**Evaluation of different ionic liquid stationary phases for the analysis of carbohydrates
by gas chromatography-mass spectrometry**

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Abstract

Five commercial ionic liquid (IL) columns have been evaluated for the first time for the gas chromatography-mass spectrometry (GC-MS) analysis of low molecular weight carbohydrate (LMWC) standards (mono-, di- and trisaccharides, inositols and iminosugars). A previous derivatization step was necessary to convert the LMWCs into their volatile and stable derivatives. Compared with conventional GC stationary phases, such as HP-1 and Supelcowax[®] 10, IL columns have shown a different selectivity in the separation of target compounds. Among the IL columns, only SLB[™]-IL82 allowed the elution of all the LMWCs studied. Its performance in terms of peak width and asymmetry, evaluated under different oven temperature conditions, was shown to be dependent on the carbohydrate class considered. As an example of application, a SLB[™]-IL82 column was successfully used to separate the complex mixtures of LMWCs in hyacinth and mulberry extracts. This column is an interesting alternative to the conventional stationary phases used in the GC analysis of LMWCs in real-world samples.

Keywords: Low molecular weight carbohydrates, gas chromatography-mass spectrometry, ionic liquids, stationary phases, hyacinth, mulberry.

1. Introduction

Carbohydrates are one of the most abundant organic substances in nature, usually occurring as complex mixtures with large isomeric diversities and a great variety of functional groups suited to their different biological roles in living organisms. In addition to other chromatographic techniques, the high resolution and sensitivity provided by gas chromatography-flame ionization detector (GC-FID) has made it one of the tools of choice for their determination. Moreover, advantages in terms of structural characterization provided by the coupling of GC to mass spectrometry (GC-MS) has also contributed to the wide use of this technique to analyze complex mixtures of low molecular weight carbohydrates (LMWCs) [1,2]. Although less explored, recently GC-vacuum ultraviolet spectroscopy (GC-VUV) followed by a deconvolution strategy has also been proposed as a promising alternative to GC-MS for the rapid and efficient differentiation of isomeric mono-, di- and trisaccharides [3].

Converting carbohydrates into volatile and stable derivatives is mandatory prior to analyzing them by GC. Several derivatization methods such as silylation, acetylation etc. have been proposed for this purpose [2]. Among them, the formation of trimethylsilyl (TMS) derivatives is one of the most common. However, as the different tautomeric forms of reducing sugars can give rise to multiple peaks, the formation of oximes to suppress the anomeric center before silylation is usually carried out [2, 4].

Derivatized carbohydrates are usually analyzed by GC using poly(dimethylsiloxane) stationary phases, since they have good thermal stability and high permeability towards most solutes [5]. However, data regarding their retention on more polar stationary phases, such as those containing different percentages of phenyl-, trifluoropropyl-, vinyl- or cyanopropyl groups in the polysiloxane chain, are also found in the literature [6-10]. High-temperature

stationary phases based on carborane-modified siloxane have also been used to determine oligosaccharides with high degrees of polymerization [11-13]. In contrast, poly(ethylene glycol) stationary phases have rarely been used for LMWC analysis due to their limited temperature range and stability [7,8,14]. Considering that selectivity is one of the most important parameters in GC separations and that it is governed by the interaction mechanisms of the compounds with the stationary phase and the relative strengths of these interactions [15], the evaluation of new stationary phases with different selectivity is of great interest for improving carbohydrate resolution.

Ionic liquids (ILs) are defined as organic salts with melting points below 100 °C, consisting of organic cations associated with organic/inorganic anions [16]. Since their first application as GC stationary phases by Barber *et al.* in 1959 [17], ILs are gaining popularity due to their unique properties, such as low volatility, high thermal stability, low column bleed, high viscosity, variable polarities and tunable selectivity [18]. IL stationary phases have been shown to have complex retention mechanisms that depend on multiple types of intermolecular forces, leading to unique retention properties with many potential applications [19,20].

Several IL-based GC capillary columns with different stationary phases and polarities have been introduced in the market (SLBTM-IL59, SLBTM-IL60, SLBTM-IL61, SLBTM-IL76, SLBTM-IL82, etc.). They have been used to analyze different compounds, including fatty acids [21], coffee volatiles [22], synthetic fragrances [23] and plasticizers [24], among others. However, little information is available regarding their use in the analysis of carbohydrates; only some mono- [25] and disaccharides [26] have recently been analyzed using a SLBTM-IL60 column. Furthermore, to the best of our knowledge, no comprehensive study comparing the IL columns currently available on the market has been performed for the analysis of diverse classes of LMWCs.

In this work, linear retention indices (I^T) of derivatized LMWCs, including mono-, di- and trisaccharides, inositols and iminosugars, on five commercial IL capillary columns have been determined. For comparative purposes, I^T data for two other columns coated with conventional GC stationary phases of different composition and polarities, such as poly(dimethylsiloxane) and poly(ethylene glycol), have also been included in this study. The effect of operation temperature on retention, peak width and asymmetry of LMWCs eluted on the most appropriate IL column has been evaluated. As an example of application, the selected IL column and corresponding optimal chromatographic conditions have been used for the GC-MS analysis of LMWCs in different plant extracts.

