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Fragmentation pattern of underivatised xylo-oligosaccharides and their alditol derivatives by electrospray tandem mass spectrometry

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Abstract

Maltopentaose and olive pulp xylo-oligosaccharides and the correspondent alditol derivatives were analysed by ESI-MS and ESI-MS/MS. The ESI-MS spectrum of maltopentaose and maltopentaose alditols showed $[M + Na]^+$and $[M + H]^+$ ions. ESI-MS spectrum of xylo-oligosaccharides and their alditols showed $[M + Na]^+$of neutral (Xyl_{3-6}) and acidic (Xyl_{2-3}MeGlcA and Xyl_{2-3}GlcA) xylo-oligosaccharides. The ESI-MS/MS spectra of maltopentaose and underivatised xylo-oligosaccharides presented fragments of glycosidic cleavages attributed to B/Z and C/Y ions. On the other hand, MS/MS spectra of the correspondent alditols showed glycosidic cleavages unambiguously identified as B-type and Y-type ions. Y-type fragment ions showed higher abundance in the MS/MS spectra of the alditol derivatives when compared to the non-reduced samples. The study of the oligoxylosyl alditols fragmentation permits to distinguish fragmentation pathways that occur both from the reducing end and from the non-reducing end of the xylan chain, allowing to obtain more information about the localization of the acidic substituent along the glucuronoxylan backbone.

Keywords: Glucuronoxylans; Alditols; Oligosaccharides; Electrospray; Tandem mass spectrometry

1. Introduction

The number of studies involving the characterisation of oligosaccharides by Mass Spectrometry has increased over the last years, as a consequence on the advances achieved on electrospray ionisation (ESI) and matrix-assisted laser desorption ionisation (MALDI). These methods promote the ionisation of intact molecules into the gas phase, allowing us to determine the molecular mass of each component present in a complex mixture. Structural information such as sugar composition, sugar sequence, branching and/or type of linkage can be obtained by the study of the fragmentation of the ions formed either in the MALDI-MS (Finke, Stahl, Pfenninger, Karas, Daniel, & Sawatzki, 1999; Garozzo, Spina, Cozzolino, Cescutti, & Fett, 2000; Harvey, 1999; Huisman, Schols, & Voragen, 2000; Mele & Malpezzi, 2000; Reis, Domingues, Ferrer-Correia, & Coimbra, 2003a; Vierhuis, Schols, Beldman, & Voragen, 2001a; Yamagaki & Nakanishi, 1998, 2001) and ESI-MS (Ekeberg, Knutsen, & Sletmoen, 2001; Harvey, 2000a; Körner, Limberg, Christensen, Mikkelsen, & Roepstorff, 1999; Lemoine, Fournet, Despeyroux, Jennings, Rosenberg, & Hoffman, 1993; Mo et al., 1999; Reinhold, Reinhold, & Costello, 1995; Reis, Coimbra, Domingues, Ferrer-Correia, & Domingues, 2002) spectra of the oligosaccharides.

The analysis of oligosaccharides by electrospray mass spectrometry has been performed either on underivatised (Chai, Luo, Lim, & Lawson, 1998; Ekeberg et al., 2001; Harvey, 2000b; Reis et al., 2002) or on derivatised samples, involving either permethylation (Brüll et al., 1998; Weiskopf, Vouros, & Harvey, 1998) or reductive amination (Harvey, 2000a; Mo et al., 1999). Apart from providing more sensitive analysis (Harvey, 2000a), derivatised samples also provide unambiguous fragment ion identifications (Viseux, Costello, & Domon, 1999) due to the mass increment observed on the *m/z* value of the fragment ions formed from the reducing end. A drawback of the derivatisation procedures are the clean-up steps that usually increase the time of manipulation of the samples.

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The reaction of the oligosaccharides with $NaBH_4$ is another derivatisation approach, leading to an increase of 2 mass units through the reduction of the hemiacetal group to an alcohol. So far, the formation of oligosaccharide alditols has been proposed for the stabilisation of the anomeric carbon during β-elimination release of oligosaccharides from *O*-glycans (Coppin, Maes, Morelle, & Strecker, 1999; Kogelberg, Chai, Feizi, & Lawson, 2001; Morelle, Guyétant, & Strecker, 1998; Morelle, Lemoine, & Strecker, 1998) prior to mild periodate oxidation, and also during the analysis by NMR of oligosaccharides derived from xyloglucans (Hantus, Pauly, Darvill, Albersheim, & York, 1997; Spronk et al., 1998; York, Kumar Kolli, Orlando, Albersheim, & Darvill, 1996). In spite of the simplicity of this derivatisation procedure, to our knowledge, the use of alditol oligosaccharides for mass spectrometry analysis has only been applied on structural studies of xyloglucan oligosaccharides by MALDI-PSD (Vierhuis et al., 2001b).

