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Analysis of sulfobutyl ether-β-cyclodextrin mixtures by ion-spray mass spectrometry and liquid chromatography–ion-spray mass spectrometry

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Abstract

The analysis of two commercial and two home-made sulfobutyl ether β-cyclodextrin (SBE-β-CD) samples by ion-spray (IS) mass spectrometry and by liquid chromatography–mass spectrometry coupling (LC–MS) is investigated in a negative ion mode. SBE-β-CD fragmentation was first investigated by direct infusion. In IS, the best conditions for SBE-β-CD ionization consisted of ammonium acetate added to an acetonitrile/water mixture as sample solvent. These conditions allowed simplification of the mass spectrum, mainly by the formation of dicharged species $[M-2H]^{2-}$, thus limiting the production of multicharged fragments. IS-MS permits fast and simple measurement of the substitution pattern and determination of the global degree of substitution for SBE-β-CD mixtures. A complementary method using LC–MS was developed for the analysis of these mixtures. The substitution patterns obtained by LC–MS are in good agreement with those determined by direct MS analysis. The LC–MS coupling enabled separation of the mixtures versus the charge in anion-exchange chromatography (AEC) whereas no separation of the different substitution isomers potentially present in the SBE-β-CD mixture was displayed. The AEC methodology described can be successfully used for fractionation of SBE-β-CD derivatives at the semi-preparative scale.

Keywords: Mass spectrometry; Sulfobutyl ether; Cyclodextrins

1. Introduction

Sulfobutyl ether derivatives of β cyclodextrin have found industrial applications (drug formulation, cosmetics) [1–3] but are also widely used as chiral selectors for the separation of enantiomers by capillary electrophoresis [4–7]. The sulfobutyl ether β-cyclodextrins (SBE-β-CDs) are said to be randomly substituted because they are mixtures due to number, position and glucose unit, thus one has to differentiate between degree of substitution, positional isomerization (2, 3 or 6) and regioisomerization (glucose unit 1–7 for beta CD). A generalized structure of the SBE-β-CD derivatives is provided in Fig. 1. There is an analytical requirement to determine the composition and purity of these derivatised CDs because their composition has an influence on enantioselectivity [8–10] and may also be of interest in industrial applications.

Anion-exchange chromatography (AEC) is a wide-

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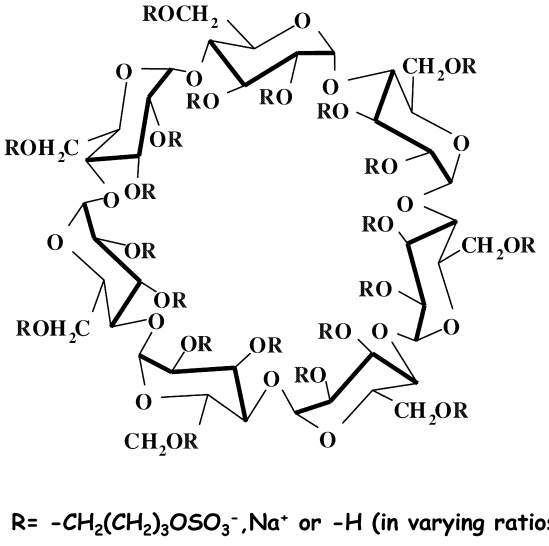

Fig. 1. Generalized structure of sulfobutyl ether β-cyclodextrin sodium salt, in which butylsulfonic acid groups replace up to 12 of the hydroxyl hydrogens in β-cyclodextrin.

spread technique for the separation of oligosaccharides [11–14]. Recently, an analytical method based on AEC using volatile eluent ion and evaporative light scattering detection was developed for the analysis of mixtures of SBE-β-CDs [15]. This method provides an efficient and characteristic liquid chromatography fingerprint, which depicts the SBE-β-CD mixture complexity. However, in the absence of commercially available pure SBE-β-CDs, a complementary LC–MS method has to be developed to provide structural information about the eluted compounds.

Concerning mass spectrometry (MS), ion-spray is a mild and highly efficient ionization method [16], suitable for the analysis of polar, ionic, high-molecular-mass and thermally labile compounds [17]. Thus, this technique has already been used for the characterization of anionic oligosaccharides [11]. Given the polarity and the multicharged nature of the sulfonated cyclodextrins, on-line AEC–MS with ion-spray ionization would appear to be a very powerful tool in the separation and rapid determination of the various constituents of SBE-β-CD mixtures.