2. Materials and Methods

2.1. Standards

2.1.1. Mono-, di- and trisaccharides

Analytical standards of α,α -, α,β - and β,β -trehalose (α -Glc-(1 $\rightarrow$ 1)- α -Glc, α -Glc-(1 $\rightarrow$ 1)- β -Glc and β -Glc-(1 $\rightarrow$ 1)- β -Glc, respectively), arabinose, erlose (α -Glc-(1 $\rightarrow$ 4)- α -Glc-(1 $\rightarrow$ 2)- β -Fru), fructose, galactose, glucose, isomaltose (α -Glc-(1 $\rightarrow$ 6)-Glc), 1-kestose (β -Fru-(2 $\rightarrow$ 1)- β -Fru-(2 $\rightarrow$ 1)- α -Glc), kojibiose (α -Glc-(1 $\rightarrow$ 2)-Glc), lactulose (β -Gal-(1 $\rightarrow$ 4)-Fru), lactose (β -Gal-(1 $\rightarrow$ 4)-Glc), laminaribiose (β -Glc-(1 $\rightarrow$ 3)-Glc), maltose (α -Glc-(1 $\rightarrow$ 4)-Glc), maltotriose (α -Glc-(1 $\rightarrow$ 4)- α -Glc-(1 $\rightarrow$ 4)-Glc), melezitose (α -Glc-(1 $\rightarrow$ 3)- β -Fru-(2 $\rightarrow$ 1)- α -Glc), raffinose (α -Gal-(1 $\rightarrow$ 6)- α -Glc-(1 $\rightarrow$ 2)- β -Fru), ribose, sucrose (α -Glc-(1 $\rightarrow$ 2)- β -Fru), turanose (α -Glc-(1 $\rightarrow$ 3)- β -Fru) and phenyl- β -D-glucoside (internal standard) were obtained from Sigma-Aldrich. (St. Louis, USA). A mixture of linear alkane standards from heptane (C₇H₁₆) to tetracontane (C₄₀H₈₂) was also purchased from Supelco Inc. (Bellefonte, PA, USA).

2.1.2. Inositols

chiro-Inositol (*cis*-1,2,4-*trans*-3,5,6-cyclohexanehexol), *myo*-inositol (*cis*-1,2,3,5-*trans*-4,6-cyclohexanehexol), *scyllo*-inositol (*cis*-1,3,5-*trans*-2,4,6-cyclohexanehexol), pinitol (3-*O*-methyl-*D-chiro*-inositol) and galactinol (1-*O*- α -D-galactopyranosyl-*L-myo*-inositol) were purchased from Sigma Chemical Co.

2.1.3. Iminosugars

1-Deoxymannojirimycin (DMJ, (2R,3R,4R,5R)-2-(hydroxymethyl)-3,4,5-piperidinetriol) and 1-deoxynojirimycin (DNJ, (2R,3R,4R,5S)-2-(hydroxymethyl)-3,4,5-piperidinetriol) were obtained from Sigma Chemical Co. α -Homonojirimycin (α -HNJ, (2R,3R,4R,5S,6R)-2,6-bis(hydroxymethyl)-3,4,5-piperidinetriol) and 2,5-dideoxy-2,5-imino-*D*-mannitol (DMDP, (2R,3R,4R,5R)-2,5-bis(hydroxymethyl)-3,4-pyrrolidinediol) were acquired from Dextra Laboratories (Reading, UK), while miglitol ((2R,3R,4R,5S)-1-(2-hydroxyethyl)-2-(hydroxymethyl)-3,4,5-piperidinetriol) (Diastabol, Sanofi-Aventis) was purchased in a local pharmacy.

2.2. Samples and extraction procedure

Hyacinth (*Hyacinthus orientalis*) bulbs and mulberry (*Morus alba*) leaves were acquired in a local market in Madrid (Spain). Carbohydrates were extracted ($n = 3$) from these matrices as described by Rodríguez-Sánchez *et al.* [27]. In brief, air-dried samples (1 g) were ground in a domestic mill (Moulinex, Barcelona, Spain) and sieved ($< 500 \mu\text{m}$) before extraction (stirring for 2 hours at ambient temperature) into 10 mL of Milli-Q water (Millipore, Billerica, MA, USA). Extracts were then filtrated through Whatman no. 4 paper and kept at -20°C until analysis.

2.3. Derivatization

Carbohydrates were derivatized following the procedure described by Sanz *et al.* [5]. One mL of carbohydrate standard solution (0.006-0.1 mg mL⁻¹ in methanol:water (70:30, v/v)) or 1 mL of plant extracts was evaporated under vacuum and treated with 350 µL of a 2.5% solution of hydroxylamine hydrochloride (Sigma Chemical Co.) in anhydrous pyridine and then heated for 30 min at 75 °C. Compounds were then silylated with 350 µL of hexamethyldisilazane (Sigma Chemical Co.) and 35 µL of trifluoroacetic acid (Sigma Chemical Co.) at 45 °C for 30 min. After reaction, samples were centrifuged at 4400 g for 10 min. In order to remove excess silylation reagents and avoid damaging the GC stationary phase, 50 µL of supernatant were treated with 50 µL of heptane and 100 µL of water. The organic phase containing the derivatized carbohydrates was recovered and 1 µL was injected into the injection port of the GC-MS system.

This derivatization procedure produced a single chromatographic peak for non-reducing sugars and inositols, corresponding to the *O*-persilylated derivative, whereas two peaks (the *syn* (*E*) and *anti* (*Z*) isomers of the oxime) were obtained for reducing sugars. The isomer *E* was more abundant than isomer *Z* for aldohexoses, whereas the isomers of carbohydrates having a ketose as the reducing moiety presented an approximate 1:1 area ratio, labelled as peak 1 and 2 [28]. As previously reported by Rodríguez-Sánchez *et al.* [29], only one peak corresponding to the *O*-persilylated form of iminosugars was obtained under these derivatization conditions.

2.4. GC-MS analysis

147 GC-MS analyses were carried out using a 7890A gas chromatograph coupled to a 5975C
148 quadrupole mass detector (Agilent Technologies, Palo Alto, CA, USA) using helium at ~ 0.8
149 mL min^{-1} as carrier gas.

150 Five IL columns (SLBTM-IL59, SLBTM-IL60, SLBTM-IL76, SLBTM-IL82 and SLBTM-IL100),
151 provided by Supelco, were evaluated (Rodriguez-Sánchez *et al.* [30]). Their characteristics
152 are summarized in **Table 1**. For comparative purposes, two additional stationary phases with
153 different polarities were included: a poly(dimethylsiloxane) column (HP-1, Agilent
154 Technologies, Palo Alto, CA, USA; $25 \text{ m} \times 0.25 \text{ mm i.d.} \times 0.25 \text{ }\mu\text{m } d_f$) and a bonded
155 poly(ethylene glycol) capillary column (SUPELCOWAX[®] 10, Supelco; $30 \text{ m} \times 0.25 \text{ mm i.d.}$
156 $\times 0.25 \text{ }\mu\text{m } d_f$).

