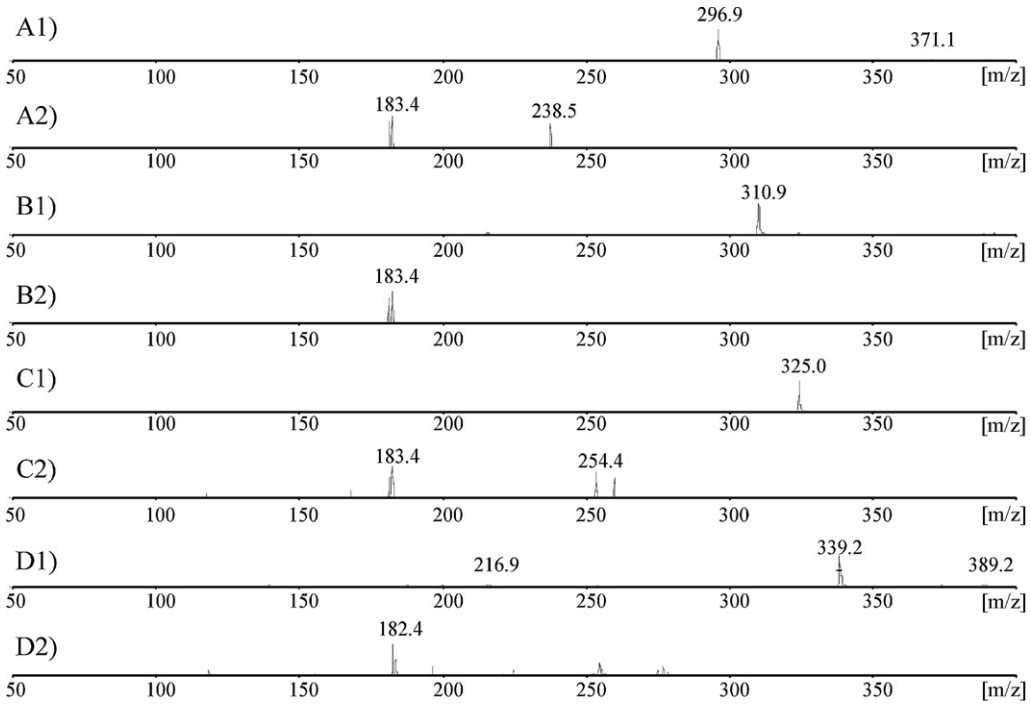

Fig. 3. MS spectra and their according MS/MS spectra of four LC–MS peaks of a separation of technical LASs. A1: MS spectrum, A2: MS/MS spectrum of the LC–MS peak at RT: 29.15 min; B1: MS spectrum, B2: MS/MS spectrum of the LC–MS peak at RT: 34.85 min; C1: MS spectrum, C2: MS/MS spectrum of the LC–MS peak at RT: 41.25 min; D1: MS spectrum, D2: MS/MS spectrum of the LC–MS peak at RT: 48.43 min; RT = retention time.

48.43 min (LC–MS retention times correspond to the LC-UV retention times shown in Fig. 2)) are shown in Fig. 3.

By observing the $[M-Na]^-$ peak in the mass spectra a distinction between groups of LASs with different numbers of CH_2-groups in the alkyl chain is easy to achieve (Fig. 3). C_{10}-LASs species show a $[M-Na]^-$ peak with *m/z* of 297, C_{11}-LASs species with *m/z* of 311, C_{11}-LASs species with *m/z* of 325 and C_{13}-LASs species with *m/z* of 339. The MS/MS spectra offer a typical LASs fragment ion $[C_8H_7O_3S]^-$ with *m/z* of 183 which can be found by other mass spectrometric techniques as well [43]. No isomer specific mass fragments could be observed. The individual retention times in HPLC-MS, their according $[M-Na]^-$ MS peaks and MS/MS fragments are summarised in Table 3.

3.3. NMR

NMR signal assignment is based on the nomenclature shown in Fig. 4. In this case 6-C_{11}-LAS is used as an example. Generally, LASs species are described by the expression *x*-C_y-LAS, where *x* describes the substitution position of the aromatic ring at the alkyl chain and *y* refers to the total number of carbons of the alkyl chain.