The analysis of oligosaccharides released from xylans has been done mainly by NMR, and also by mass spectrometry using MALDI-MS (Deery, Stimson, & Chapell, 2001; Huisman et al., 2000; Jacobs, Larsson, & Dahlman, 2001; Kabel et al., 2002; Reis et al., 2003a; Vierhuis et al., 2001a) and ESI-MS (Reis et al., 2002; Reis, Domingues, Domingues, Ferrer-Correia, & Coimbra, 2003b; Samuelsen, Cohen, Paulsen, Brüll, & Thomas-Oates, 2001). Recently, positive tandem mass spectrometry, using ESI, was proposed for the characterisation of underivatised neutral (Xyl_n) and acidic (Xyl_n-MeGlcA and Xyl_nGlcA) xylo-oligosaccharides formed by partial acid hydrolysis (Reis et al., 2002, 2003b). The fragment ions observed in the MS/MS spectra of sodium adducts of acidic xylo-oligosaccharides allowed to identify the substituting residue attached to the xylan chain. However, the occurrence of fragment ions formed from both reducing and non-reducing end made difficult to propose the location of the substituting residue along the xylan backbone. The possible sites for the location of the acidic residues increase with the increasing of the molecular weight. Therefore, the study of the fragmentation of acidic xylo-oligosaccharides alditol derivatives should give a more precise assessment of the fragment ions formed upon collision-induced dissociation.

To evaluate the validity of alditol derivatisation on the unambiguous identification of the fragmentation of linear oligosaccharides, such as xylo-oligosaccharides, an initial study of the fragmentation of maltopentaose has been performed and compared with the fragmentation of the maltopentaose alditol derivative. This study discusses and compares the fragmentation pattern of molecular ions of underivatised maltopentaose and xylo-oligosaccharides and the correspondent alditol derivatives formed under ESI. Based on the identification of the fragment ions, the use of the oligoxylosyl alditol derivatives allowed to distinguish the different fragmentation pathways that occur either from the reducing end or from the non-reducing end of the xylan chain, allowing to obtain additional structural information.

2. Experimental

2.1. Samples

Maltopentaose was purchased from Fluka and sodium borohydride was purchased from Riedel-de-Haën. All solvents used were HPLC grade, and the solutions used were prepared with MilliQ high purity water. Olive pulp xylo-oligosaccharides were obtained from olive fruit glucuronoxylan extracted with 1 M KOH, as described by Coimbra, Waldron and Selvendran (1994). The extract, with 82% of sugar material, was composed mainly of xylose (78 mol%), uronic acid (11 mol%), glucose (4 mol%) and arabinose (3 mol%). The xylo-oligosaccharides were obtained by partial acid hydrolysis using TFA 50 mM at 100 °C for 45 min followed by size-exclusion chromatography on a Biogel P6 (6000–800 Da; BioRad) gel media column using ammonium formate 100 mM (pH 3.6) as eluent. The elution profile was monitored using an evaporative light scattering detector (ELSD) and UV, as described by Reis et al. (2003a). The low molecular weight fraction was analysed by ESI mass spectrometry in the positive mode.

2.2. Preparation of oligosaccharide alditols

The maltopentaose solution (5 mg/ml) was prepared in MilliQ high purity water, and the corresponding alditol derivatives were prepared by addition of 50 μl of sodium borohydride solution (15% in NH_4OH, 3 M) to 100 μl of maltopentaose, and left to react for 1 h at 30 °C in a screw cap tube. The excess of reducing agent was removed by addition of 5 μl aliquots of glacial acetic acid until no bubbles were observed. The content of borate salts was decreased in solution by evaporation of methanol under reduced pressure. The addition of methanol was repeated until the absence of salts was observed in the screw cap tube (Selvendran, March, & Ring, 1979).

The dried hydrolysate containing approximately 0.50 mg of the xylo-oligosaccharides mixture was solubilised in 100 μl of MilliQ high purity water and the xylo-oligosaccharide alditols were prepared as described for the maltopentaose.

2.3. Electrospray mass spectrometry

Electrospray analysis of maltopentaose and maltopentaose alditols was performed by dissolving the dried oligosaccharides in 1 ml of water and further diluted by a factor of 1:100 in MeOH:H_2O (1:1, v/v) solution with 1.0% (v/v) of formic acid. The reduced and non-reduced xylo-oligosaccharides were dissolved in 100 μl of MeOH:H_2O

(1:1, v/v) solution with 1.0% (v/v) of formic acid without further dilution. Samples were introduced into the mass spectrometer using a flow rate of 5 μl/min.

Positive ion mode ESI-MS and MS/MS spectra were acquired using a Q-TOF 2 instrument (Micromass, Manchester, UK), setting the needle voltage at 3000 V with the ion source at 80 °C and desolvation temperature at 150 °C maintaining the cone voltage at 30 V. Tandem mass spectra (MS/MS) of molecular ions were obtained using collision induced dissociation (CID), using Argon as the collision gas and varying collision energy between 30–60 eV for the sodium adducts, and between 15–20 eV for the protonated molecules. Each spectrum was produced by accumulating data during 1–2 min. In MS and MS/MS experiments TOF resolution was set to approximately 10,000 (FWHM definition). In MS/MS experiments Q1 peak width (FWHM) was set to approximately to 0.7 Th.

