Characterization of Mixtures of Alkyl Polyglycosides (Plantacare) by Liquid Chromatography-Electrospray Ionization Quadrupole Time-of-Flight Mass Spectrometry

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Purpose. Industrial products of alkyl polyglycosides (APGs) are widely used as well tolerated surfactants in washing and cleaning agents. APGs belong to the class of nonionic surfactants and are mixtures of molecules consisting of a hydrocarbon chain with *Y* carbon atoms linked to *X* sugar residues. Physicochemical properties of APG products strongly depend on the molecular composition. Therefore, a detailed analytical investigation of technical-grade APGs is presented.

Methods. Complex mixtures of alkyl/alkenyl polyglycosides (APGs, Plantacare) and mixtures of APGs with other surfactants were analyzed by reversed-phase liquid chromatography-electrospray ionization quadrupole time-of-flight mass spectrometry (LC/ESI-QTOF-MS) using methanol-water as mobile phase and gradient elution.

Results. Analytes were separated according to the chain length of the alkyl homologs. Under the soft ionization of the ESI technique, mainly $[M+Na]^+$ ions were observed, which proved to be very stable. Additionally $[M+H]^+$ ions were detected. The QTOF mass spectrometer allows to identify even high molecular mass components in the mixtures, and APGs up to eight sugar residues were found. From these data, the alkyl/alkenyl chain lengths and the degree of oligomerization of the sugar moiety for different technical-grade APGs were calculated. Using MS/MS experiments, additional structural and chemical information was obtained.

Conclusions. The presented LC/ESI-QTOF-MS approach allows to analyze and characterize various APG products, such as Plantacare. The ability of this LC/ESI-QTOF-MS approach to analyze mixtures of APGs with other surfactants is demonstrated.

KEY WORDS: alkyl polyglycosides; LC/ESI-MS; mass spectrometry; Plantacare; surfactants.

INTRODUCTION

Alkyl polyglycosides (APGs) represent a nonionic surfactant class based on sugar and fatty alcohol (Fig. 1). The industrial process follows the Fischer synthesis to convert natural fats and starches into APGs (1). In contrast to stereospecific pathways using protective groups, which results in well defined components, the industrial process leads to a complex mixture of alkyl oligoglycosides. Due to the formation of α- and β-anomers, furanosides, and pyranosides, as well as binding isomers, technical products contain a large number of different components and stereoisomers, and for this they are called alkyl polyglycosides. The products are characterized by the length of the alkyl chain and the average number of monosaccharide units linked to it (degree of oligomerization; DO).

APGs are widely used in cleaning and personal care products due to their excellent environmental and skin compatibility (2–4). They have a better foaming performance compared to other nonionic surfactants and are perfectly suitable for mild cosmetic products.

Efficient analytical methods are required to determine the composition of various APG products. Basic characteristics such as viscosity (5) and foaming behavior afford information on the average molecular weight of the products.

Detailed measurements such as high-performance liquid chromatography (HPLC) (6–8) provide substantially more information on the components. Using reversed-phase isocratic or gradient HPLC, APGs are separated according to their alkyl chain length. The application of on-line LC/^{1}H NMR to characterization of alkyl diglucosides has been described (9). To identify high mass components in the mixtures, matrix-assisted-laser-desorption-ionization mass spectrometry (MALDI-TOF-MS) is very suitable (10,11).

Due to the limited number of available pure substances and to the lack of any chromophoric group in the molecules, coupling of chromatographic separation with mass spectrometric detection is required, such as is possible with reversed-phase thin-layer chromatography (TLC) and identification of APGs by time-of-flight secondary-ion mass spectrometry (TOF-SIMS) (12). For examination of APGs using gas chromatography-mass spectrometry (GC/MS), the molecules have to be silylated prior to analysis due to their low volatility. Operating in electron impact mode (EI) identification of analytes is possible only by characteristic fragments (13). Using liquid chromatography-electrospray ionization mass spectrometry (LC/ESI-MS) (14,15), the derivatization step can be circumvented and the soft ionization conditions generate mostly molecule ions, which makes the assignment of peaks much easier.