The C_{10}-LASs group contain the lowest number of LASs isomers, namely four. All four species are showing the best chromatographic separation of all LASs groups. Therefore the following assignment via NMR is exemplified for the four C_{10}-LASs isomers. Their H-H-COSY-spectra are shown in Fig. 5. All proton signals for all four C_{10} isomers have been assigned. The following strategy for characterisation was used: At first a starting point for the assignment needs to be defined. In this case the α-proton is used as starting point, because of its highest downfield shift of all aliphatic protons (the α-proton is bonded to a tertiary carbon). From this resonance the assignments for the signals of the aliphatic protons were identified using the H-H-COSY-correlations. The colour coding of the cross peaks compares to the assignment of protons visualised in Fig. 4. The differentiation can be done by identifying the specific H-H-COSY-correlation for each isomer. It can be detected between the

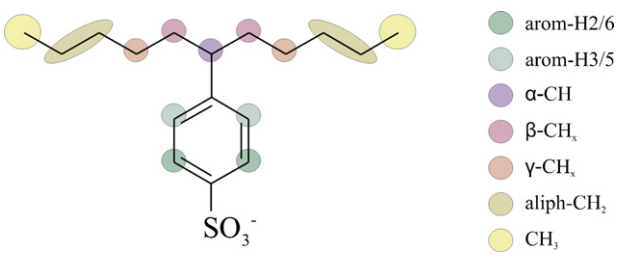

Fig. 4. Nomenclature for LASs, exemplified for 6-C_{11}-LAS. In every LAS isomer the tertiary carbon of the alkyl chain and its bonded hydrogen is defined as the α-CH group.

Table 3
LC-ESI-MS-retention times, LC-ESI-MS *m/z* peaks of the parent ions ($[M-Na]^-$) in mass spectra and detectable fragment ions in MS/MS spectra of all major LASs species. RT = retention time; displayed data were produced using the LC–MS/-SPE-NMR method described in Section 2.

LASs group	RT [min]	*m/z*	
		MS $[M-Na]^-$	MS/MS $[C_8H_7O_3S]^-$
C10	27.25	296.9	182.4
C10	27.67	297.0	182.5
C10	28.08	297.0	182.5
C10	29.15	296.9	182.5
C11	32.13	310.9	182.5
C11	32.89	311.0	182.4
C11	33.62	310.9	182.5
C11	34.85	310.9	182.5
C12	38.22	325.0	182.4
C12	39.32	325.0	182.5
C12	39.95	325.0	182.5
C12	41.25	325.0	182.5
C13	44.95	330.1	182.5
C13	46.18	339.2	182.4
C13	46.87	339.2	182.5
C13	48.43	339.2	182.4