157 Preliminary experiments were carried out with each of the columns to select the temperature
158 programs that provided the best separation of derivatized carbohydrate standards. The
159 optimized conditions for the IL columns (ramp 1) were $80 \text{ }^\circ\text{C}$ (5 min)- $10 \text{ }^\circ\text{C min}^{-1}$ - $220 \text{ }^\circ\text{C}$ (10
160 min)- $10 \text{ }^\circ\text{C min}^{-1}$ - $250 \text{ }^\circ\text{C}$ (50 min). The only exception was the SLBTM-IL100 column, whose
161 maximum operating temperature is only $230 \text{ }^\circ\text{C}$ and, therefore, required a slight variation in
162 the temperature program as follows: $80 \text{ }^\circ\text{C}$ (5 min)- $10 \text{ }^\circ\text{C min}^{-1}$ - $220 \text{ }^\circ\text{C}$ (60 min). The
163 optimized conditions for the HP-1 column were $100 \text{ }^\circ\text{C}$ (0 min)- $15 \text{ }^\circ\text{C min}^{-1}$ - $200 \text{ }^\circ\text{C}$ (15 min)-
164 $15 \text{ }^\circ\text{C min}^{-1}$ - $300 \text{ }^\circ\text{C}$ (20 min); and for the SUPELCOWAX[®] 10 column they were $100 \text{ }^\circ\text{C}$ (0
165 min)- $10 \text{ }^\circ\text{C min}^{-1}$ - $220 \text{ }^\circ\text{C}$ (10 min)- $10 \text{ }^\circ\text{C min}^{-1}$ - $260 \text{ }^\circ\text{C}$ (10 min).

166 In addition to the oven temperature programs described above, a linear gradient (from $80 \text{ }^\circ\text{C}$
167 to $250 \text{ }^\circ\text{C}$ at $10 \text{ }^\circ\text{C min}^{-1}$ and held 10 min; ramp 2) and different isothermal conditions (140,
168 160, 180, 230 and $250 \text{ }^\circ\text{C}$) were also evaluated for the elution of target compounds in the
169 SLBTM-IL82 column.

Samples were injected in split mode (1:20) at 240 °C. The transfer line and ionization source were thermostated at 280 and 230 °C, respectively. Mass spectra were recorded in electron impact (EI) mode at 70 eV within the 35-650 m/z range. Acquisition was done using HPChem Station software (Agilent Technologies, Palo Alto, CA, USA). All analyses were done in triplicate ($n=3$).

I^T were calculated from the retention times of derivatized carbohydrates and suitable n -alkanes. These system-independent constants allow to correct the differences on retention associated with the slight variations in the dimensions of the columns used. Resolution (R_s) was calculated as follows: $R_s = 2 \cdot (t_{R2} - t_{R1}) / (w_{b1} + w_{b2})$, where 1 and 2 refer to two consecutive eluting peaks and w_b is the peak width at base. Peak width (w_h) was measured at half height of the peak and symmetry, measured as tailing factor (TF), was calculated using the equation $TF = (A05_i + B05_i) / (2 \cdot A05_i)$, where $A05_i$ is the left half-width of the peak at 5% height and $B05_i$ is the right half-width at 5% height. Peak capacity (n_c), defined as the maximum number of non-overlapping peaks in a given interval [31], was calculated as follows: $n_c = 1 + (t_g / \bar{w}_b)$, where t_g is the difference in t_R within a given interval and $\bar{w}_b$ is the mean peak width value at base. Three t_R intervals, according to the different carbohydrate standards analyzed in this study, were considered for n_c estimation: (i) monosaccharides, inositols (except for galactinol) and iminosugars, (ii) disaccharides and galactinol and (iii) trisaccharides.

Qualitative analysis of LMWC in hyacinth and mulberry extracts was based on the comparison of experimental I^T and mass spectra with those of standards. When they were not available, data reported in the literature [32,33] were also used for tentative characterization/identification of target analytes.

3. Results and discussion

3.1. Effect of stationary phase composition on LMWC retention

Commercial LMWC standards were derivatized and analyzed in the different columns under study. Compounds were selected to include inositols, iminosugars, monosaccharides and reducing and non-reducing disaccharides and trisaccharides with different glycosidic linkages and monomeric compositions.

Regarding the IL columns, only SLBTM-IL82 allowed the elution of all the target carbohydrates. The SLBTM-IL59, SLBTM-IL60 and SLBTM-IL76 columns enabled the elution of mono-, di-, trisaccharides and inositols; however, iminosugars could not be determined on any of them, except for DNJ and DMJ, which were eluted only from the SLBTM-IL76 column. None of the studied carbohydrates were eluted from the SLBTM-IL100 column. The low inertness of these columns to compounds with H-bonding capabilities [20] can cause the adsorption or degradation of iminosugars and related compounds. Other authors have reported similar problems with these columns for some organochlorine pesticides [23,34], polybrominated diphenyl ethers [34] and volatile suspected allergens [23]. On the other hand, both the HP-1 and SUPELCOWAX[®] 10 columns allowed the elution of all target carbohydrates. However, a noticeable increase of the baseline signal was observed in the SUPELCOWAX[®] 10 column when using the oven temperature required to elute the most retained compounds (e.g., galactinol and trisaccharides), even when below the maximum operating temperature (280 °C); this fact limits its use for sensitive analysis of these compounds.

I^T calculated for the derivatized LMWCs are summarized in **Table 2**. As previously observed by other authors [35], I^T were noticeably lower in the SUPELCOWAX[®] 10 column (between 1331 and 3014), whereas I^T values in IL columns increased with the polarity of the stationary phase (SLBTM-IL82 provided the highest values and SLBTM-IL59 the lowest). In general,

monosaccharides, inositols (except for galactinol) and iminosugars eluted first, followed by disaccharides and galactinol. As expected, trisaccharides were the most retained compounds. It is also worth noting that available *n*-alkanes were barely retained on the SLBTM-IL76 and SLBTM-IL82 columns (e.g., retention times of 20.4 and 20.9 min for C40, respectively), which is in line with the high cohesive energy and low capability for dispersive interactions of these stationary phases [20]. Thus, I^T values for highly retained carbohydrates (t_R trisaccharides: 23.6-24.0 min and 28.5-33.67 min, respectively) could not be determined and they have been indicated as $I^T > 4000$ in **Table 2**.