The formation of microemulsions based on APGs combined with hydrophobic cosurfactants has been reported (16,17). However, technological properties of surfactants such as APGs strongly depend on their molecular composition. The dependence of the rheological behavior on the alkyl chain length, *Y*, and the degree of oligomerization, *X*, has been described (5).

Therefore, this work presents a new analytical approach for the examination of complex industrial product mixtures of APGs (Plantacare) by reversed-phase liquid chromatography-electrospray ionization quadrupole time-of-flight mass spectrometry (LC/ESI-QTOF-MS). The QTOF mass spectrometer allows to identify even high molecular mass components in the mixtures. Additional structural information was

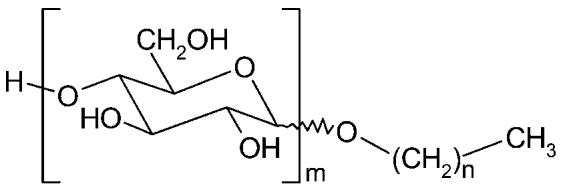

Fig. 1. General chemical structure of APGs (binding, ring and stereo isomers not indicated). m = 1 to 10; n = 5 to 15.

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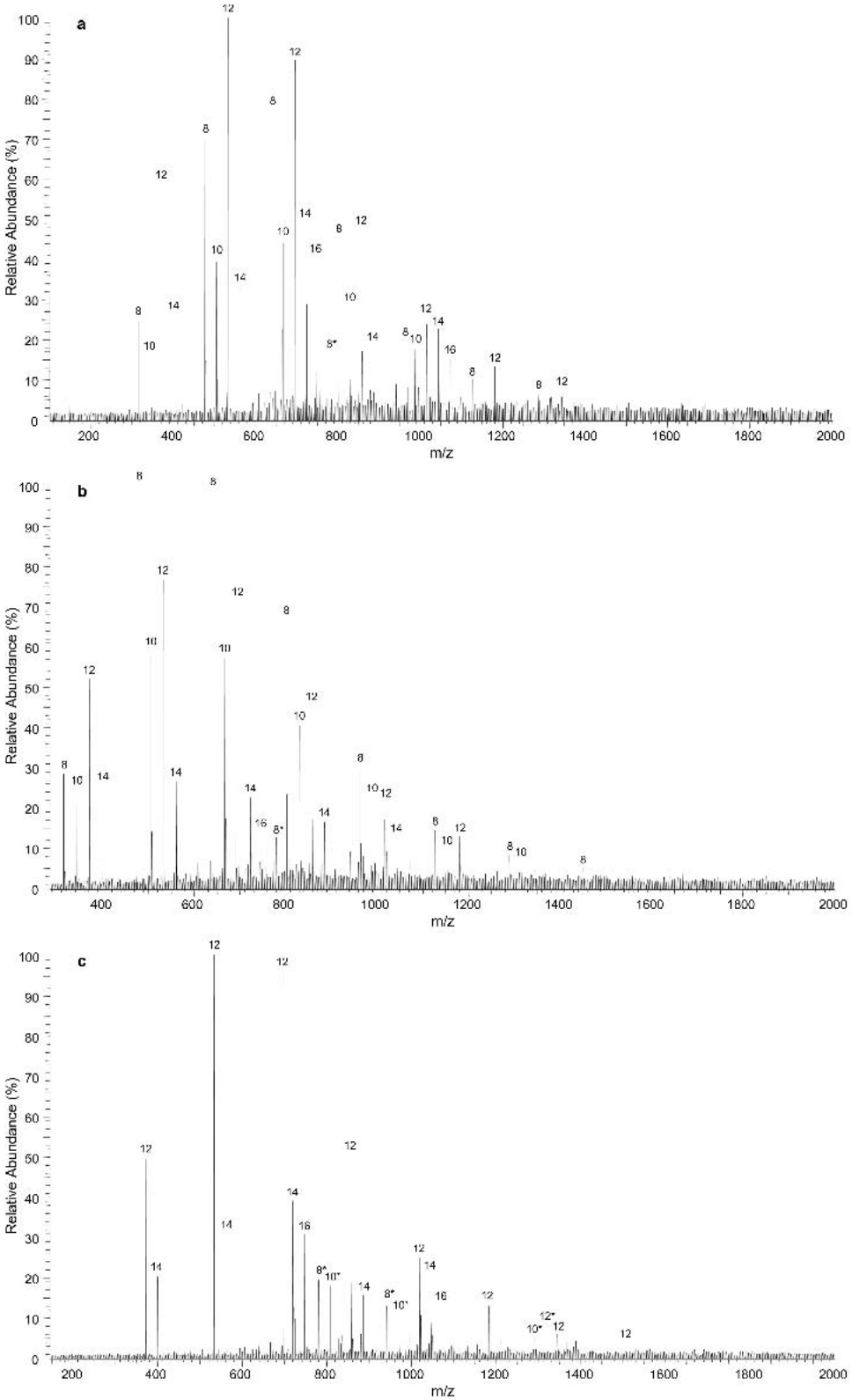