In this paper, SBE-β-CD fragmentation as well as the influence of the sample solvent on ion fragmentation and MS detection sensitivity were first investigated in a negative ion mode. MS was then evaluated as a fast method to determine the substitution pattern and the global degree of substitution of two commercial and two home-made SBE-β-CD samples. In order to obtain additional information on the composition of these four SBE-β-CD samples, a complementary AEC–MS method was then investigated.

2. Experimental

2.1. Reagents

Four different SBE-β-CD samples were studied. Samples A and B were synthesized in our laboratory according to the procedure of Rajewski and Stella [18] and as reported in our previous work [19]. Advasep® (sample C) and Captisol® (sample D) were purchased from Cydex Inc (Overland Park, KS, USA). The different mixtures were characterized by anion-exchange chromatography (AEC) using a silica quaternary ammonium exchanger (Vydac 302 IC column) with ammonium acetate as volatile salt in water/acetonitrile mixture (70:30) as mobile phase and using evaporative light scattering detection (ELSD) [15]. The average substitution degree of synthesized and commercial SBE-β-CD samples was determined to be 3.5 (sample A), 7.7 (sample B), 5.3 (sample C) and 6.4 (sample D) by this LC–ELSD method.

Eluent constituents were purchased as follows: HPLC-grade acetonitrile and methanol from J.T. Baker (Noisy Le Sec, France); water from an Elgastat UHQ II System from Elga (Villeurbanne, France). Acetic acid, formic acid and ammonium hydroxide were obtained from Fluka (St. Quentin Fallavier, France).

For MS studies, the SBE-β-CD sample was prepared at 1000 mg l^{-1} in the different sample solvents studied and for LC–MS analyses, the SBE-β-CD sample was dissolved at the same concentration in deionized water.

PHOEBUS, an application program for buffer studies (Sedere Co, Franklin, MA, USA) was used for the mobile phase preparation. The mobile phase was a mixture of ammonia and acetic or formic acid. It was prepared by pre-setting the acetate or formate concentration value and a pH value of 4. The ammonium concentration was calculated by the software. For each mobile phase, the pH value was

checked with a Beckman (Gagny, France) pH meter model Φ 10.

2.2. Apparatus

Ion-spray was performed using a Perkin-Elmer Sciex (Forster City, CA, USA) API 300 mass spectrometer triple quadrupole instrument, in negative ion mode. The ion-spray voltage was set at −4.8 kV. The orifice and the focusing ring voltages were set, respectively, at −60 and −300 V. Dry air was used as nebulizing gas while nitrogen was used as curtain gas at a flow-rate of 1.25 l min^{-1}. For the study of the MS parameters, the solution was introduced into the ion-spray source at a constant flow-rate of 5 μl min^{-1} with a Harvard Model 22 syringe pump.

Experiments in the LC–MS chromatographic system were performed on a Perkin-Elmer (Toronto, Canada) model LC-200 binary pump coupled with the Perkin-Elmer Sciex API 300 mass spectrometer. Injections were done by a Perkin-Elmer series 200 autosampler (Toronto, Canada) fitted with a 20-μl loop. For the LC–MS system, a 50 μl min^{-1} portion of column effluent was diverted to the ion source. The anion-exchanger column was a Vydac 302 IC column (250×4.6 mm I.D., 100 μequiv. g^{-1}) from Interchim (Montluçon, France). Flow-rate was 1 ml min^{-1} and under these conditions, the pressure was about 60 bar with the different mobile phases.

3. Results and discussion

3.1. Mass spectrometry study of SBE-β-CDs using ion-spray ionization

Mass spectrometry (MS) is, like gas phase light scattering, a semi-universal detection mode, the only requirement for the detection of a compound by MS being its ionization. To carry out the analysis, a negative mode of MS detection was selected because SBE-β-CDs are totally dissociated in aqueous solution above pH 2 and negatively charged. Moreover this detection mode has been successfully used for the characterization of sulfated heparin oligosaccharides [20] and sulfoalkyl cyclodextrin derivatives [21]. Samples A and B were analyzed by direct infusion and the influence of the sample solvent on ion fragmentation and detection sensitivity was evaluated. First, pure water was used as sample solvent. Under these conditions, a very low mass spectrometric response of the analytes was observed, probably due to a low ionisation of the anionic cyclodextrins. Thus, the use of water as sample solvent was not satisfactory to determine the different components of the SBE-β-CD mixtures.