Elution order of monosaccharides was similar for all the stationary phases; pentoses (arabinose and ribose) eluted before hexoses (fructose, galactose and glucose). Only slight differences in their separation were observed: e.g., galactose Z and glucose Z were not resolved on the HP-1 (I^T 2078) and SLBTM-IL76 (I^T 2068) columns, whereas they were separated ($R_S > 1$) on the SLBTM-IL59 (I^T 1943 and 1938, respectively) column.

No differences in the elution order of inositols were found among the different columns (pinitol, a methyl-inositol, eluted first followed by *chiro*-, *scyllo*- and *myo*-inositol and finally galactinol, a glycosyl-inositol); however, iminosugars were highly affected by the selectivity of the different stationary phases. As an example, **Figure 1** shows the GC-MS profiles of monosaccharides (labelled as ■), iminosugars (labelled as ▲) and inositols (labelled as ★) eluted from the SLBTM-IL82, HP-1 and SUPELCOWAX[®] 10 columns. In the HP-1 column, elution is almost independent of the functional groups, as the substances are mainly separated by dispersive interactions. Therefore, solutes tend to elute as the number of carbon atoms in the molecule or the size of the ring increase. Consequently, clusters of compounds belonging to the three studied families were not observed in this chromatogram. When a polar stationary phase was used, like that of the SUPELCOWAX[®] 10 column, new directional and more specific polar interactions were involved in the separation process. Poly(ethylene glycols) are

dipolar/polarizable stationary phases with H-bond acceptor characteristics [15], so iminosugar retention was increased with respect to monosaccharides and inositols due to additional H-bonding through the hydrogen atom of the amine group. An interesting fact is the high retention of DNJ (peak 12) compared to its isomer DMJ (peak 10); the higher number of substituents in equatorial positions of DNJ provides a greater surface area and less steric hindrance of polar interactions. In the SLBTM-IL82 column, iminosugars were in general more retained than monosaccharides and inositols. This column is less H-bond basic and more dipolar/polarizable than poly(ethylene glycols), but it is also moderately H-bond acid. This last characteristic confers a distinct selectivity to all the IL-based columns studied in this work. In particular, high retention of basic compounds, that is, compounds with heteroatoms with lone electron pairs (such as the studied carbohydrates), is expected. Moreover, the greater localization of unshared electron pairs around the oxygen atom rather than the nitrogen atom makes this type of interaction easier for iminosugars. Therefore, the increase observed in their retention in the SUPELCOWAX[®] 10 column could be explained by this additional separation mechanism.

In terms of resolution, several differences were observed for the different columns. As an example, DNJ and α -HNJ could not be resolved in the SUPELCOWAX[®] 10 column (Table 2 and peaks 12 and 14 in **Figure 1**, R_s : 0.8); however, these peaks were exceptionally separated in the SLBTM-IL82 (R_s : 51.6) and in HP-1 (R_s : 22.9) columns. However, in this last column α -HNJ and galactose *E* were not baseline resolved (peaks 14 and 6; R_s : 0.9). Similarly, arabinose 2 and DMDP coeluted in HP-1 (peaks 1 and 9 in **Figure 1**, R_s : 0.1), whereas these peaks were clearly separated in the SLBTM-IL82 (R_s : 37.0) and SUPELCOWAX[®] 10 (R_s : 18.5) columns. Taking into account that it is difficult to objectively measure overall resolution in complex mixtures of variable composition, peak capacity was considered in this study (Table 3). Similar n_c for monosaccharide, inositol and iminosugar interval was achieved by

using the HP-1 and SLBTM-IL82 (n_c : 89 and 81, respectively) columns, whereas this value was notably lower for the other columns (n_c : 53-69).

Regarding disaccharides, sucrose was the first to elute in all the evaluated columns and, in general, isomaltose (with 1→6 glycosidic linkage) was the last. This chromatographic behavior has been previously observed in dimethyl- and phenylmethyl-silicone stationary phases [35]. However, as a result of the different selectivity of IL stationary phases, some variations in the retention order of disaccharides were also observed. **Figure 2** shows the GC profiles of disaccharides eluted from the SLBTM-IL82, SUPELCOWAX® 10 and HP-1 columns as an example. Changes in retention order were clearly observed for α,α -, α,β - and β,β -trehaloses. In the SUPELCOWAX® 10 column, α,α -trehalose (peak 2) was the first to elute followed by α,β - and β,β -trehaloses (peaks 10 and 8, respectively); this elution order with a much poorer resolution was also observed in the HP-1 column (**Table 2**). Retention of these compounds was different in IL columns in which α,β -trehalose eluted latter and was baseline separated from β,β -trehalose (except for the SLBTM-IL60 column where they almost coeluted, $R_s < 1$). Differences in the retention of these carbohydrates among the studied columns could be attributed to the additional capability of these IL stationary phases to act as H-bond acids [20]. They can interact with the lone electron pairs of oxygen atoms of disaccharides, increasing their retention. However, the directional nature of hydrogen bonding would promote a greater retention of α,β -trehalose, due to its more planar conformation of one of the rings, which means less steric hindrance between this disaccharide and the stationary phase.

With respect to resolution, it is worth noting that isomers *E* and *Z* of reducing glucosyl-glucose disaccharides (e.g., maltose, laminaribiose, kojibiose and isomaltose) were highly separated in the HP-1 (R_s : 2.6-6.0), SLBTM-IL59 (R_s : 1.4-3.5) and SLBTM-IL60 (R_s : 1.8-4.8)

292 columns, whereas resolution values for these isomers were lower in the SLBTM-IL76 (R_s : 0.7-
293 1.7) and SLBTM-IL82 (R_s : 0.6-1.4) columns. Poorer resolution was achieved for turanose (R_s :
294 0.0-1.4) and for lactose (R_s : 0.0-0.8) in all the IL columns. Regarding peak capacity for
295 disaccharides (**Table 3**), the best results were obtained with the HP-1 column (n_c : 53),
296 whereas the smallest values resulted from using the SUPELCOWAX[®] 10 and SLBTM-IL59
297 columns (n_c : 29 and 30, respectively).