Fig. 2. ESI-MS of (a) Plantacare 818 UP, (b) Plantacare 2000 UP, and (c) Plantacare 1200 UP obtained using the Finnigan LCQ ion trap mass spectrometer. Peaks are labeled according to the carbon number in the alkyl chains; $[M+Na]^+$ and $*[M+H]^+$.

gathered by MS/MS (tandem MS) experiments. Finally we demonstrate the capability of this method for determination of APGs in the presence of other surfactants.

MATERIALS AND METHODS

Materials

Methanol was supplied by J. T. Baker (Deventer, The Netherlands). Plantacare 1200 UP, Plantacare 2000 UP, Plantacare 818 UP and Plantacare K 55 were kindly provided by H. U. Krächter (Cognis Deutschland GmbH, Düsseldorf, Germany). Formic acid (98–100%) was obtained from VWR International GmbH (Dresden, Germany). All other chemicals used were purchased from Sigma (Taufkirchen, Germany).

MS and Tandem MS

Mass spectrometry was performed using a Finnigan LCQ ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) via the electrospray interface. The electrospray voltage applied to the ESI needle was +4.0 kV. Spectra were recorded in positive ESI mode. Samples (100 μg/ml in Methanol) were introduced at a flow rate of 10 μL/min. The heated capillary was maintained at 260°C.

LC/ESI-QTOF-MS

Determination of APGs was performed using a LC Packings HPLC equipped with a Famos μ-autosampler, Thermos column oven and Ultimate pumpsystem (LC Packings, Amsterdam, The Netherlands). This was coupled via an electrospray interface to a Q-TOF 2 orthogonal hybrid quadrupole time-of-flight mass spectrometer (Micromass, Manchester, UK) in Micromass Z-spray geometry. MS conditions: ESI voltage +4 kV (positive ion mode), cone potential 70 V, desolvation temperature 220°C. Spectra were acquired over *m/z* 150–2000, at 2 s per scan. The separations were carried out on a YMC-Pack ODS-AQ (150 mm × 1 mm, i.d. 3 μm) column (YMC, Schermbeck, Germany) which was thermostated at 30°C. A gradient was used in the separation. Mobile phase A consisted of 0.1% (v/v) formic acid in water, and mobile phase B consisted of 0.1% (v/v) formic acid in methanol. The gradient was initially held constant at 100% A for 2 min, then progressed within 13 min to 100% B. In the following 12 min B was held constant at 100%. Finally the eluent composition was reset within 2 min to the starting conditions and held until equilibration. For analysis of Plantacare K 55 the step of progression to 100% B was prolonged to 20 min. The flow rate was 30 μl/min and the injection volume of sample solution (100 μg/ml in methanol) was 5 μl.