3.1.1. Effect of organic modifier

The addition of an organic modifier such as acetonitrile or methanol to water was considered. Indeed, it is well established that the addition of an organic modifier to the sample solvent is an important parameter in ensuring good sensitivity and stability of MS detection [22]. As previously noted [15], SBE-β-CDs are soluble in water–acetonitrile or water–methanol mixtures at a percentage of organic modifier equal to or less than 50%. Fig. 2 shows the ion-spray mass spectra of two SBE-β-CD samples (A and B) dissolved in a (70:30) water/acetonitrile mixture. The mass range studied was 400–1400 because above the m/z ratio 1400, the intensities of the detected peaks are too weak for any fragment identification. For the two analytes A and B, we noted a complex ion formation composed of mono- to tetracharged ions, resulting from a high fragmentation of the solutes. The natural levels of Na^+ impurities in the samples are sufficient (samples A and B are isolated as sodium salts) to produce these ions as already noted with cyclodextrins and neutral derivatives thereof analysed by ion-spray mass spectrometry [23]. In order to assign the main MS signals in Fig. 2, Table 1 reports for each SBE_n-derivative, the different m/z ratio values for the main molecular $[M–H]^-$ ions formed and for the potential fragments observed in collisionally-induced dissociation (CID)-MS after the $[M–H]^-$ ions have passed through the curtain gas of the interface. For sample A, seven cyclodextrin derivatives (SBE_1 to SBE_7) were identified, as illustrated in Fig. 2. For SBE_1 only the monocharged species was detected at m/z 1270.0. For SBE_2, the dicharged $[M–2H]^{2-}$ ion at m/z 702.5 was obtained and for SBE_3 the tricharged $[M–3H]^{3-}$ ion at m/z 513.3 and the dicharged $[M–3H+Na]^{2-}$ ion at m/z 781.0 were observed (Table 1). For each higher derivative (SBE_4 to SBE_7), the presence of

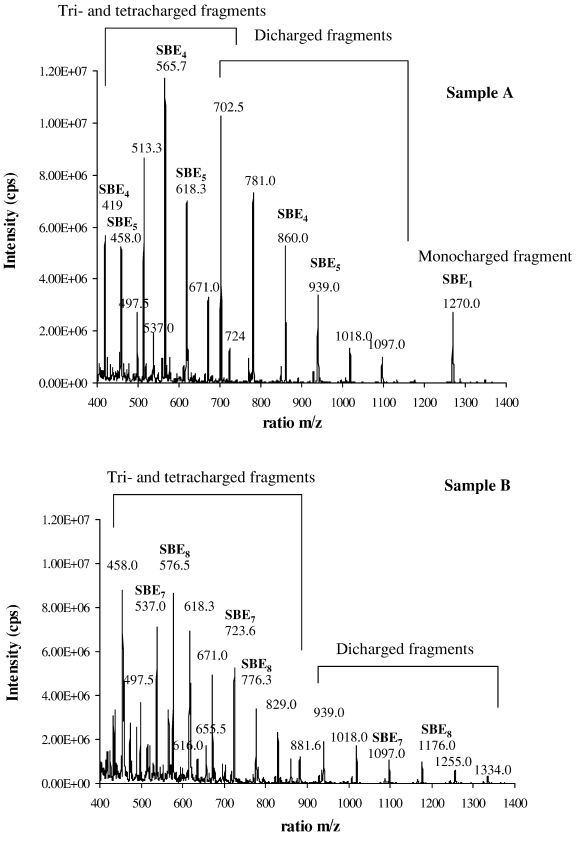

Fig. 2. Mass spectra by ion-spray of two home-made SBE-β-CD samples. The samples were dissolved in a water/acetonitrile (70:30) mixture and were detected in the mass range m/z=400–1400; MS conditions: ion-spray voltage=−4.8 kV, orifice voltage=−60 V, focusing ring voltage=−300 V, dry air was used as nebulizing gas while nitrogen was used as curtain gas at a flow-rate of 1.25 l min^{-1}; flow-rate of the syringe pump: 5 μl min^{-1}.

three main fragments (di-, tri- or tetracharged) was detected (in bold in Table 1). Concerning sample B, six cyclodextrin derivatives (SBE_5 to SBE_{10}) were detected and the presence of three main fragments di-, tri- and tetracharged was observed for each derivative (in bold in Table 1). The dissolution of sample A and sample B in various mixtures of water/acetonitrile (10–50% of organic solvent) induced the same multiple MS fragmentation for each SBE derivative and a similar relative abundance of each fragment was observed whatever the acetonitrile percentage. Moreover, the replacement of ACN by MeOH decreased all the MS fragment intensities.