3. Results and discussion

3.1. Maltopentaose mass spectrometry

The electrospray mass spectra obtained for the maltopentaose and for the reduced maltopentaose are shown in Fig. 1. The predominant ion observed in the mass spectrum of maltopentaose prior to $NaBH_4$ reduction (Fig. 1a) at *m/z* 851 corresponds to the sodium adduct $[M + Na]^+$, although it can also be seen, with low relative abundance, the contribution of the protonated molecule $[M + H]^+$, at *m/z* 829, as well as the potassium adduct $[M + K]^+$, at *m/z* 867. The mass spectrum of the maltopentaose alditol (Fig. 1b) showed the predominance of the sodium adduct at *m/z* 853, over the protonated molecule (*m/z* 831) or the potassium adduct (*m/z* 869). By comparison of the mass spectrum of maltopentaose alditol (Fig. 1b), with the spectrum of the native maltopentaose (Fig. 1a), it can be seen that the ions observed had an increase of two mass units, which is in accordance with the expected value resultant of the reduction of the aldehyde group to an alcohol. The absence in the maltopentaose alditols mass spectrum (Fig. 1b) of ions at *m/z* 829, 851, and 867, corresponding to the protonated molecule, to the sodium adduct, and to the potassium adduct, respectively, indicates that the reaction with $NaBH_4$ was complete.

The MS/MS mass spectra obtained for the sodium adducts of maltopentaose and maltopentaose alditols are shown in Fig. 2. The predominant fragment ions observed in the MS/MS spectrum of maltopentaose (Fig. 2a) show a 162 mass difference that is attributed to the glucosyl unit that composes the oligosaccharide. The fragmentation process of underivatised oligosaccharides in the positive mode may lead to glycosidic cleavages with charge retention at the reducing end originating Y- or Z-type fragments, or with charge retention at the non-reducing end thus leading to B- or C-type fragment ions, were named according to the nomenclature developed by Domon and Costello (1988) for carbohydrates (Scheme 1). The ions at *m/z* 671, 509, 347, and 185 correspond to glycosyl cleavages B/Z-type fragment ions.

The fragment ions with low relative abundance, at *m/z* 689, 527, 365, and 203 correspond to C/Y-type fragment ions. The ion at *m/z* 185, according to Scheme 2a, corresponds to the mass of 163 of the glucosyl residue, plus 23 mass units of the Na^+, with removal of an H from the fragment ion. The ion at *m/z* 203, according to Scheme 2a, corresponds to the mass of 179 of the glucosyl residue, plus 23 mass units of the Na^+, with addition of an H to the fragment ion. This pattern is also observed for the other ions, allowing to conclude that the formation of B/Z-type fragment ions involves the removal of one hydrogen from the fragment ion, and the formation of C/Y-type fragment ions involves migration of one hydrogen to the fragment ion. The fragment ions at *m/z* 791, observed with low relative abundance, formed by loss of 60 mass units, were attributed to cross-ring cleavages corresponding to $^{0,2}A/^{2,4}X$-type fragment ions, probably by loss of $C_2H_4O_2$, as suggested by Harvey (2000b).

The MS/MS spectra of the sodium adduct of maltopentaose alditol (Fig. 2b) showed the presence of

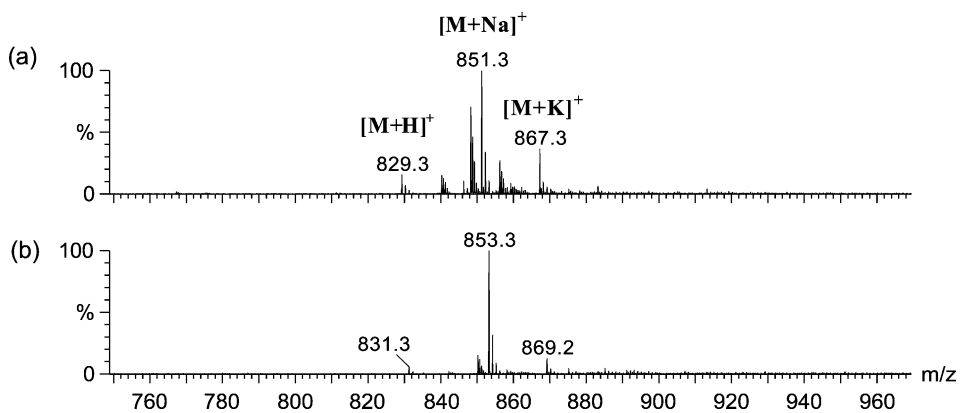


Fig. 1. ESI-MS/MS spectra of maltopentaose, (a) before and (b) after reduction with $NaBH_4$.