298 Non-reducing trisaccharides eluted first in all the columns studied, followed by maltotriose
299 (reducing trisaccharide), except for the SUPELCOWAX[®] 10 column, in which maltotriose
300 eluted before erlose (**Table 2**). However, differences in the elution order of non-reducing
301 trisaccharides were also observed among the columns tested. Melezitose was the first eluting
302 trisaccharide in the SLBTM-IL82 (t_R : 28.5 min), SLBTM-IL76 (t_R : 23.6 min) and
303 SUPELCOWAX[®] 10 (t_R : 29.4 min) columns, followed by kestose (t_R : 29.4, 23.7 and 29.5
304 min, respectively) and raffinose (t_R : 29.9, 24.0 and 29.8 min, respectively). However, kestose
305 eluted first from the SLBTM-IL59 and SLBTM-IL60 columns followed by raffinose and
306 melezitose, whereas raffinose was the first trisaccharide to elute in the HP-1 column followed
307 by kestose, erlose and melezitose (**Table 2**). In the HP-1 column, trisaccharides were found to
308 elute according to the size of rings (as dispersion interactions with the stationary phase rise
309 accordingly), with an increase in the retention when the fructose ring was in the middle
310 position. However, the opposite effect was found when the polar stationary phases were
311 involved. Trisaccharides with a fructose in the middle position have globular structures with
312 more TMS groups bound outwards, hindering polar interactions. Therefore, a decrease in
313 retention of melezitose and kestose was found as stationary phase polarity increases, resulting
314 in a very different elution pattern than the HP-1 column.

As **Table 3** shows, the highest peak capacity for trisaccharides was obtained in the HP-1 column (n_c : 69), whereas extremely small values were obtained for both SLBTM-IL76 (n_c : 3) and SLBTM-IL59 (n_c : 9) columns.

In light of the results discussed above, the SLBTM-IL82 phase can be considered useful for analyzing carbohydrates, mainly monosaccharides, inositols and iminosugars and, therefore, it was selected for further study.

3.2. Influence of temperature on peak width and tailing factor

The effect of temperature conditions (isothermal and temperature program) on the w_h and TF , as a measure of peak asymmetry, of target LMWCs was evaluated for the SLBTM-IL82 column. Isothermal conditions were chosen on the basis of the optimal temperature for elution of each type of carbohydrate on this column: 140 and 160 °C for monosaccharides, inositols and iminosugars; 180, 230 and 250 °C for disaccharides and 230 and 250 °C for trisaccharides.

Table 4 shows peak widths of monosaccharides, inositols and iminosugars eluted under the isothermal and temperature program conditions (ramp 1 and ramp 2) indicated in Section 2.4. using the SLBTM-IL82 column.

Under isothermal conditions, w_h values varied from 0.03 to 0.15 min, with the exception of α -HNJ at 140 °C, which was a highly retained and broad peak (w_h : 0.26 min). Slightly narrower peaks were obtained under temperature program conditions, mainly for carbohydrates eluting in the optimal ramp (ramp 1; w_h : 0.03-0.07 min).

Regarding the tailing factor, values higher and lower than 1 were obtained depending on the type of carbohydrate. In general, good TF values were obtained for monosaccharides, with the exception of galactose eluted using ramp 1 and fructose 1 and glucose *E* eluted at 160 °C,

which presented TF values < 1 . TF s lower than 1 indicate that solute-stationary phase interactions are weaker than those among solutes or among stationary phase molecules, but they can also be caused if the column is overloaded when injecting large sample sizes [36]. Considering that in this work the amount of sample injected (0.006 to 0.1 mg mL⁻¹) was selected precisely to avoid overloading, this behavior is likely to be attributed to the first fact. On the contrary, iminosugars and inositols gave TF values higher than 1, which indicates peak tailing due to adsorption phenomena in the column. A similar behavior was previously observed by Rodríguez-Sánchez *et al.* [20] for amino-compounds analyzed in the SLBTM-IL82 column; the low inertness of this stationary phase towards compounds with hydrogen bonding capabilities could justify these results.

As expected, peak widths for di- and trisaccharides (**Table 5**) were higher under isothermal elution conditions, mainly at 180 and 230 °C (w_h : 0.05-0.30 min), than under temperature program conditions (0.02-0.24 min and 0.02-0.11 min for ramp 1 and 2, respectively). Whereas peak widths of disaccharides were appropriate using both temperature programs (w_h : 0.02-0.04 min), trisaccharides, the most retained compounds, eluted as broader peaks in ramp 1 (w_h : 0.05-0.24 min).

Peak fronting ($TF < 1$) was observed for disaccharides eluted from the SLBTM-IL82 column at 180 °C. However, peak tailing was, in general, detected at higher temperatures (230 and 250 °C), while most of the TF values were close to 1 under temperature program elution. A similar behavior was observed for trisaccharides. Peak fronting was found for trisaccharides eluted at 230 °C, except for kestose and melezitose, whereas peak tailing was, in general, detected at 250 °C. As commented before for inositols and iminosugars, peak tailing could be attributed to the existence of adsorption phenomena in the column. Appropriate TF values were obtained for most of the trisaccharides eluted using either temperature programs (mean TF values of 1.03 and 1.04 for ramp 1 and 2, respectively).

Therefore, it can be concluded that the SLBTM-IL82 column is useful for the analysis of LMWCs with different structural characteristics (mono-, di- and trisaccharides, inositols and iminosugars), once the most appropriate elution conditions for each type of carbohydrate are selected.

3.3. Analysis of real-world samples

As an example of application, complex mixtures of carbohydrates present in real-world samples were analyzed using the SLBTM-IL82 column under optimal temperature program conditions (ramp 1). Hyacinth bulbs and mulberry leaf extracts were chosen for this purpose, due to their complex composition in different mono- and disaccharides, iminosugars and inositols [27,37].