RESULTS AND DISCUSSION

The mass spectra of Plantacare 1200 UP, Plantacare 2000 UP and Plantacare 818 UP clearly illustrate differences in the composition of the examined products (Fig. 2). The spectra contain several series of $[M+Na]^+$ ions separated by $\Delta m/z$ 162 (hexose unit). Calculated monoisotopic *m/z* values for $[M+Na]^+$ ions of APGs are shown in Table I. The observation of patterns with spacings of $\Delta m/z$ 2–4 can be explained by the existence of alkenyl polyglycosides in the mixtures (18,19). Apart from the $[M+Na]^+$ ions, the spectra contain several $[M+H]^+$ ions (peaks labeled with an asterisk), which might be explained by differences in the affinities of α- and β-anomers, furanosides and pyranosides toward Na^+ (15). In the spectrum of Plantacare 1200 UP the adduct ions assignable to the lauryl (oligo-) glycosides clearly appeared to dominate as following the product specifications.

For further chemical characterization, lauryltriglycoside was analyzed by tandem mass spectrometry. MS/MS experiments in positive ion mode revealed loss of one monosaccharide unit and led to the formation of lauryldiglycoside (Scheme 1). The cleavage of the glycosidic bond between sugar and dodecylalcohol resulted in a trisaccharide obtaining a ketone (enol) structure. In MS^3, the lauryldiglycoside further split off another monosaccharide unit to give the laurylmonoglycoside, and in a second pathway a disaccharide having the ketone (enol) structure was formed (with the consequent loss of dodecylalcohol).

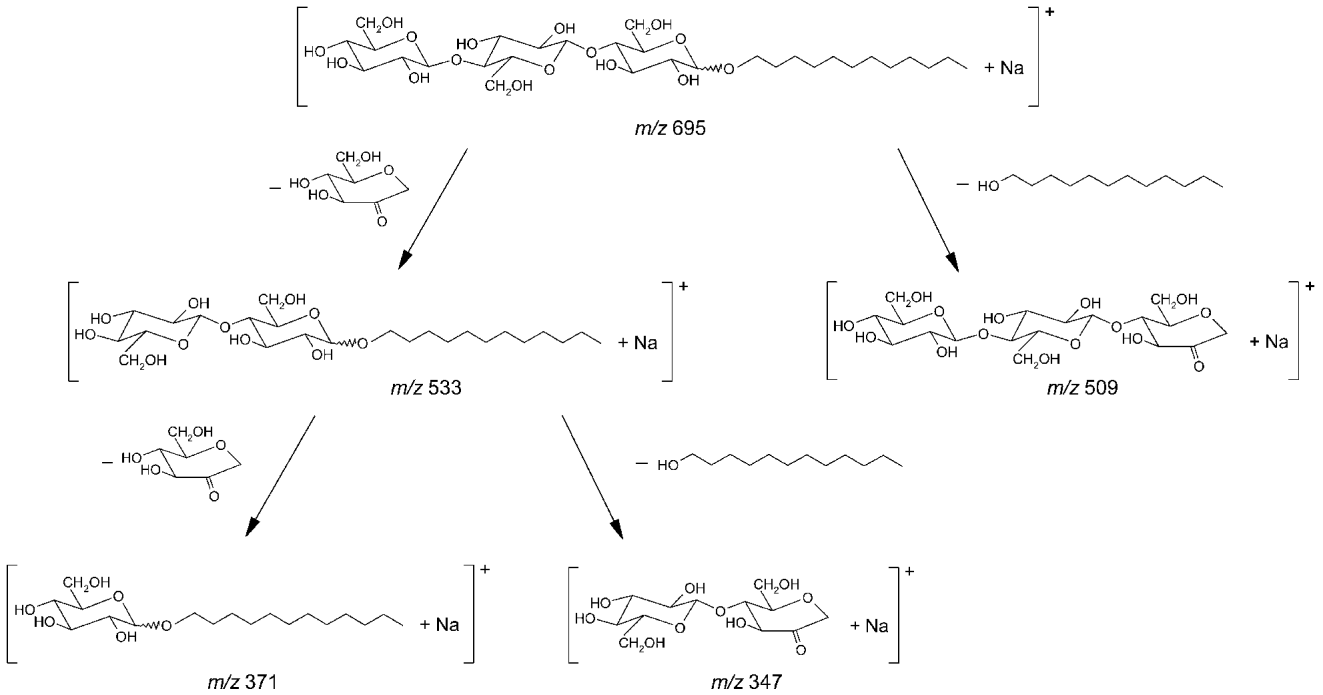
Because no multiply charged ions appeared in the spectra of all experiments, the sodium ion was previously suggested to be associated with the glycosidic oxygen connecting the sugar and the alkyl chain (14). This can not explain the formation of $[M+Na]^+$ ion for the trisaccharide structure after cleavage of laurylalkohol. The hydroxy groups and ring oxygens, therefore, have to be considered to act as acceptor for the sodium ion.