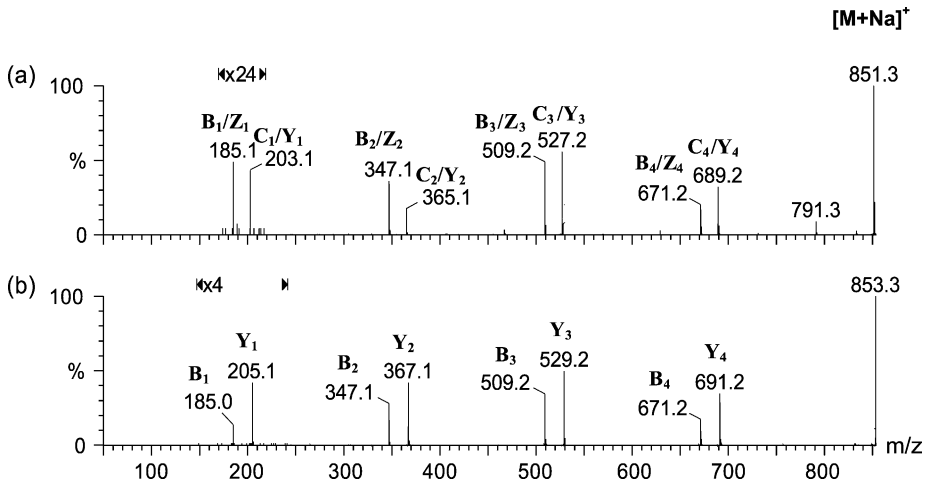


Fig. 2. ESI-MS/MS spectra of sodium adduct $[M + Na]^+$of maltopentaose (a) before and (b) after reduction with $NaBH_4$.

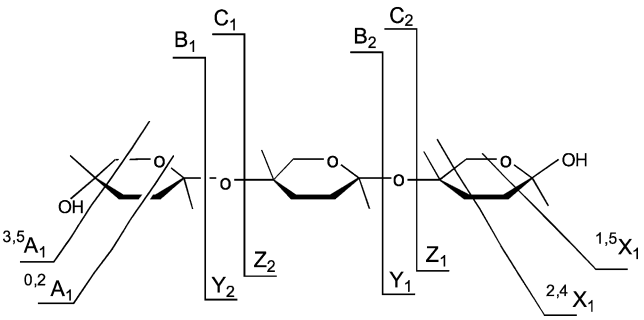
abundant fragment ions at *m/z* 671, 509, 347, and 185 that maintained the same *m/z* value, allowing to assign these fragment ions as B-type. In opposition, the fragment ions at *m/z* 691, 529, 367, and 205, showed a mass increase of 2 mass units relative to the fragment ions observed in the previous spectrum. This increase is due to charge retention at the alditol side of the oligosaccharide (Scheme 2b), which allows to, unambiguously, assign the fragment ions as Y-type. These results are in accordance with the presence of abundant Y-type fragments and weak B-type fragment ions for the sodium cationised adducts of a large range of xylosyl disaccharide alditols obtained by high-energy decompositions with FAB-MS (Kovacik, Hirsch, & Heerma, 1997). No cross-ring fragment ions were observed in the MS/MS spectrum of maltopentaose alditols.

The ESI-MS/MS mass spectra obtained for the protonated molecule of maltopentaose and maltopentaose alditols are shown in Fig. 3. The predominant fragment ions observed in the MS/MS spectrum of maltopentaose (Fig. 3a) show, as observed for the sodium adducts, a 162 mass difference, attributed to the glycosidic cleavages of the oligosaccharide. The ions at *m/z* 649, 487, 325, and 163 corresponded to B/Z-type fragment ions, and the fragment ions with lower relative abundance, at *m/z* 667, 505, 343, and 181 correspond to C/Y-type fragment ions. The MS/MS spectra of the protonated molecule of maltopentaose alditol (Fig. 3b) showed the presence of abundant fragment ions at *m/z* 669, 507, 345, and 183, containing a 2 mass units increase relative to non-reduced fragment ions. This result allowed to unambiguously assign the fragment ions as Y-type. On the other hand, the fragment ions at *m/z* 649, 487, 325, and 163 showed the same *m/z* value even after reaction with $NaBH_4$, which is consistent with the charge retention at the non-reducing end, corresponding to B-type fragment ions. Both B- and Y-type fragment ions showed high relative abundance.

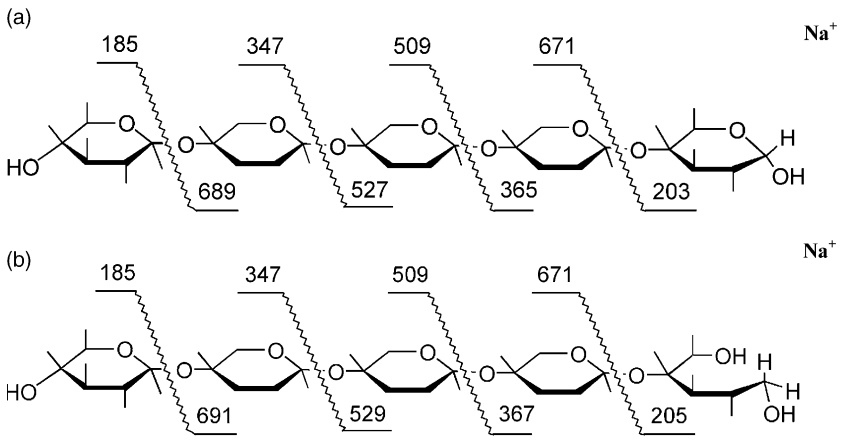
The results obtained from both sodium adducts and protonated molecules MS/MS spectra of the maltopentaose alditol showed that the relative abundance of the Y-type fragment ions was higher when compared to the non-derivatised maltopentaose.