GC-MS analysis of the hyacinth extract using the SLBTM-IL82 column allowed all the LMWCs in this sample to be separated (**Figure 3A**). Different iminosugars (DMJ, DNJ, HMJ and α -HNJ, peaks 3, 5, 6 and 7, respectively), as well as monosaccharides (fructose and glucose, peaks 1 and 2, respectively), and sucrose (peak 9) were identified. *Myo*-inositol (peak 4) and different unknown glycosyl-inositols and disaccharides (labeled as peaks 10) were also detected.

Regarding the GC-MS analysis of the mulberry leaf extract (**Figure 3B**), fructose, galactose, glucose, and sucrose (peaks 2, 3, 4 and 9, respectively), *myo*-inositol (peak 5) and minor amounts of iminosugars [DNJ (peak 6) and fagomine ((2R,3R,4R)-2-(hydroxymethyl)-3,4-piperidinediol, peak 1)] were detected.

4. Conclusions

Five commercial IL capillary columns have been evaluated for the first time, comparing their performance in the analysis of mono-, di-, trisaccharides, inositols and iminosugars with that of conventional polyethylene glycol and poly(dimethylsiloxane) stationary phases. Although the conventional HP-1 stationary phase was, in most cases, the most appropriate for LMWC separation, the complex carbohydrate mixtures generally found in plant extracts could benefit from employing GC stationary phases with a different selectivity, such as that provided by IL columns. Among the IL columns evaluated, SLBTM-IL82 showed the best results for the analysis of the different LMWC classes evaluated (mono-, di-, trisaccharides, inositols and iminosugars). Therefore, it can be concluded that IL stationary phases are worthy of consideration for the resolution of challenging separations in this field. In addition, the evaluation of novel IL chemical designs is a research line with great future prospect which could serve as a driver for further developments regarding the improved separation of complex carbohydrate mixtures.

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Conflict of interest

The authors declare no conflict of interest in the publication of this manuscript.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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FIGURE CAPTIONS

Figure 1. GC-MS chromatographic profiles obtained for derivatized carbohydrate standards analyzed in SLBTM-IL82 (using ramp 1), SUPELCOWAX[®] 10 and HP-1 columns: 1) arabinose (*E* and *Z*), 2) pinitol, 3) ribose (*E* and *Z*), 4) *chiro*-inositol, 5) fructose (1 and 2), 6) galactose (*E* and *Z*), 7) *scyllo*-inositol, 8) glucose (*E* and *Z*), 9) DMDP, 10) DMJ, 11) *myo*-inositol, 12) DNJ, 13) miglitol and 14) α -HNJ. ■: Monosaccharides; ★: inositols; ▲: iminosugars.

Figure 2. GC-MS chromatographic profiles obtained for derivatized disaccharide standards analyzed in SLBTM-IL82 (using ramp 1), SUPELCOWAX[®] 10 and HP-1 columns: 1) sucrose, 2) α,α -trehalose, 3) lactulose (1 and 2), 4) lactose (*E* and *Z*), 5) maltose (*E* and *Z*), 6) turanose (1 and 2), 7) kojibiose (*E* and *Z*), 8) β,β -trehalose, 9) laminaribiose (*E* and *Z*), 10) α,β -trehalose, 11) galactinol and 12) isomaltose (*E* and *Z*).

Figure 3. GC-MS chromatographic profile obtained using SLBTM-IL82 column and ramp 1 for **A**) a hyacinth extract [1) fructose (1 and 2), 2) glucose (*E* and *Z*), 3) DMJ, 4) *myo*-inositol, 5) DNJ, 6) HMJ, 7) α -HNJ, 8) phenyl- β -D-glucoside (I.S.), 9) sucrose, 10) disaccharides + glycosyl-inositols] and **B**) a mulberry extract [1) fagomine, 2) fructose (1 and 2), 3) galactose (*E* and *Z*), 4) glucose (*E* and *Z*), 5) *myo*-inositol, 6) DNJ, 7) glycosyl-glycerol, 8) phenyl- β -D-glucoside (I.S.) and 9) sucrose. All compounds detected as TMSO/TMS derivatives.

Table 1. Characteristics of the commercial ionic liquid columns studied.

Column	Stationary phase	Chemical structure	Polarity	Temperature limits	Dimensions ^a
SLB TM -IL100	1,9-Di(3-vinylimidazolium)nonane bis(trifluoromethylsulfonyl)imide		4357 ^b	Subambient to 230 °C	30 m × 0.25 mm × 0.2 μm
SLB TM -IL82	1,12-Di(2,3-dimethylimidazolium)dodecane bis(trifluoromethylsulfonyl)imide		3741 ^b	50 to 300 °C	30 m × 0.25 mm × 0.2 μm
SLB TM -IL76	Tri(triethylphosphoniumhexanamide) triethylamine bis(trifluoromethylsulfonyl)imide		3509 ^b	Subambient to 270 °C	30 m × 0.25 mm × 0.2 μm
SLB TM -IL60	1,12-di(triethylphosphonium)dodecane bis(trifluoromethylsulfonyl)imide ^c		2684 ^d	35 to 300 °C	30 m × 0.25 mm × 0.2 μm
SLB TM -IL59	1,12-di(triethylphosphonium)dodecane bis(trifluoromethylsulfonyl)imide		2760 ^b	Subambient to 300 °C	30 m × 0.25 mm × 0.2 μm

^a Column length × internal diameter × film thickness; ^b Data from [20]; ^c Modified (deactivated) version of SLBTM-IL59; ^d Unpublished results.

Table 2. Linear retention indices (I^T) calculated for the TMSO/TMS derivatives of carbohydrate standards analyzed in the different columns assayed.

