Structure elucidation of di- and trisaccharides by liquid chromatography coupled to tandem mass spectrometry

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Currently, there is a high interest in the incorporation of bioactive carbohydrates into diet to yield benefits for human health [1]. Carbohydrates either obtained from nature or chemically or enzymatically synthesized, usually appear as complex mixtures of different glycosidic linkages, monomeric units and degrees of polymerization [2]. Advances in analytical chemistry help to increase the knowledge about these complex structures. Among analytical techniques, liquid chromatography coupled to mass spectrometry (LC-MS) provides an acceptable resolution, high sensitivity and identification capacity, which is required for the analysis of these complex mixtures [3]. However, considering the similar structure of target carbohydrates, their different abundance in real samples and the few commercially available standards, their characterization is still very challenging. Moreover, there is scarce information on correlation among chemical structures, LC data and mass spectra of carbohydrates.

Therefore, the aim of this work was to establish some specific criteria that allowed the characterization of oligosaccharides. For this purpose reliable information about chromatographic data (retention times, peak symmetry, resolution and peak width), using a porous graphitized column and a hydrophilic interaction liquid chromatography column, combined with the characteristic MS^2 fragmentation of 17 disaccharides with different linkages and monomeric units (glucose, galactose and fructose) was obtained. Stepwise discriminant analyses were applied to the different standard samples grouping them according to the linkage in which the monosaccharide units are attached, to the reducing character and to the presence of different monosaccharide moieties. F-statistic was used to estimate the significance of the relation between the different fragments and the chemical structure.

The correlation of chromatographic retention data and characteristic *m/z* ions observed by MS^n using the discriminant function was successful in establishing some specific criteria that allowed the characterization of trisaccharides with different structural features.

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