3.1.2. Effect of salt additive

The complexity of the mass spectrum obtained with water–acetonitrile mixture as sample solvent made the identification of the different ion species difficult. Therefore, the addition of salts in the water–acetonitrile mixture was investigated in order to limit the multicharged ion formation and to enhance the sensitivity of the detection. First, ammonium acetate was tested. This ammonium salt is compatible with MS detection [24–26] and has been successfully used for the characterization of aromatic sulfonated [27] and sulfated oligosaccharides [20,26] by LC–MS. Furthermore, it has been observed that a mixture consisting of organic modifier and aqueous buffer salts such as ammonium acetate helps the pneumatic dispersion of the analyte solution into droplets small enough for the emission of ions. The ions formed under these solvent conditions can escape from droplets more easily than in an aqueous solvent because of a decrease in the medium dielectric constant [22,28]. Fig. 3 shows the mass spectra of sample A and sample B dissolved in water/acetonitrile (70:30) containing 100 m*M* of ammonium acetate. The mass spectra were registered in full scan in the same mass range (from m/z 400 to 1400) as that given in Section 3.1.1. It can be clearly seen that the mass spectra of samples A and B in Fig. 3 are different from those in Fig. 2. From Table 2, it appears that ions equivalent to $[M–2H]^{2-}$ were the most abundant peaks observed in Fig. 3 for each SBE_n derivative (except for SBE_1). The production of tri- and tetracharged ions was drastically limited by the addition of ammonium salts in solvent. As for the sodium adducts $[M–nH+(n-2)Na]^{2-}$, they were detected for each derivative in addition to the doubly charged ions $[M–2H]^{2-}$. No ammonium adduct was observed in spite of the use of ammonium buffer. This is probably due to the higher stability of the sodium salts than of the ammonium salts, as already noted with sulfated heparin oligosaccharides analysed by electrospray mass spectrometry [20]. The major mass peaks in Fig. 3 have been identified as corresponding to mono- up to nine-SBE substitutions for sample A and to three- up to 12-SBE substitutions for sample B. The distribution of the peaks is centered around the largest peak corresponding to SBE_2-derivative for sample A and SBE_6-derivative for sample B, respectively. Comparing Fig. 3 with

Table 1
Potential fragmentation in CID-MS in a negative mode ionization for the onefold to the 10-fold substituted SBE_n-β-CD[a] dissolved in water/acetonitrile (70:30)

SBE_n derivative	*m/z* (amu)									
	$[M-H]^-$ molecular ion	$[M-nH]^{(n)-}$ /*n*	$[M-nH+Na]^{(n-1)-}$ /(*n*−1)	$[M-nH+2Na]^{(n-2)-}$ /(*n*−2)	$[M-nH+3Na]^{(n-3)-}$ /(*n*−3)	$[M-nH+4Na]^{(n-4)-}$ /(*n*−4)	$[M-nH+5Na]^{(n-5)-}$ /(*n*−5)	$[M-nH+6Na]^{(n-6)-}$ /(*n*−6)	$[M-nH+7Na]^{(n-7)-}$ /(*n*−7)	$[M-nH+8Na]^{(n-8)-}$ /(*n*−8)
SBE_1	1270.0	**1270.0**								
SBE_2	1406.0	**702.5**	1428.1							
SBE_3	1542.0	**513.3**	**781.0**	1585						
SBE_4	1678.0	**419.0**	**565.7**	**860.0**	1743.0					
SBE_5	1814.0	362.1	**458.0**	**618.3**	**939.0**	1901.0				
SBE_6	1950.0	324.1	393.4	**497.5**	**671.0**	**1018.0**	2059.0			
SBE_7	2086.0	297.1	350.3	425.0	**537.0**	**723.6**	**1097.0**	2217.0		
SBE_8	2222.0	277.0	319.5	376.6	456.6	**576.5**	**776.3**	**1176.0**	2375.0	
SBE_9	2358.0	261.1	296.5	342.1	403.0	488.2	**616.0**	**829.0**	**1255.0**	2533.0
SBE_{10}	2494.0	248.5	278.5	316.2	364.7	429.3	519.8	**655.5**	**881.6**	**1334.0**

The most abundant fragments observed in the *m/z* ratio range 400–1400 are shown in bold. M, molar mass of the SBE_n-β-CD where each negative SBE group is associated with H^+.
[a] *n* varying from 1 to 10.