As described above, for determination of APGs in the mixtures chromatographic separation is required.

The eluent has to ensure both an optimal chromatographic separation and an appropriate ionization and desolvatation. The use of methanol/water (containing formic acid) instead of acetonitrile/water as mobile phase increased the signal intensity. It seems that the stabilization of charge in the molecule is much more affected by methanol than acetonitrile and, additionally, the desolvation process is accelerated. Otherwise as reported before (15), the adduct formation was re-

Table I. Calculated Monoisotopic *m/z* Values for $[M+Na]^+$ Ions of APGs

Alkyl chain (C_x)	Hexose units									
	1	2	3	4	5	6	7	8	9	10
6	287.1	449.2	611.3	773.3	935.4	1097.4	1259.5	1421.5	1583.6	1745.6
8	315.2	477.2	639.3	801.3	963.4	1125.4	1287.5	1449.5	1611.6	1773.7
10	343.2	505.3	667.3	829.4	991.4	1153.5	1315.5	1477.6	1639.6	1801.7
12	371.2	533.3	695.3	857.4	1019.5	1181.5	1343.6	1505.6	1667.7	1829.7
14	399.3	561.3	723.4	885.4	1047.5	1209.5	1371.6	1533.6	1695.7	1857.7
16	427.3	589.4	751.4	913.5	1075.5	1237.6	1399.6	1561.7	1723.7	1885.8



Scheme 1. Fragmentation pathway for lauryl triglycoside of Plantacare 818 UP obtained by ESI-MS/MS and MS^3 experiments in positive ion mode (binding, ring and stereo isomers not indicated).

producible, although the concentration of sodium is not adjusted to a certain level. The following studies were carried out in positive-ion mode.

Figure 3 displays LC/ESI-MS extracted ion chromatograms of Plantacare 2000 UP.