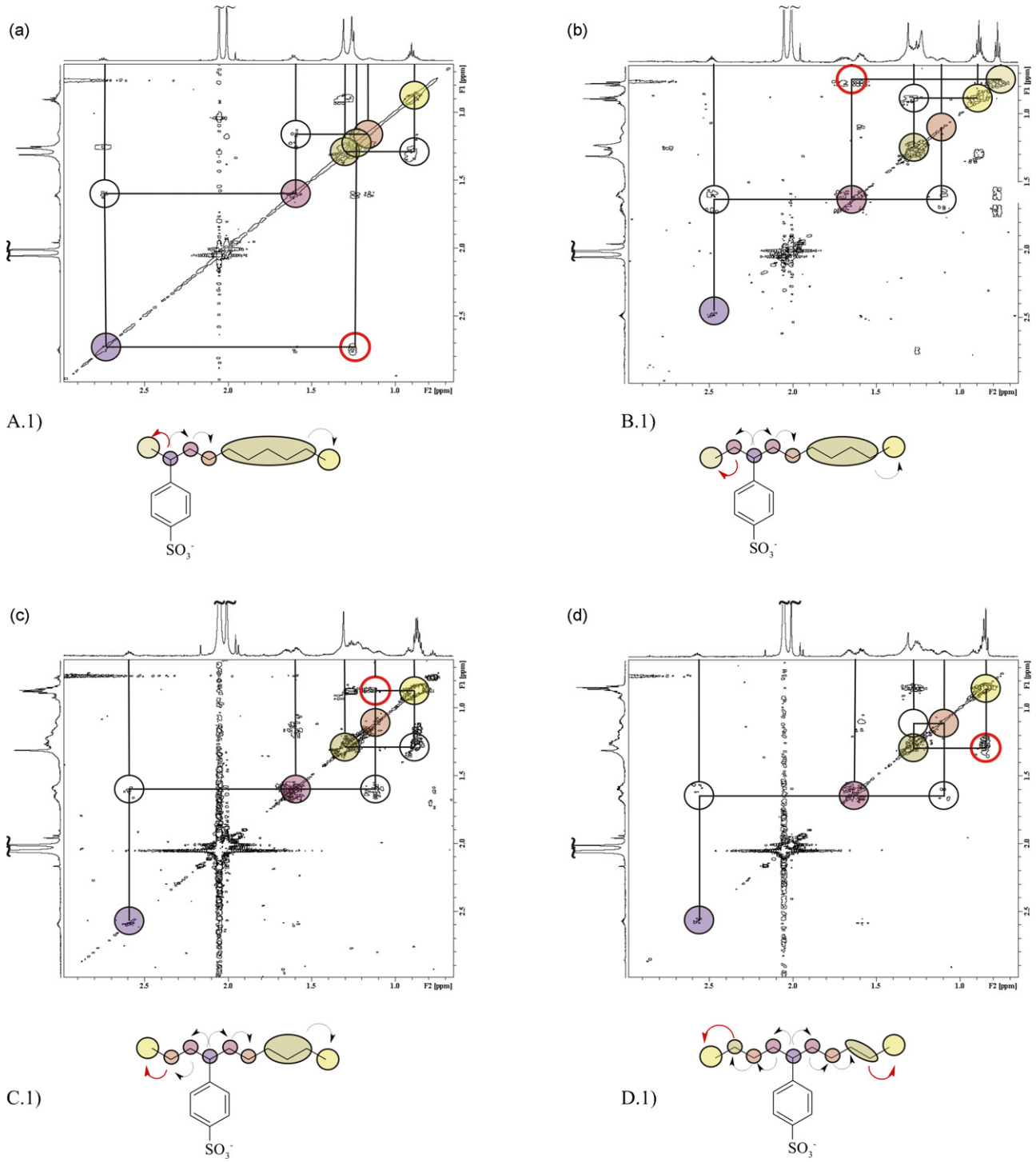

Fig. 5. A) H-H-COSY spectra of 2-C_{10}-LAS; A1) Structure and H-H-COSY correlations of 2-C_{10}-LAS; B) H-H-COSY spectra of 3-C_{10}-LAS; B1) Structure and H-H-COSY correlations of 3-C_{10}-LAS; C) H-H-COSY spectra of 4-C_{10}-LAS; C1) Structure and H-H-COSY correlations of 4-C_{10}-LAS; D) H-H-COSY spectra of 5-C_{10}-LAS; D1) Structure and H-H-COSY correlations of 5-C_{10}-LAS; Species specific correlations are highlighted in red.

protons of the methyl group of the shorter alkyl chain and its vicinal proton or proton group, because every such methyl group has a vicinal proton or group of protons which differ regarding their chemical shifts.

Spectrum A shows the COSY spectrum of 2-C_{10}-LAS (Fig. 5, A1). The α-proton (2.75 ppm) shows a correlation to the β-CH_2-protons which is not specific for any LASs species (1.61 ppm) together with a correlation to a methyl group (1.25 ppm) in β-position which is specific for the 2-isomer only. Furthermore a correlation between the β-CH_2-protons and the γ-CH_2-protons (1.15/1.23 ppm) and a correlation between the aliphatic CH_2-groups (1.25–1.30 ppm) and the methyl group (0.90 ppm) of the longer alkyl chain are visible. However, due to the small intensity and their broad peak forms no correlation between the γ-CH_2-protons and the aliphatic CH_2-groups are detectable.