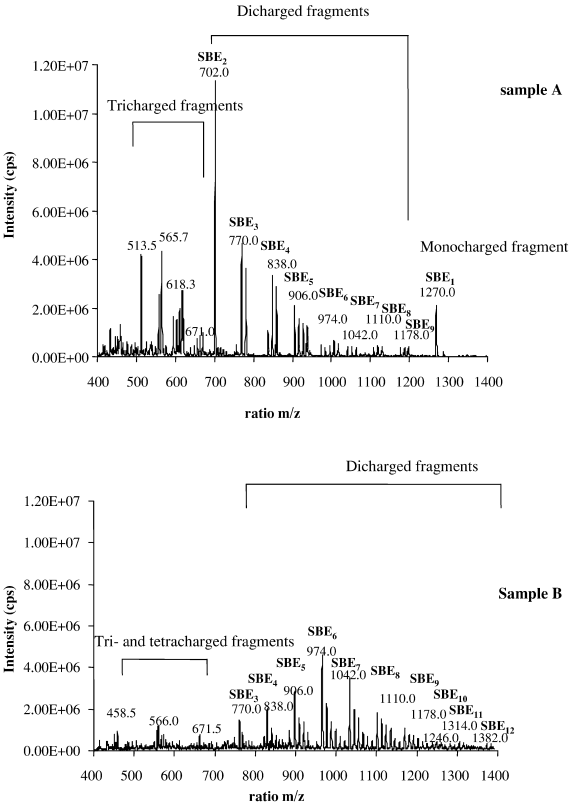

Fig. 3. Mass spectra by ion-spray of two home-made SBE-β-CD samples. The samples were dissolved in 100 m*M* ammonium acetate in mixture water/acetonitrile (70:30). MS conditions as described in Fig. 2.

Fig. 2, it can be noted that two additional (SBE_8 and SBE_9) or four additional (SBE_9 to SBE_{12}) derivatives were detected for sample A and sample B, respectively. Thus, the addition of ammonium acetate in mixture water/acetonitrile (70:30) led to limiting the production of multicharged species and to improving the detection limits.

To study the effect of ammonium acetate concentration and organic modifier percentage on analyte response, a series of mixtures was prepared. These consisted of acetonitrile–water mixtures with different percentages of acetonitrile (20, 30 or 50%) containing ammonium acetate in the concentration range 10–300 m*M*. It was observed that the nature of the main ions detected for each SBE-β-CD sample was not affected by acetonitrile percentage or salt concentration. However, increasing the concentration

Table 2
m/z values of the main CID fragments observed for SBE_n-β-CD derivative dissolved in 100 m*M* of ammonium acetate in water–acetonitrile (70:30)

SBE_n derivative	m/z (amu)	
	$(M-H)^{1-}$	$[(M-2H)/2]^{2-}$
SBE_1	1270.0	
SBE_2		702.0
SBE_3		770.0
SBE_4		838.0
SBE_5		906.0
SBE_6		974.0
SBE_7		1042.0
SBE_8		1110.0
SBE_9		1178.0
SBE_{10}		1246.0
SBE_{11}		1314.0
SBE_{12}		1382.0

M, molar mass of the SBE_n derivatives where the negative charges are neutralized with H^+.

of the salt additive (above 200 m*M*) led to a small loss in response and with 20% of the organic modifier, the detection sensitivity is lower than with 30 and 50%.

3.1.3. Effect of electrolyte nature

Flow injection analyses of samples A and B were achieved by replacing ammonium acetate with ammonium formate in sample solvent. A 100 m*M* salt concentration added to mixture water/acetonitrile (70:30) was used. Similar ionic species from sample A and sample B (mainly $[M-2H]^{2-}$) were formed, with ammonium formate or ammonium acetate in media. Fig. 4 shows a comparison of the absolute MS response of each SBE_n derivative present in sample A and sample B, respectively versus the nature of the ammonium salt. It appears that ammonium acetate gave the best overall response for the SBE_n derivatives. Moreover, the relative ratios of each derivative in the mixture were the same whatever the nature of the ammonium salt. A distribution centered around SBE_2 derivative for sample A and around SBE_6 for sample B was noted. The MS value given in Fig. 4 is obtained by summing up all the mass peak intensities observed for each SBE_n derivative.

In conclusion, it is with a sample solvent constituted by ammonium acetate (10–200 m*M*) in water–acetonitrile (70:30 to 50:50) that the best sensitivity for the SBE-β-CD analysis was obtained.