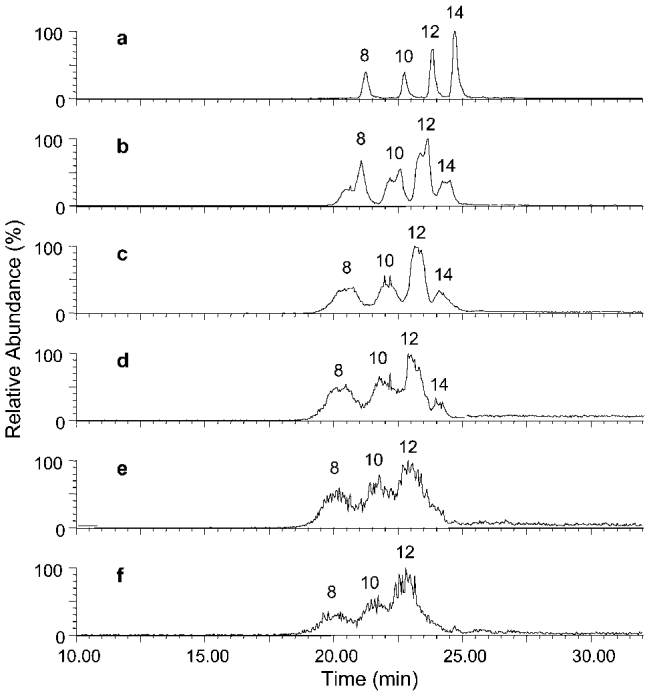


Fig. 3. LC/ESI-QTOF-MS of Plantacare 2000 UP. Extracted ion chromatograms of (a) monoglycosides, (b) diglycosides, (c) triglycosides, (d) tetraglycosides, (e) pentaglycosides, and (f) hexaglycosides; sodium adduct ions throughout. Peaks are labeled according to the carbon number in the alkyl chains.

Analytes were separated according to the chain length of the alkyl homologs: increased chain length resulted in increased retention time. A separation with regard to the glycoside moiety was achieved partially, since the used column shows both hydrophobic and hydrophilic areas for interactions with analytes. Higher degrees of oligomerization led to weaker retardation on the C_{18} column owing to higher hydrophilicity of the molecules. Due to the higher number of possible isomers (including ring isomers, stereoisomers and binding isomers) the peak pattern of a specific alkyl homolog is much more complex for alkyl oligoglycosides compared to the corresponding monoglycoside. Since no double peaks were observed for the monoglycosides, α- and β- anomers were not separated under the conditions used.

The results for the technical grade surfactants obtained using LC/ESI-QTOF-MS are shown in Fig. 4. Differences in data presented in Figs. 2 and 4 can be explained by slightly different ionization behavior of the analytes depending on the used mass spectrometer (LCQ/QTOF). Calculations were based on the sodium adducts of the alkyl oligoglycosides, whereas the proton adducts and adducts of the alkenyl oligoglycosides were not included. Under these conditions, the main part of the investigated surfactant mixtures consists of molecules with two or three hexose groups. The number of molecules with seven or more hexose groups is close to the detection limit. In comparison, the distribution of the number of hexose groups of tetradecyl (oligo-) glycosides shows a larger extent of monoglycosides. Plantacare 1200 UP contains mostly dodecyl- and tetradecyl (oligo-) glycosides. The small amount of hexadecylglycosides was not detectable using LC/ESI-MS.

For analysis of Plantacare K 55 (mixture of APGs and cocamidopropyl betaine, CAPB) the gradient was modified in order to separate the APGs from CAPB. CAPB is a mixture of amphoteric surfactants (Fig. 5) commonly used in personal