Spectrum B shows the COSY spectrum of 3-C_{10}-LAS (Fig. 5, B1). The α-proton (2.48 ppm) shows a correlation to the vicinal protons of the CH_2-groups in β-position (1.59/1.69 ppm). The β-protons

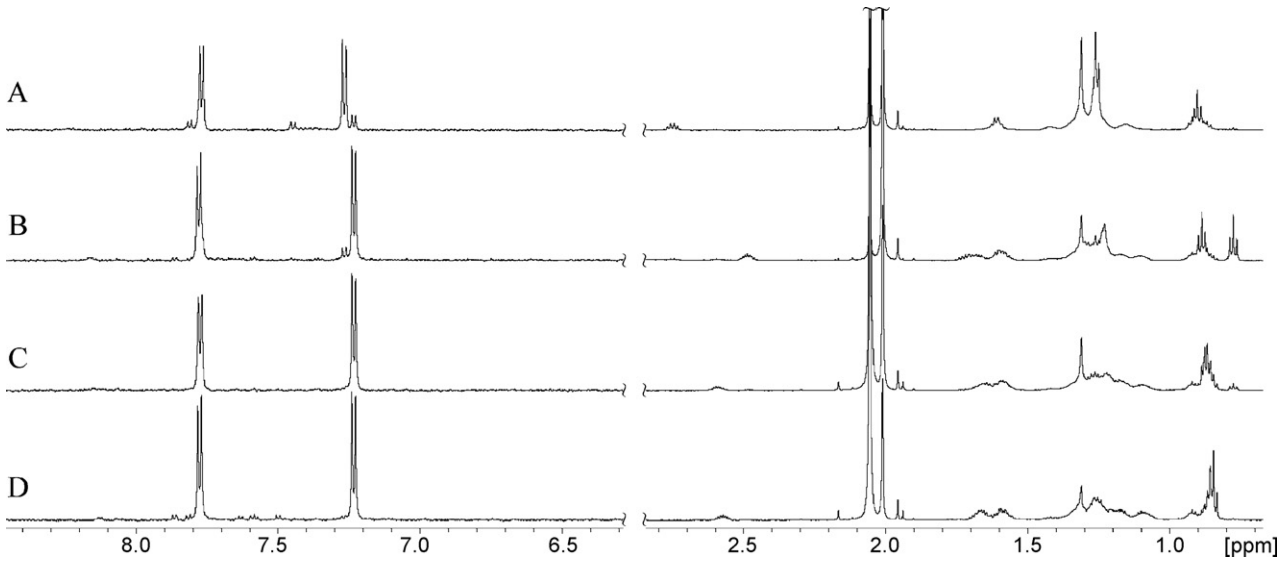

Fig. 6. ^{1}H spectra of A) 2-C_{10}-LAS; B) 3-C_{10}-LAS; C) 4-C_{10}-LAS; D) 5-C_{10}-LAS.

offer correlations to the γ-CH_2-group (0.89 ppm) of the longer alkyl chain and a correlation to the CH_2-group in γ-position (0.78 ppm) which is only observable within the 3-isomer. Additionally a correlation from the aliphatic CH_2-groups (1.20–1.30 ppm) to the methyl group (0.98 ppm) of the longer alkyl chain is detected.

Spectrum C displays the COSY spectrum of the 4-C_{10}-LAS (Fig. 5, C1). The α-proton (2.59 ppm) correlates with the β-protons (1.59/1.65 ppm), which correlate with the vicinal γ-protons (1.01/1.17 ppm). The γ-protons show a correlation to a methyl group (0.85 ppm), which is specific for the 4-isomer. Furthermore a correlation between the aliphatic CH_2-groups (1.19–1.30 ppm) and the methyl group (0.85 ppm) of the longer alkyl chain is detected.

Spectrum D shows the H-H-COSY spectrum of 5-C_{10}-LAS (Fig. 5, D1). The α-proton (2.57 ppm) correlates with the β-protons (1.59/1.66 ppm), which additionally correlate with the vicinal γ-protons. Furthermore the γ-protons (1.01/1.17 ppm) show a correlation to the protons of the aliphatic CH_2-groups (1.19–1.30 ppm). The 5-isomer specific correlation is detectable between the aliphatic CH_2-groups and the methyl groups (0.84 ppm), whereas no other correlation to the methyl groups exists.