3.2. Xylo-oligosaccharide alditols mass spectrometry

The positive ESI-MS spectrum obtained for the xylo-oligosaccharides mixture (Xyl_{3-6}, $Xyl_{2-3}GlcA$, Xyl_{2-3}-MeGlcA) (Reis et al., 2003a,b) are summarised in Table 1. The predominant oligosaccharide ions observed at *m/z* 437, 569, 701, and 833 were identified as sodium adducts of neutral oligosaccharides (Xyl_n) for $n = 3–6$, respectively; the ions at *m/z* 481 and 613, with a 44 mass units increase relative to the neutral oligosaccharides, were identified as xylo-oligosaccharides substituted by one glucuronic acid (GlcA) residue, corresponding to $Xyl_{2-3}GlcA$, and the ions observed at *m/z* 495 and 627, with a 58 mass units increase relative to the neutral oligosaccharides, were identified as xylo-oligosaccharides substituted with one 4-*O*-methyl-glucuronic acid (MeGlcA) residue corresponding



Scheme 1. Nomenclature for fragment ions from carbohydrates according to Domon and Costello (1988).



Scheme 2. Schematic fragmentation pathways of the sodium adducts of (a) maltopentaose and (b) maltopentaose alditol.

to $Xyl_{2\text{-}3}$MeGlcA. Ammonium and potassium adducts were observed with low relative abundance. The ESI-MS spectrum of the xylo-alditol oligosaccharides showed the presence of the ions identified as sodium adducts with a 2 Da mass increase when compared to the previous spectrum. The fact that other adducts, namely ammonium and potassium adducts, are not observed may be due to the use of sodium ions in the reducing agent, reflecting an increment of the amount of sodium ions in the sample. The xylo-oligosaccharides observed in the ESI-MS spectra obtained before and after $NaBH_4$ reaction are summarised in Table 1. The absence of ions of non-reduced molecules in the ESI-MS of xylo-oligosaccharide alditols showed that the reduction with $NaBH_4$ was complete.

The ESI-MS/MS spectra of sodium adducts for the neutral and acidic xylo-oligosaccharides, as previously reported (Reis et al., 2002, 2003b), showed the presence of C/Y-type glycosidic ions and, in lower abundance, A-type cross-ring fragment ions. The ESI-MS/MS spectra of the acidic oligosaccharides substituted with MeGlcA and GlcA showed the loss of the substituting residue as the predominant fragmentation.

The MS/MS spectrum of sodium adduct of Xyl_3Xylol (Xyl_4 alditol), at *m/z* 571, is depicted in Fig. 4b, which can be compared to the MS/MS spectrum of the correspondent sodium adduct of Xyl_4, at *m/z* 569 (Fig. 4a). The MS/MS spectrum of Xyl_4 showed the presence of ions at *m/z* 437, 305, and 173, attributed to C/Y-type fragment ions, at *m/z* 419 and 287, attributed to B/Z-type ions, and at *m/z* 509 and 377, attributed to ^{0,2}A-type ions. The ESI-MS/MS spectrum obtained for Xyl_3Xylol showed the presence of fragment ions at *m/z* 439, 307, and 175, showing a 132 Da mass difference, corresponding to glycosidic fragments, with a 2 Da mass increase relative to the non-reduced Xyl_4, which allows assigning these as Y-type fragment ions. The fragment ions observed at *m/z* 419 and 287 are also observed in the MS/MS spectrum of Xyl_4. This fact is consistent with the charge retention at the non-reducing end, thus corresponding to B-type fragment ions. The fragment ions observed at *m/z* 377 and 245 that exhibited a 60 Da mass difference relative to the glycosidic ions at *m/z* 437 and 305 in the Xyl_4 mass spectrum, maintained the same *m/z* value even after reaction with $NaBH_4$, leading to the identification of the ions as corresponding to ^{0,2}A-type ions.

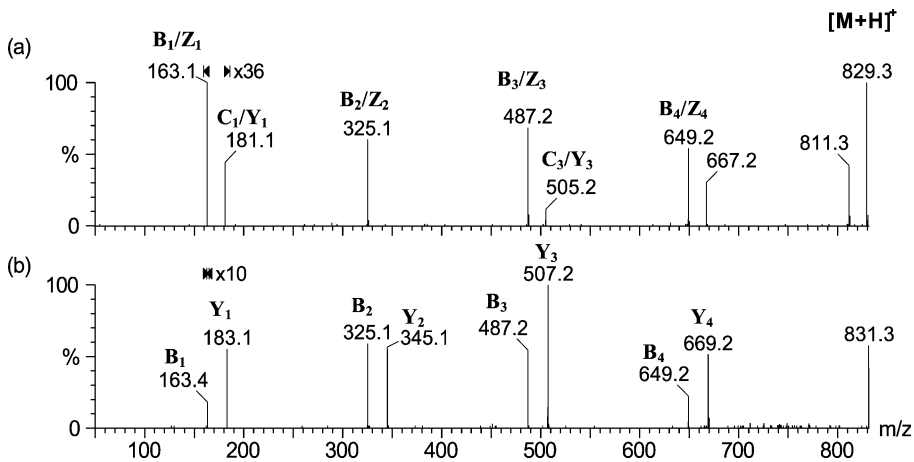


Fig. 3. ESI-MS/MS spectra of the protonated molecule $[M + H]^+$of maltopentaose, (a) before (*m/z* 829) and (b) after reduction with $NaBH_4$ (*m/z* 831).