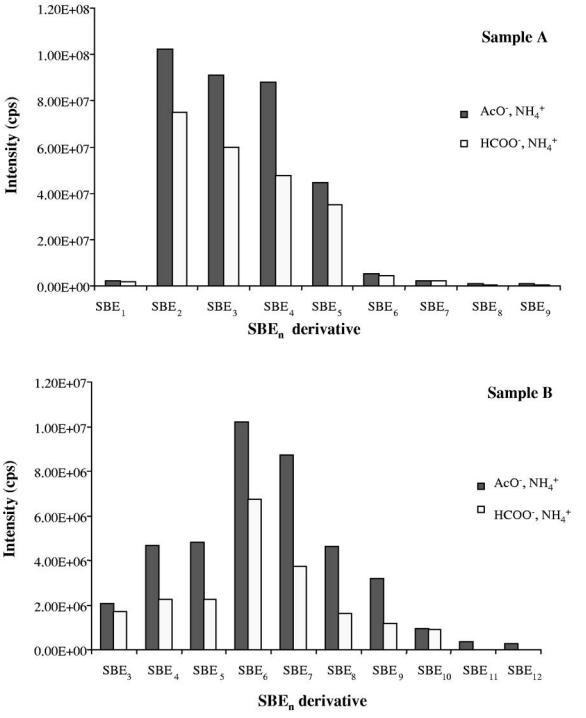

Fig. 4. Absolute MS response of each SBE_n derivative present in sample A and sample B, respectively, versus the nature of ammonium salt at a concentration of 100 m*M* in a (70:30) water–acetonitrile mixture. Peak areas of all ion species were summed for each SBE_n derivative.

3.1.4. Determination of the average degree of substitution of SBE-β-CD mixtures

In order to characterize the two SBE-β-CD mixtures, an average degree of substitution (DS) of each sample was calculated. The DS expresses the average number of substituted hydroxyl groups per cyclodextrin molecule in the mixture. The DS values were obtained from the relation: $DS=\Sigma(n\times A_n\ (\%)/100)$, where n is the SBE group number in a given SBE_n derivative and A_n (%) represents the percent contribution of all peak areas, attributable to a given SBE_n derivative, to the total peak areas of a given sample. It has already been established that the MS response of sulfated analytes decreases by increasing the number of sulfated moieties [27]. However, as pure SBE-β-CD standards with a well-established substitution degree were not commercially available,

an average degree of substitution has been calculated by considering an equal MS response coefficient for each SBE_n derivative. Table 3 reports the DS values calculated by IS-MS for samples A and B dissolved in different sample solvents, composed of ammonium acetate (10–300 m*M*) in mixture water/acetonitrile (20, 30 and 50% acetonitrile). Whatever the sample solvent conditions, very similar DS values were noted for each given sample; therefore, the composition in acetonitrile percentage or salt concentration of the sample solvent does not significantly affect the DS value.

Direct injection in MS is a fast method to characterize SBE-β-CD samples and to determine an average degree of substitution. It depicts differences in the composition of SBE-β-CD mixtures. The method described here can certainly be used as a fast quality control in the manufacture of scale compound mixtures; however, it cannot distinguish the potential SBE_n derivative isomers. To obtain additional information on the composition of the SBE-β-CD samples it was therefore necessary to use several complementary LC chromatographic systems, which can also separate the different substitution isomers. Moreover, only a separative technique such as liquid chromatography allows the production of pure SBE-β-CD components on a micro- or macroscale.

3.2. Analysis of the sulfobutyl ether β-cyclodextrin in LC–MS

The mass spectrometer needs a volatile mobile phase when LC–MS coupling is investigated. This is also a requirement for evaporative light scattering detection in order to avoid high background noise. As a consequence, LC methodology previously developed with ELSD is directly compatible with MS detection. In a recent paper [15], we have proposed an anion-exchange chromatography method using volatile salt (ammonium acetate) in mixture water/acetonitrile as mobile phase and evaporative light scattering detection to obtain suitable fingerprints for the SBE-β-CD samples. Moreover, this mobile phase composition is in good agreement with the conditions required to obtain the best MS response for SBE-β-CD mixtures. Thus, the analysis of two home-made (samples A and B) and two commercial sulfobutyl ether β-cyclodextrin mixtures (samples C and D) was achieved by LC–MS under similar LC–ELSD conditions to those used in Ref. [15]. Samples A and C, characterized by the LC–ELSD method with the lowest average DS (3.5 and 5.3, respectively) were analysed under the same gradient elution composed of an initial concentration of acetate of 10 m*M* in water–acetonitrile (70:30) for 5 min and a final concentration of acetate of 250 m*M* in water/acetonitrile (70:30) reached in 20 min. Samples B and D, more charged, required a higher concentration of salt added in the mobile phase to be eluted and were analysed under the gradient elution conditions based on increasing acetate concentration from 50 to 300 m*M* in water/acetonitrile (70:30) in 35 min.