After the chromatographic and spectroscopic identification of the LASs isomers of the C_{10}-LASs group is achieved, the identification of all LASs isomers via comparisons of ^{1}H-spectra of homologues LASs isomers can be concluded. For these procedures the ^{1}H spectra of all 20 major LASs are used.

The aromatic protons of all LASs species show two multiplets in the ^{1}H spectra, which correspond to the AAïBBï spin system of the 4 protons arom-H3/5 and arom-H2/6. The protons arom-H3/5 of all isomers have the same chemical shift (7.77 ppm). The chemical shift of the arom-H2/6 protons of the 2-isomer (7.27 ppm) shows just a little downfield shift (0.04 ppm) in comparison to the signals of the other isomers. Due to the multiplicity of scalar coupling the α-protons of all species are split up into multiplets. Considering their chemical shifts the 2-isomer α-proton (2.75 ppm) differs from the 3-isomer α-proton (2.48 ppm) and the 4-/5-isomers α-protons (2.57 ppm). The chemical shifts of the β-CH_2-protons of all isomers are in the same range (1.59–1.66 ppm). Except the 2-

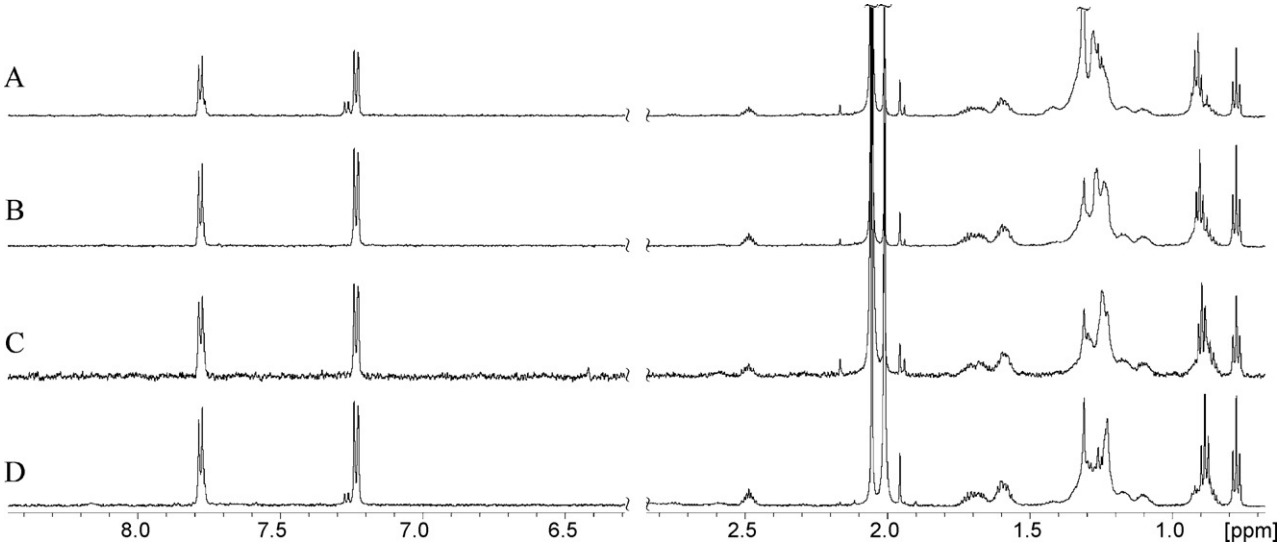

Fig. 7. ^{1}H spectra of A) 3-C_{13}-LAS; B) 3-C_{12}-LAS; C) 3-C_{11}-LAS; D) 3-C_{10}-LAS.

Table 4
LC–UV-retention times, chemical shifts, multiplicities and coupling constants of the signals in ^{1}H spectra of all major LASs species. RT = retention time; arom = aromatic; aliph = aliphatic; d = doublet; t = triplet; m = multiplet; displayed data were produced using the LC–MS/-SPE-NMR method described in Section 2.