STANDARD		I^T					
		SLB TM -IL82	SLB TM -IL76	SLB TM -IL60	SLB TM -IL59	SUPELCOWAX [®] 10	HP-1
Monosaccharides	Arabinose 1	1879	- [*]	1727	1684	-	1795
	Arabinose 2	1891	-	1731	1693	1331	1806
	Ribose 1	1905	1836	1762	1714	1335	1821
	Ribose 2	1924	1840	1767	1720	1343	-
	Fructose 1	2076	1995	1883	1840	1402	2003
	Fructose 2	2083	2000	1894	1844	1404	2012
	Galactose <i>E</i>	2140	2043	1950	1899	1430	2047
	Galactose <i>Z</i>	2159	2068	1993	1943	1438	2078
	Glucose <i>E</i>	2155	2060	1966	1914	1438	2058
	Glucose <i>Z</i>	2155	2068	1990	1938	-	2078
Disaccharides	Sucrose	2995	2828	2644	2530	1841	2681
	α,α -Trehalose	3072	2917	2739	2596	1936	2722
	β,β -Trehalose	3278	3092	2891	2735	2184	2743
	α,β -Trehalose	3316	3143	2895	2925	2065	2741
	Lactulose 1	3202	-	-	-	2029	2744
	Lactulose 2	3221	-	-	-	2057	2748
	Lactose <i>E</i>	3217	3039	2808	2649	2039	2749
	Lactose <i>Z</i>	3217	3042	2816	2657	2039	2755
	Laminaribiose <i>E</i>	3284	3108	2870	2706	2200	2768
	Laminaribiose <i>Z</i>	3301	3127	2905	2745	2223	2785
	Turanose 1	3264	3098	2872	2709	2118	2782
	Turanose 2	3264	3098	2872	2709	2126	2786
	Maltose <i>E</i>	3248	3079	2863	2701	2115	2784
	Maltose <i>Z</i>	3257	3087	2880	2722	2115	2793
	Kojibiose <i>E</i>	3277	3099	2869	2704	2173	2789
	Kojibiose <i>Z</i>	3297	3122	2907	2744	2216	2812
	Isomaltose <i>E</i>	3484	3254	3004	2857	2553	2835
	Isomaltose <i>Z</i>	3508	3278	3042	2906	2594	2857
Trisaccharides	Raffinose	>4000 ^{**}	>4000	3546	3372	2967	2857
	Kestose	>4000	>4000	3540	3365	2955	3084
	Erlose	>4000	-	3612	3497	3014	3100
	Melezitose	>4000	>4000	3571	3401	2954	3122
	Maltotriose <i>E</i>	>4000	-	>4000	-	2996	3212
	Maltotriose <i>Z</i>	>4000	-	>4000	-	-	3226
Inositols	Pinitol	1895	1851	1779	1734	1334	1882
	<i>chiro</i> -Inositol	2047	1981	1914	1859	1380	2019
	<i>scyllo</i> -Inositol	2142	2081	2027	1971	1422	2085

	<i>myo</i> -Inositol	2207	2169	2109	2052	1451	2146
	Galactinol	3375	3209	3012	2868	2337	2851
Iminosugars	DMDP	2170	-	-	-	1377	1808
	DMJ	2173	2227	-	-	1368	1831
	DNJ	2253	2300	-	-	1465	1959
	α -HNJ	2557	-	-	-	1463	2051
	Miglitol	2521	-	-	-	1503	2119

Relative standard deviations ($n=3$) were lower than 0.4% for all compounds and columns evaluated.

* Peaks were not detected.

** Retention times higher than those of available *n*-alkanes (tetracontane, C₄₀H₈₂).

Table 3. Retention times (t_R) of first and last peak, mean peak width ($\overline{w_b}$) and peak capacity (n_c) for the different elution intervals considered in the separation of TMSO/TMS derivatives of carbohydrate standards using the columns under study: (i) monosaccharides, inositols (except for galactinol) and iminosugars, (ii) disaccharides and galactinol, and (iii) trisaccharides.

Elution interval	Column	t_R (min) first peak	t_R (min) last peak	$\overline{w_b}$ (min)	n_c
Monosaccharides, inositols and iminosugars	HP-1	8.22	14.71	0.074	89
	SUPELCOWAX® 10	6.25	9.46	0.048	68
	SLB™-IL59	7.21	11.77	0.068	68
	SLB™-IL60	12.00	15.43	0.051	69
	SLB™-IL76	10.10	13.92	0.073	53
	SLB™-IL82	10.75	15.75	0.062	81
Disaccharides and galactinol	HP-1	27.67	30.24	0.049	53
	SUPELCOWAX® 10	13.99	17.95	0.141	29
	SLB™-IL59	14.1	17.12	0.103	30
	SLB™-IL60	19.14	22.33	0.078	42
	SLB™-IL76	17.01	19.29	0.074	32
	SLB™-IL82	18.14	20.85	0.083	34
Trisaccharides	HP-1	30.24	41.47	0.164	69
	SUPELCOWAX® 10	18.99	29.82	0.324	34
	SLB™-IL59	24.42	26.22	0.230	9
	SLB™-IL60	30.71	31.12	0.139	26
	SLB™-IL76	23.66	24.05	0.216	3
	SLB™-IL82	28.48	33.67	0.248	22

Relative standard deviations ($n=3$) for the different carbohydrates under study were lower than 0.08% and 3.85% for t_R and w_b , respectively.

Table 4. Retention times (t_R), peak widths (w_h) and tailing factors (TF) obtained for the TMSO/TMS derivatives of monosaccharide, inositol and iminosugar standards analyzed under different conditions in SLBTM-IL82 column.