To increase MS signal intensity, the different acquisitions of the analytes in LC–MS were performed using the selected ion monitoring mode (SIM). The m/z ratios corresponding to the main $[M-2H]^{2-}$ dianions were selected for each potential SBE_n derivative. The $[M-H]^{1-}$ ion for the onefold derivative of samples A and B was also selected. No sodium adduct was detected in LC–MS because Na^+ present in the SBE-β-CD solutions was eluted in void volume on the Vydac 302 IC column, which is an anion-exchanger support.

Figs. 5 and 6 show the selected ion monitoring (SIM) of samples A–D obtained by LC–MS. The four SBE-β-CD samples typically showed a complex profile of resolved peaks. The chromatographic

Table 3
Average degrees of substitution ($DS=\Sigma(n\times A_n\ (\%)/100)$[a] calculated by MS for sample A and sample B with acetate ammonium concentrations varying from 10 to 300 m*M* in water/acetonitrile mixtures at different percentages of acetonitrile

	Percentage of acetonitrile and acetate concentration (m*M*)						Average value of DS
	20%, 100	30%, 10	30%, 50	30%, 100	30%, 300	50%, 100	
Sample A	3.48	3.49	3.53	3.51	3.47	3.56	3.50
Sample B	7.79	7.74	7.82	7.85	7.81	7.83	7.80

[a] A_n was obtained by adding the peak areas of all species detected for each substituted SBE_n derivative.

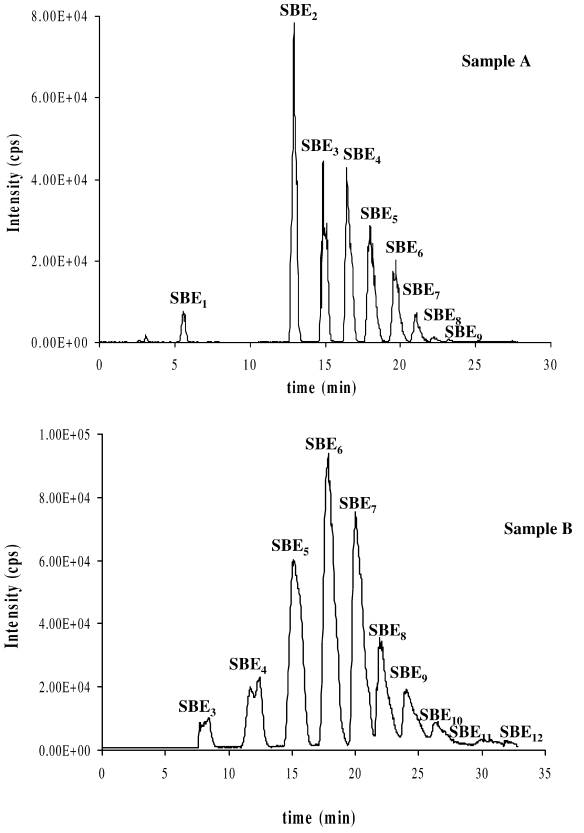

Fig. 5. Selected ion-monitoring of two home-made SBE-β-CD samples separated on an anion-exchange column under gradient elution conditions and detected on-line as $[M-2H]^{2-}$ with IS-MS. Column Vydac 302 IC (250×4.6 mm I.D.). Flow-rate: 1 ml min^{-1}. Split: 1/20. Gradient conditions for sample A: Eluent A, 10 m*M* of ammonium acetate in water/acetonitrile (70:30). Eluent B, 250 m*M* of ammonium acetate in water/acetonitrile (70:30). From 100% A for 5 min to 100% B in 20 min. Gradient conditions for sample B: Eluent A, 50 m*M* of ammonium acetate in water/acetonitrile (70:30). Eluent B, 300 m*M* of ammonium acetate in water/acetonitrile (70:30). From 100% A to 100% B in 35 min. Mass spectrometry: IS: −4.8 kV, OR: −60 V, RNG: −300 V, intensity of selected ions: counts per s (cps).