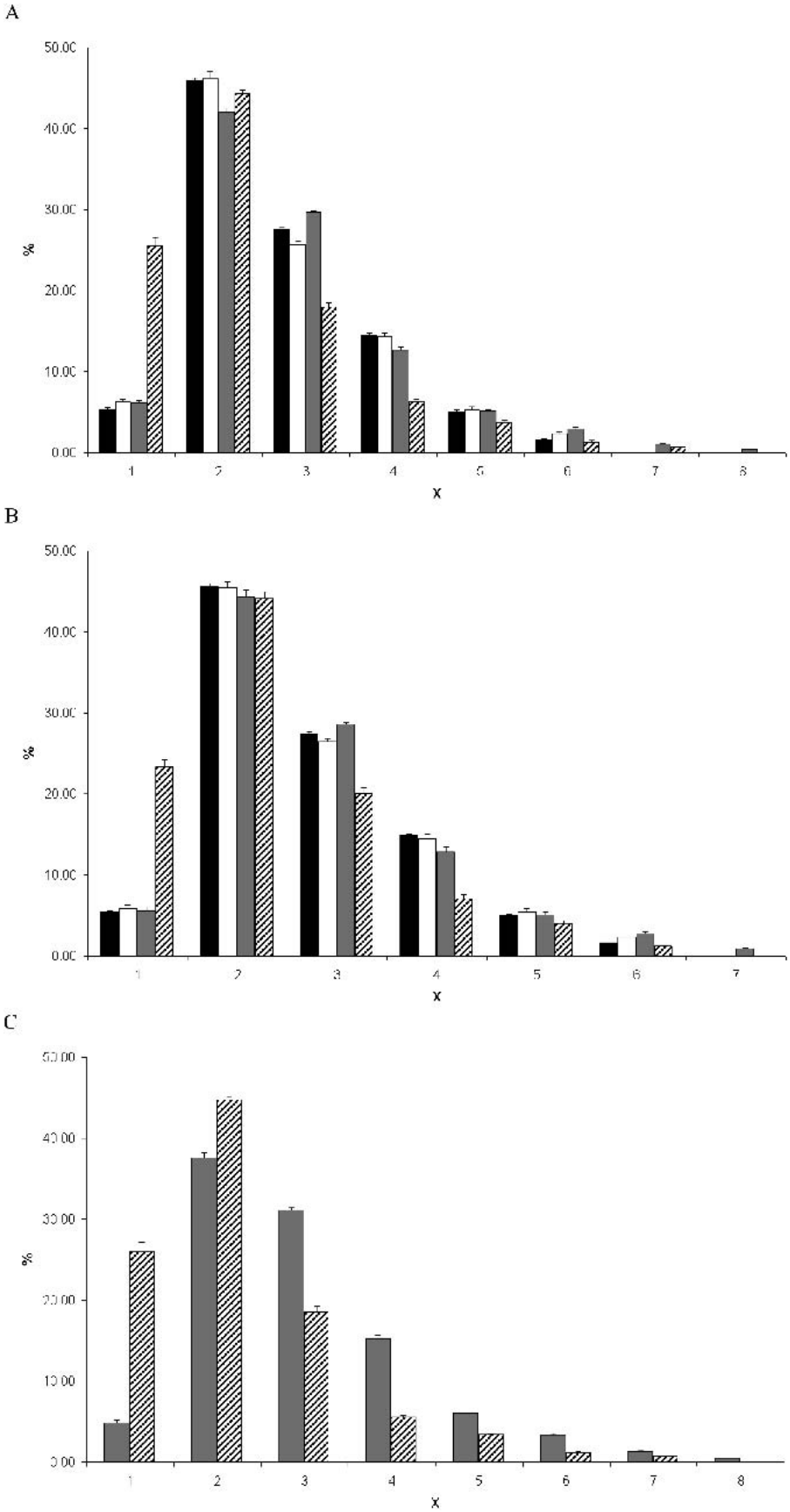


Fig. 4. Distribution of the number of glycose groups, *X*, as function of the *n*-alkyl chain length, *Y*, (black: C_8; white: C_{10}; gray: C_{12}; and hatched: C_{14}) obtained by LC/ESI-QTOF-MS. (A) Plantacare 818 UP, (B) Plantacare 2000 UP, and (C) Plantacare 1200 UP. Values are averages of at least five consecutive measurements.

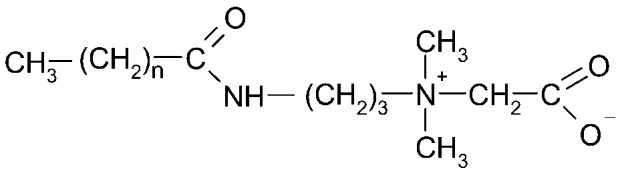


Fig. 5. General chemical structure of CAPB; n: 10, 12, 14, 16.

care products and surface cleaners. However, clinical allergy to CAPB due to impurity content has been reported (20).