Table 1
Cationised adducts of xylo-oligosaccharides and xylo-oligosaccharide alditols observed in the ESI-MS spectra

	Xylo-oligosaccharide			Xylo-oligosaccharide alditols
	$[M + NH_4]^+$	$[M + Na]^+$	$[M + K]^+$	$[M + Na]^+$
Xyl_3	432	437	453	439
Xyl_2GlcA	476	481	497	483
$Xyl_2MeGlcA$	490	495	511	497
Xyl_4	564	569	585	571
Xyl_3GlcA	608	613	629	615
$Xyl_3MeGlcA$	622	627	643	629
Xyl_5	696	701	717	703
Xyl_6	828	833	849	835

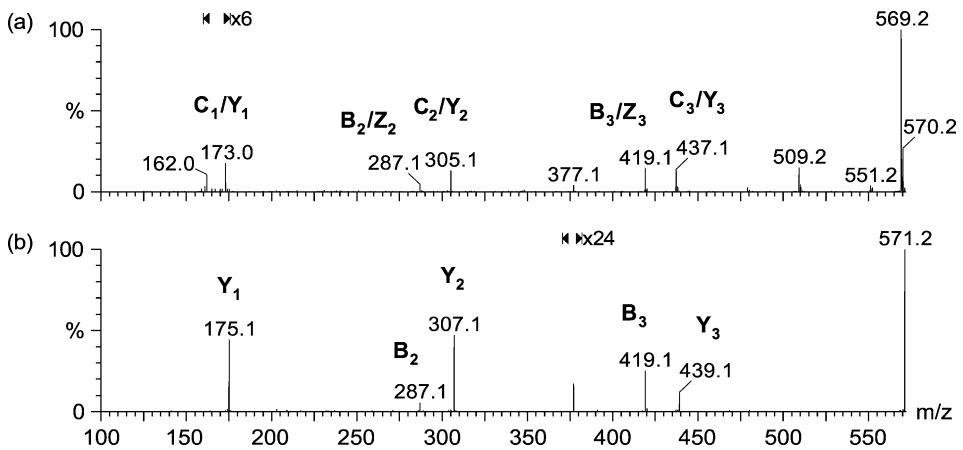


Fig. 4. ESI-MS/MS spectra of sodium adduct $[M + Na]^+$of neutral xylo-oligosaccharide Xyl_4, (a) before (*m/z* 569) and (b) after reduction with $NaBH_4$ (*m/z* 571).

The MS/MS spectra of xylo-oligosaccharide alditols Xyl_4Xylol, at *m/z* 703, and Xyl_5Xylol, at *m/z* 835, showed fragmentation pathways similar to that observed for Xyl_3-Xylol, summarised in Table 2. By the reduction of the neutral xylo-oligosaccharides unambiguous fragment ions assignment was achieved (Table 2). The MS/MS spectrum of sodium adduct of XylGlcAXylol (Xyl_2GlcA alditol), at *m/z* 483, is depicted in Fig. 5b, which can be compared to the MS/MS spectrum of the correspondent sodium adduct of Xyl_2GlcA, at *m/z* 481 (Fig. 5a). The MS/MS spectrum of XylGlcAXylol showed the presence of a fragment ion at *m/z* 307, formed by the loss of the substituting residue (176 Da) leaving the xylan chain, which prevents to obtain any structural information regarding the location of the GlcA in the chain. The presence of the fragment ion at *m/z* 331, formed by loss of 152 (149 of terminal Xyl + 2 of the reduction +1 of migration of H to the reducing end) as a B-type fragmentation, suggests the presence of the GlcA at the non-reducing end. On the other hand, the identification of the ion at *m/z* 351, formed by loss of 132, due to the loss of the xylosyl residue from the non-reducing as a Y-type fragmentation, confirms the presence of the GlcA also at the reducing end. This is also observed in

Table 2
Fragment ions observed in the ESI-MS/MS spectra for sodium adducts of neutral xylo-oligosaccharides at *m/z* 701 (Xyl_5) and *m/z* 833 (Xyl_6), and their alditol derivatives, at *m/z* 703 (Xyl_4Xylol) and *m/z* 835 (Xyl_5Xylol)