Carbohydrate	Isothermal						Temperature program					
	140 °C			160 °C			Ramp 1			Ramp 2		
	t_R (min)	w_h (min)	TF	t_R (min)	w_h (min)	TF	t_R (min)	w_h (min)	TF	t_R (min)	w_h (min)	TF
Arabinose 1	2.67**	0.10	1.00	2.15**	0.07	0.93	10.75	0.04	1.00	7.41	0.03	1.00
Arabinose 2							10.87	0.04	1.50	7.47	0.03	1.10
Ribose 1	2.87**	0.07	0.80	2.18**	0.07	0.88	11.01	0.03	1.00	6.21	0.04	1.00
Ribose 2							11.19	0.04	1.50	6.40	0.04	1.80
Fructose 1	3.84	0.05	0.81	2.52	0.04	0.63	12.40	0.03	0.87	7.52	0.03	1.20
Fructose 2	3.92	0.06	1.40	2.54	0.03	1.50	12.46	0.03	1.30	7.57	0.03	1.30
Galactose E	4.53	0.07	1.06	2.73	0.05	0.81	12.96	0.07	0.68	7.97	0.03	1.10
Galactose Z	4.78	0.07	0.94	2.82	0.04	0.86	13.11	0.04	0.71	8.11	0.03	1.00
Glucose E	4.68	0.06	1.00	2.77	0.06	0.64	13.08	0.04	0.94	8.08	0.03	1.00
Glucose Z	4.72	0.04	0.88	2.80	0.03	1.50	-*	-	-	-	0.03	1.15
Pinitol	2.82	0.06	1.06	2.18	0.05	1.00	10.91	0.03	1.50	6.22	0.03	1.25
chiro-Inositol	3.58	0.06	1.06	2.44	0.10	1.40	12.12	0.03	1.50	7.24	0.03	1.25
scyllo-Inositol	4.55	0.06	1.10	2.74	0.10	1.30	12.97	0.04	1.60	8.01	0.04	1.50
myo-Inositol	5.56	0.07	1.08	3.10	0.11	1.30	13.49	0.03	1.50	8.51	0.04	1.30
DMDP	5.12	0.06	1.29	2.95	0.05	1.50	13.20	0.03	1.33	8.50	0.05	1.30
DMJ	5.29	0.06	1.29	3.05	0.07	1.25	13.22	0.03	1.83	8.62	0.10	1.90
DNJ	6.67	0.07	1.11	3.58	0.08	0.94	13.82	0.03	1.33	9.14	0.06	1.50
α-HNJ	18.68	0.26	2.75	6.49	0.10	2.08	15.75	0.03	1.17	10.96	0.04	1.33
Miglitol	18.07	0.15	2.04	5.89	0.07	1.43	15.54	0.03	1.17	10.75	0.04	1.50

Relative standard deviations ($n=3$) for the different carbohydrates and chromatographic conditions were lower than 0.09% and 2.70% for t_R and w_h , respectively.

* Not detected.

** Coelution of isomers.

Table 5. Retention times (t_R), peak widths (w_h) and tailing factors (TF) obtained for the TMSO/TMS derivatives of disaccharides, trisaccharides and galactinol analyzed under different conditions in SLBTM-IL82 column.

Carbohydrate	Isothermal									Temperature program					
	180 °C			230 °C			250 °C			Ramp 1			Ramp 2		
	t_R (min)	w_h (min)	TF	t_R (min)	w_h (min)	TF	t_R (min)	w_h (min)	TF	t_R (min)	w_h (min)	TF	t_R (min)	w_h (min)	TF
Sucrose	7.05	0.19	0.55	2.15	0.06	0.75	-*			18.14	0.03	1.00	13.10	0.02	1.00
α,α -Trehalose	8.22	0.11	1.50	2.27	0.05	1.13	1.93		1.13	18.54	0.03	1.10	13.53	0.04	1.10
α,β -Trehalose	17.65	0.11	0.89	2.59	0.07	0.94	2.03	0.05	1.14	19.82	0.04	1.20	14.79	0.03	1.60
β,β -Trehalose	13.8	0.11	0.87	2.52	0.05	1.17	2.00	0.08	1.40	19.62	0.03	1.10	14.60	0.04	1.10
Lactulose 1	11.83	0.12	0.72							19.22	0.03	0.86	14.17	0.03	1.10
Lactulose 2	12.42	0.12	0.71	2.47	0.08	0.62	2.00	0.2	1.24	19.32	0.03	1.10	14.27	0.03	1.00
Lactose $E + Z^{**}$	12.05	0.09	0.81	2.46	0.10	1.78	2.00	0.10	2.00	19.30	0.04	1.20	14.24	0.04	1.10
Laminaribiose E	14.27	0.11	0.70							19.65	0.04	1.10	14.51	0.03	1.10
Laminaribiose Z	14.68	0.08	1.19	2.54	0.06	1.32	2.02	0.09	3.47	19.74	0.02	1.00	14.57	0.03	1.00
Turanose 1, 2 ^{**}	12.40	0.10	1.55	2.55	0.10	1.25	2.03	0.06	1.33	19.55	0.03	1.20	14.48	0.03	1.00
Maltose E	12.89	0.30	0.56							19.46	0.04	1.10	14.40	0.02	0.88
Maltose Z	13.14	0.15	0.66	2.51	0.09	1.30	2.04	0.06	1.42	19.51	0.04	1.10	14.44	0.03	1.00
Galactinol	18.07	0.18	0.59	2.77	0.08	1.80	2.14	0.10	1.65	20.13	0.04	1.10	15.02	0.04	1.00
Kojibiose E	14.06	0.18	0.58	2.57	0.11	1.00				19.61	0.04	0.87	14.54	0.04	0.93
Kojibiose Z	14.60	0.11	0.75	2.59	0.12	2.10	2.03	0.07	1.25	19.72	0.03	1.00	14.63	0.04	1.00
Isomaltose E				3.05	0.07	0.63	2.22	0.06	0.69	20.72	0.03	1.00	15.42	0.04	1.10
Isomaltose Z				3.11	0.05	0.90	2.25	0.04	1.50	20.85	0.04	1.00	15.51	0.03	1.00
Raffinose				7.92	0.27	0.61	3.88	0.15	1.08	29.87	0.07	1.05	18.56	0.04	1.00
Kestose				7.46	0.21	1.13	3.73	0.07	1.23	29.40	0.16	0.69	18.42	0.09	0.82
Erlose				9.26	0.17	0.88	4.24	0.09	1.96	31.20	0.10	0.90	19.11	0.07	1.24
Melezitose				6.97	0.17	1.53	3.62	0.11	1.50	28.48	0.24	0.77	18.25	0.08	0.82
Maltotriose E				13.35	0.30	0.77	5.62	0.13	0.83	33.44	0.05	1.10	20.65	0.11	0.76
Maltotriose Z				13.91	0.20	0.90	5.79	0.19	1.69	33.67	0.07	1.04	20.85	0.08	1.20

Relative standard deviations ($n=3$) for the different carbohydrates and chromatographic conditions were lower than 0.10% and 3.55% for t_R and w_h , respectively.

* Not detected.

** Coelution of isomers.

1 **Figure 1.**

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5 **Figure 2.**

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9 **Figure 3.**

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