Figure 6 displays LC/ESI-MS extracted ion chromatograms of Plantacare K 55. With respect to the alkyl/acyl chain lengths protonated CAPB were detected at same *m/z* as the sodium adducts of the corresponding smaller alkyl monoglycosides (Figs. 6c, 6d). Therefore, good separation is required. Additionally $[M+Na]^+$ ions of CAPB were generated under these conditions (Fig. 6b), which permits a definite assignment of peaks according to the observed retention time even without reference substances. The separation of CAPB components followed the chain length of the acyl homologs. For analytes having alkyl/acyl chains with the same number of carbon atoms the CAPB molecules eluted earlier.

Determination of APGs in the mixture revealed a composition similar to Plantacare 1200 UP (data not shown).

CONCLUSIONS

The presented LC/ESI-QTOF-MS approach allows to analyze complex mixtures of industrial APG products, such as Plantacare. Using gradient controlled HPLC, good separation of APGs from other surfactants (CAPB) was achieved. Tandem MS experiments provide additional structural and chemical information. From these data, the alkyl/alkenyl chain lengths and the degree of oligomerization of the sugar moiety can be calculated and correlated to physico-chemical properties of APG products. The Identification of analytes by their mass spectra was possible even without reference substances. However, there is no information about structural assignment available to elucidate stereochemistry of APGs. The stereochemical characterization can be obtained by NMR spectroscopy (9).

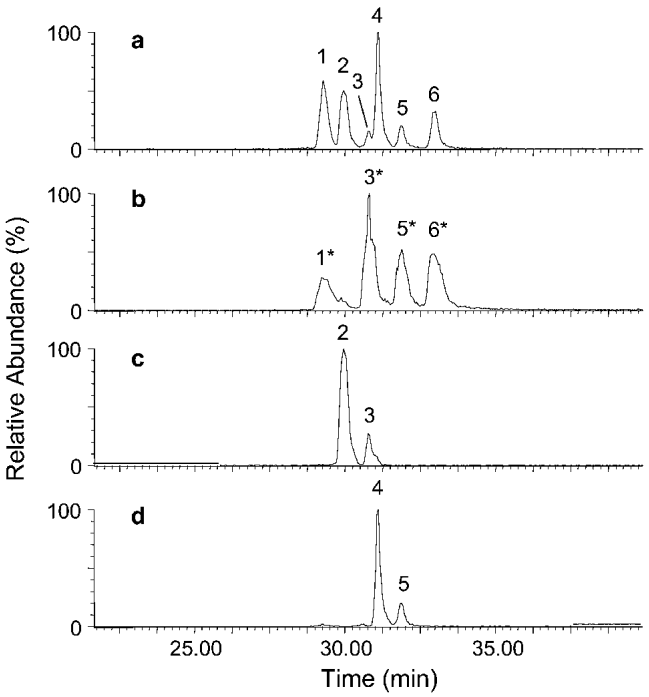


Fig. 6. LC/ESI-QTOF-MS of Plantacare K 55. Extracted ion chromatograms of (a) alkyl monoglycosides (C_{12}-monoglycoside, $[M+Na]^+$, **2**, and C_{14}-monoglycoside, $[M+Na]^+$, **4**) and CAPB, $[M+H]^+$ ions, n: 10 (**1**), 12 (**3**), 14 (**5**), 16 (**6**); (b) CAPB, $[M+Na]^+$ ions, n: 10 (**1***), 12 (**3***), 14 (**5***), 16 (**6***); (c) C_{12}-monoglycoside ($[M+Na]^+$, **2**) and CAPB (n: 12, $[M+H]^+$, **3**), *m/z* 371; and (d) C_{14}-monoglycoside ($[M+Na]^+$, **4**) and CAPB (n: 14, $[M+H]^+$, **5**), *m/z* 399.

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