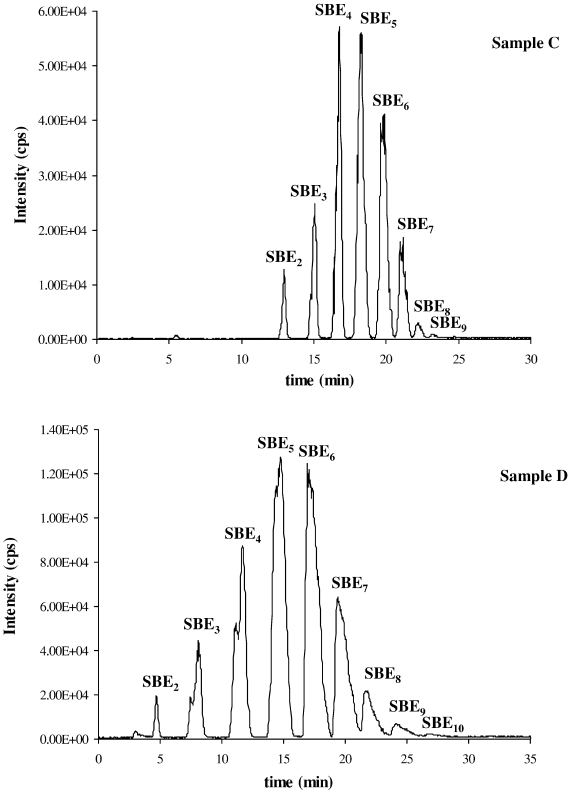

Fig. 6. Selected ion-monitoring of two commercial SBE-β-CD samples separated on an anion-exchange column under gradient elution conditions and detected on-line as $[M-2H]^{2-}$ with IS-MS. Column Vydac 302 IC (250×4.6 mm I.D.). Flow-rate: 1 ml min^{-1}. Split: 1/20. Sample C analysed under the same gradient conditions as sample A and sample D analysed under the same gradient conditions as sample B in Fig. 5. Mass spectrometry: conditions as in Fig. 5.

profiles obtained by LC–MS or LC–ELSD are comparable in terms of retention time but resolutions are sometimes lower in LC–MS than in LC–ELSD, probably due to differences in the apparatus. The LC–MS analysis confirms that on a silica anion-exchanger, the more charged the cyclodextrin is, the more retained it is and the substitution isomers potentially present in the mixture were not separated.

An average degree of substitution (DS) based on the percent contribution of each chromatographic peak to the total peak area composition of a LC profile can also be calculated for the four SBE-β-CD mixtures. Table 4 reports the average DS values for

Table 4
Average DS of different SBE-β-CD samples calculated in LC–MS and eluted on Vydac 302 IC column (250×4.6 mm I.D.)[a]

SBE-β-CD sample	DS=Σ(n×A_n (%)/100)[b]
Sample A	3.5
Sample B	7.8
Sample C	5.0
Sample D	6.4

[a] Under gradient elution conditions as depicted in Figs. 4 and 5.
[b] A_n is the percent contribution of each peak to the total peak area composition of the LC profile.

each sample. These calculated DS values were similar to those obtained by direct MS analysis. Under these salt conditions, the relatively low formation of tri- and tetracharged species did not contribute highly to the DS determination. Indeed, by selecting all the multicharged ions for each SBE derivative in MS or by selecting only the dianions $[M–2H]^{2-}$ and the monoanion $[M–H]^{1-}$ in LC–MS, the value of the average DS was nearly equal.

Furthermore, the DS values calculated by LC–MS are in good agreement with those obtained by LC–ELSD which are, respectively, 3.5 for sample A, 7.7 for sample B, 5.3 for sample C and 6.4 for sample D. It has been established that MS response is linked to the solute ionization which decreases by increasing the charges in the parent molecule [27] whereas ELSD response is directly linked to the sample volatility which decreases the more ionized the solute is. For the SBE-β-CD mixtures, the DS values obtained by LC–ELSD and LC–MS methods are in agreement, which may be attributed to similar ionization and volatility conditions for the different SBE_n derivatives.

4. Conclusion

This paper reports the first investigations in which SBE-β-CD mixtures were analyzed both in MS and in LC–MS using ion-spray negative mode. These methods are powerful tools in the rapid molecular mass determination of the individual substituted derivatives of the cyclodextrin samples without the use of standards and enable an average degree of substitution of the samples to be calculated. In both methods, the DS values of the SBE-β-CD mixtures obtained are equal. The advantage of the MS method is that it provides a fast and simple means of checking batch-to-batch variability in the manufacture of SBE-β-CD mixtures. LC–MS allows the separation of the sample versus the different substitution degrees of each component and consequently can be used for purification. The present study leads only to qualitative results, which could be rendered quantitative, if needed, by conducting precise measurements of the variation of the ionization efficiency with the number of sulfobutyl ether groups. Obtaining pure SBE-β-CD with a given degree of substitution is now possible using the AEC methodology with a volatile mobile phase as described in this paper.

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