Xyl_n			$Xyl_{n-1}Xylol$		
Fragments	Xyl_5 (*m/z* 701)	Xyl_6 (*m/z* 833)	Fragments	Xyl_5Xylol (*m/z* 703)	Xyl_6Xylol (*m/z* 835)
			$-H_2O$		817 (5)
		773 (60)			
		701 (45)	Y_5		703 (40)
B_5/Z_5	683 (45)	683 (100)	B_5		683 (70)
$^{0,2}A_5$	611 (22)		$^{0,2}A_5$	611 (10)	611 (<2)
C_4/Y_4	569 (50)	569 (55)	Y_4	571 (25)	571 (60)
B_4/Z_4	551 (62)	551 (80)	B_4	551 (72)	551 (40)
$^{0,2}A_4$		509 (15)	$^{0,2}A_4$	509 (5)	509 (<2)
C_3/Y_3	437 (100)	437 (60)	Y_3	439 (70)	439 (75)
B_3/Z_3	419 (70)	419 (25)	B_3	419 (25)	419 (30)
$^{0,2}A_3$	377 (22)		$^{0,2}A_3$	377 (<2)	377 (<2)
C_2/Y_2		305 (42)	Y_2	307 (100)	307 (100)
B_2/Z_2	287 (50)		B_2	287 (15)	287 (55)
$^{0,2}A_2$	245 (25)		$^{0,2}A_2$		
			Y_1	175 (50)	175 (60)

Relative abundances shown as % of fragment ions, normalised relative to the base peak.

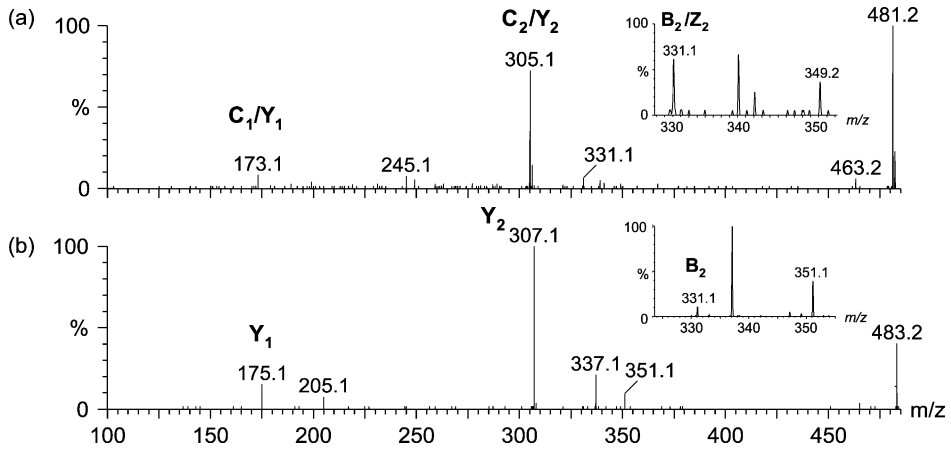


Fig. 5. ESI-MS/MS spectra of sodium adduct $[M + Na]^+$of acidic xylo-oligosaccharide Xyl_2GlcA, (a) before (*m/z* 481) and (b) after reduction with $NaBH_4$ (*m/z* 483).

Fig. 5a by the occurrence of the fragment ions at *m/z* 331 and 349, which allows to identify unambiguously the fragmentation pattern of these ions, as B-type and Y-type fragmentation, respectively.

Similarly to what was observed for the fragmentation pattern of XylGlcAXylol, the ESI-MS/MS spectrum of sodium adducts of XylMeGlcAXylol, at *m/z* 497 (data not shown), showed the predominant fragment ion at *m/z* 307, formed by the loss of the MeGlcA residue (190 Da). The presence of the fragment ions at *m/z* 345 and 365, correspondent to the loss of 152 (B-type fragment) and 132 (Y-type fragment) mass units, confirms the presence of both isomers in the mixture.

The MS/MS spectrum of sodium adduct of Xyl_2-MeGlcAXylol ($Xyl_3MeGlcA$ alditol), at *m/z* 629, is depicted in Fig. 6b, which can be compared to the MS/MS spectrum of the correspondent sodium adduct of $Xyl_3MeGlcA$, at *m/z* 627 (Fig. 6a). In both MS/MS spectra, the main fragmentation pathway correspond to the loss of 190 mass units (MeGlcA residue), with formation of the ions at *m/z* 439 and 437, respectively. The ions observed at *m/z* 477 and 345, formed by loss of the xylitol (152 Da) plus one xylosyl residue (132 Da) from the precursor ion, allowed attributing these fragment ions to B-type fragments. The predominant fragmentation pathways of the different alditol isomers are shown in Scheme 3. The ions observed at *m/z* 497 and 365 in the MS/MS spectrum of the alditol derivative (Fig. 6b), formed by loss of one and two xylosyl residues (132 Da) from the precursor ion, allowed attributing these fragment ions to Y-type fragments. This is also confirmed in Fig. 6a by the occurrence of the fragment ions at *m/z* 477 and 345, and the ions at *m/z* 495 and 363, which allows to identify unambiguously the fragmentation pattern of these ions, as B-type and Y-type fragmentation, respectively. In Fig. 6b, the identification of fragment ion at *m/z* 365 is