LAS-Spices	RI [min]	Chemical shifts [ppm]/multiplicity								
		H2 arom	H3 arom	α-CH aliph	β-CH_2 aliph	β-CH_3 aliph	γ-CH_2 aliph	γ-CH_3 aliph	CH_2 aliph	CH_3 aliph
5-C_{10}-LAS	27.25	7.78/d	7.24/d	2.57/m	1.59/1.66/m	–	1.01/1.17/m	–	1.20-1.30/m	0.84/t
4-C_{10}-LAS	27.67	7.77/d	7.23/d	2.59/m	1.59/1.66/m	–	1.01/1.17/m	–	1.19-1.30/m	0.85/t
3-C_{10}-LAS	28.08	7.78/d	7.23/d	2.48/m	1.59/1.66/m	–	1.01/1.18/m	0.78/t/7.3	1.20-1.30/m	0.89/t
2-C_{10}-LAS	29.15	7.77/d	7.27/d	2.75/m	1.61/q	1.25/d	1.15/1.23/m	–	1.25-1.30/m	0.90/t
6-C_{10}-LAS	32.13	7.78/d	7.23/d	2.57/m	1.59/1.66/m	–	1.10/1.18/m	–	1.25-1.35/m	0.84/0.87/t
5-C_{10}-LAS	32.13	7.78/d	7.23/d	2.57/m	1.59/1.66/m	–	1.10/1.18/m	–	1.25-1.35/m	0.84/0.87/t
4-C_{10}-LAS	32.89	7.77/d	7.23/d	2.59/m	1.59/1.65/m	–	1.10/1.17/m	–	1.20-1.30/m	0.84/0.89/t
3-C_{10}-LAS	33.62	7.78/d	7.23/d	2.49/m	1.59/1.69/m	–	1.10/1.17/m	0.78/t/7.3	1.20-1.30/m	0.90/t
2-C_{10}-LAS	34.85	7.77/d	7.27/d	2.75/m	1.61/q	1.25/d	1.15/1.23/m	–	1.25-1.30/m	0.91/t
6-C_{10}-LAS	38.22	7.78/d	7.23/d	2.57/m	1.59/1.66/m	–	1.10/1.17/m	–	1.20-1.30/m	0.84-0.88/t
5-C_{10}-LAS	38.22	7.78/d	7.23/d	2.57/m	1.59/1.66/m	–	1.10/1.17/m	–	1.20-1.30/m	0.84-0.88/t
4-C_{10}-LAS	39.32	7.77/d	7.23/d	2.60/m	1.59/1.65/m	–	1.10/1.17/m	–	1.20-1.30/m	0.87/0.89/t
3-C_{10}-LAS	39.95	7.78/d	7.23/d	2.49/m	1.59/1.69/m	–	1.10/1.17/m	0.78/t/7.4	1.20-1.30/m	0.90/t
2-C_{10}-LAS	41.25	7.77/d	7.27/d	2.75/m	1.61/q	1.25/d	1.25/1.35/m	–	1.25-1.35/m	0.91/t
7-C_{10}-LAS	44.95	7.78/d	7.23/d	2.57/m	1.59/1.66/m	–	1.10/1.17/m	–	1.20-1.30/m	0.84/0.87/t
6-C_{10}-LAS	44.95	7.78/d	7.23/d	2.57/m	1.59/1.66/m	–	1.10/1.17/m	–	1.20-1.30/m	0.84/0.87/t
5-C_{10}-LAS	45.37	7.77/d	7.23/d	2.57/m	1.59/1.66/m	–	1.10/1.17/m	–	1.20-1.30/m	0.84/0.87/t
4-C_{10}-LAS	46.18	7.77/d	7.23/d	2.59/m	1.59/1.65/m	–	1.10/1.17/m	–	1.20-1.30/m	0.84/0.87/t
3-C_{10}-LAS	46.87	7.78/d	7.23/d	2.50/m	1.59/1.69/m	–	1.10/1.17/m	0.78/t/7.4	1.20-1.30/m	0.91/t
2-C_{10}-LAS	48.43	7.77/d	7.27/d	2.75/m	1.61/q	1.25/d	1.15/1.23/m	–	1.25-1.35/m	0.91/t