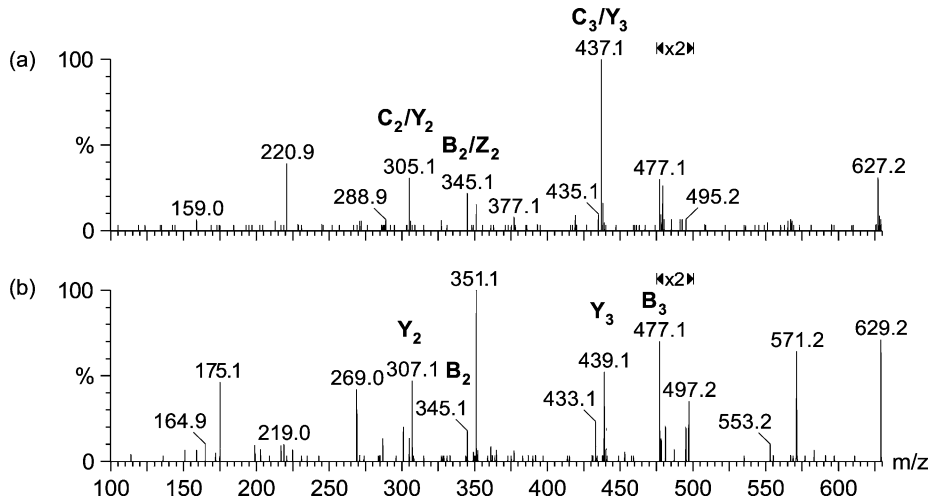
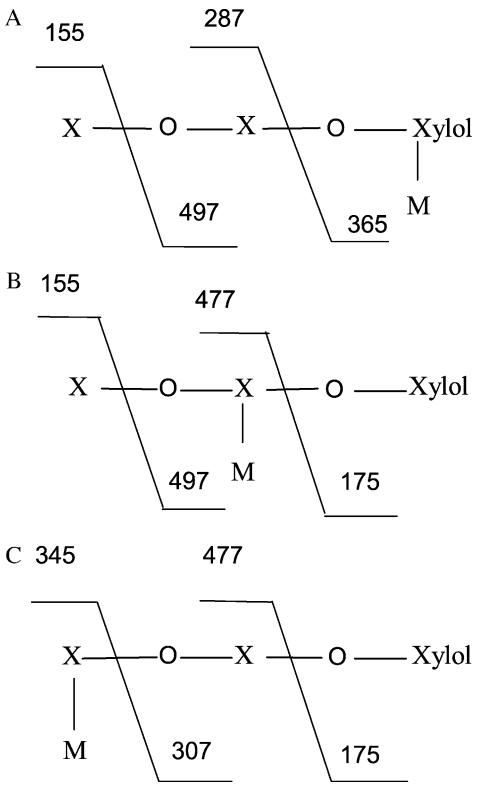


Fig. 6. ESI-MS/MS spectra of sodium adduct $[M + Na]^+$of acidic xylo-oligosaccharide $Xyl_3MeGlcA$, (a) before (*m/z* 627) and (b) after reaction with $NaBH_4$ (*m/z* 629).



Scheme 3. Schematic fragmentation pathways of $Xyl_2MeGlcAXylol$ isomers. (X represents xylosyl residue, M represents 4-*O*-methylglucuronic acid residue and Xylol represents xylitol).

diagnostic of the occurrence of isomer A (Scheme 3) while the fragment at *m/z* 345 is diagnostic of the occurrence of isomer C. The presence of the isomer B cannot be clearly determined due to peak overlapping of all fragments, with isomer A, at *m/z* 497 and 155, with isomer C, at *m/z* 477 and 175. The relative abundance of these diagnostic ions, namely the Y-type fragment ions, is higher in the MS/MS spectra of the alditol derivatives when compared with the non-reduced ones.

Similarly to what was observed for the fragmentation pattern of $Xyl_2MeGlcAXylol$, the ESI-MS/MS spectrum of sodium adducts of $Xyl_2GlcAXylol$, at *m/z* 615 (data not shown), showed the predominant fragment ion at *m/z* 439, formed by the loss of the GlcA residue. The ions observed at *m/z* 463 and 331 (data not shown), formed by loss of the xylitol followed by one xylosyl residue from the precursor ion, were attributed to B-type fragment ions and the fragment ions at *m/z* 483 and 351, formed by loss of one and two xylosyl residues, were identified as Y-type fragment ions. The occurrence in the MS/MS spectra of the non-reduced Xyl_3GlcA oligosaccharides of the fragment ions at *m/z* 463 and 331, and the ions at *m/z* 481 and 349, although in very low relative abundance, allows to identify unambiguously the fragmentation pattern of these ions, as B-type and Y-type fragmentation, respectively.

4. Conclusions

Alditol derivatisation associated with ESI-MS/MS proved to be a simple methodology to be used in the study of xylo-oligosaccharides, providing additional information in the acidic xylo-oligosaccharides, due to an unambiguous identification of the type of fragmentation pattern as B-type and Y-type fragmentations. The relative abundance of the ions resultant from the glycosidic cleavages, namely the Y-type fragment ions, is higher in the MS/MS spectra of the alditol derivatives when compared with the non-reduced samples, which is an advantage. This advantage is even more relevant in the case of the analysis of acidic xylo-oligosaccharides. Ions produced by glycosidic cleavages along the xylan chain are not always detected in the non-reduced oligosaccharides due to the high abundance of the ion resultant of the loss of the acid substituent.

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