isomer signal, which is split into 1 multiplet, all other β-protons show 2 multiplet signals. The chemical shifts of the γ-CH_2-protons of all isomers are in the range between 1.01 and 1.23 ppm and are split up into 2 multiples. Furthermore they are partially overlapping with the aliphatic CH_2-signals, which are showing broad signals in the range of 1.20–1.35 ppm and differ only by their peak form. The terminal CH_3-groups however, show isomer specific differences. The 2-isomer methyl group in β-position is split into a doublet and shifted downfield (1.25 ppm). Any other methyl groups are bonded to methylene groups and therefore split into triplets. Their protons resonate in a range between 0.84 and 0.90 ppm and are difficult to differentiate. The 3-isomer methyl group signal is an exception. It is bonded to the CH_2-group in the β-position and shows a little high field drift (0.78 ppm). A compilation of ^{1}H spectra obtained from all four possible positional isomers of C_{10}-LASs is displayed in Fig. 6.

The comparison of the ^{1}H spectra of all 3-isomers is displayed in Fig. 7. All signals offer the same chemical shifts. Only the broad aliphatic CH_2-signals (1.20–1.30 ppm) differ in their peak form and integral, due to the varying number of CH_2-groups in the alkyl chain.

The chemical shifts of the proton signals, their multiplicity and the retention times (cf. Fig. 2) in the LC-UV chromatogram of all major LASs species are combined in Table 4.

4. Conclusions

We were able to measure mass spectra of the 20 major LASs species and isolate them into 17 fractions using post-column enrichment on SPE-cartridges for subsequent measurements of ^{1}H-1D and H-H-COSY spectra employing LC-SPE-NMR/MS. The LC–MS data were used to ensure the elution order of LASs groups showing a difference in the number of CH_2-groups in the alkylchain and hence mass-to-charge ratio. Via ^{1}H-1D and H-H-COSY NMR a complete assignment of all proton signals within the spectra was performed. The NMR-spectroscopic characterisation of all 20 major LASs species was reported here for the fist time. In addition, an individual assignment of each LAS isomer to its retention time in the HPLC-UV-chromatogram was done.

The following conclusions can be drawn from our investigations:

1. Groups of LASs with a different amount of CH_2-groups in the alkylchain elute in the order: C_{10}, C_{11}, C_{12}, C_{13}.
2. The LASs species of a group exhibiting the same number of CH_2-groups within the alkylchain elute according to the linkage of the aromatic ring to the alkyl chain, while inner isomers (the linkage of the aromatic moiety is located at the inner carbons of the alkyl chain) showing a decreased retention.
3. Species of a group exhibiting the same number of CH_2-groups within the alkylchain which are not well separated or co-elute by HPLC (mostly inner isomers) show very similar ^{1}H spectra. In a mixture spectrum of such a group only the 2-isomer (via the chemical shifts of arom-H3/5, α-CH and β-CH_3) and the 3-isomer (via the chemical shifts of α-CH and γ-CH_3) can be identified unequivocally by comparing their ^{1}H spectra. All other isomers show similar ^{1}H spectra.
4. Homologues LASs species like all 2-isomeres show very similar ^{1}H-spectra with marginal differences only. In a mixture spectrum of those homologues species none of them would be distinguishable from each other by NMR. The difference is caused by varying numbers of methylene groups in the alkylchain which results in slightly varying peak forms and integrals of the broad aliphatic CH_2-resonances.

The application of the LC-SPE-NMR/MS method is of high information benefit, especially for the identification of single analytes out of complex mixtures or for the differentiation of positional isomers like LASs. This is especially true when the analytes exhibit the same molecular masses and no specific fragmentation patterns can be observed. In this case LC–MS and LC–MS/MS cannot be used to differentiate between isomers. LC-SPE-NMR offers here a powerful alternative technique, due to the amount of structural information present in the NMR-spectra.

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