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Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Development of a carbohydrate silylation method in ionic liquids for their gas chromatographic analysis

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HIGHLIGHTS

- A silylation procedure of carbohydrates in ionic liquids, particularly in [EMIM][DCA], is optimized.
- Trimethylsilylimidazole was the most effective silylation reagent.
- Other silylation reagents used under ultrasonic agitation provide variable results depending on the carbohydrate nature.
- Ionic liquids can be used as alternative to non-volatile organic solvents for carbohydrate derivatization.

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:

Received 25 January 2013
 Received in revised form 20 May 2013
 Accepted 23 May 2013
 Available online 3 June 2013

Keywords:

Carbohydrate derivatization
 Silylation
 Ionic liquids
 Gas chromatography
 Conformational isomerism

ABSTRACT

This paper reports on the feasibility of silylation of low molecular weight carbohydrates dissolved in different ionic liquids (ILs) for their further analysis by gas chromatography (GC). Derivatization reagents (nature and amounts), temperature and time of reaction and stirring conditions were evaluated for different carbohydrates (i.e., glucose, mannose, fructose and lactose) dissolved in 1-ethyl-3-methylimidazolium dicyanamide [EMIM][DCA]. Evaluation of conformational isomerism of glucose dissolved in [EMIM][DCA] revealed the effect of the time of dissolution in the equilibration of α - and β -furanoses (up to 3% and 6%, respectively, after 70 h of incubation) and that 21 h sufficed to obtain results similar to those provided by the reference method involving pyridine. Once optimized, the proposed derivatization procedure provided satisfactory yields (i.e., close to 100%) using 100 μ L of trimethylsilylimidazole (TMSI) at mild conditions (25 °C) for a relatively short time (1 h) for most of the investigated carbohydrates. Under these experimental conditions, linear responses (i.e., R^2 better than 0.974) were obtained in the tested range of 0.25–1 mg of the derivatized target compounds. Other reagents, such as *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS), were successfully used under ultrasonic conditions for aldose monosaccharides and disaccharides derivatization, while BSTFA was useful for ketose monosaccharides. The possibility of using the proposed method for the derivatization of selected carbohydrates dissolved in different ILs and the efficiency of the method applied to the analysis of carbohydrates present in real samples (fruit juices) have also been investigated.

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1. Introduction

Room temperature ionic liquids (or simply ionic liquids, ILs) are low melting point salts that exist in liquid phase at relatively

low temperatures (generally speaking, below 150 °C). ILs possess unique and attractive properties such as low volatility, variable viscosity, chemical and thermal stability, and tunable solubility properties, among others [1,2]. These properties make them to be considered a promising recyclable alternative to traditional volatile organic solvents for a high number of applications [1,3–5], including carbohydrate synthesis and processing [6,7]. The low solubility of these compounds in a large majority of conventional organic

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solvents makes the use of ILs a good alternative in carbohydrate chemistry.

Gas chromatography (GC) is a powerful separation technique for the analysis of complex mixtures of low molecular weight carbohydrates (LMWC), for which shows high resolution and sensitivity. Nevertheless, for GC carbohydrate analysis a previous derivatization step is mandatory. Silylation is the most frequently used procedure for the derivatization of carbohydrates which provides them with the required volatility and stability [8]. The formation of per-trimethylsilyl (TMS) ethers is generally carried out using individual or combined silylation agents, such as trimethylsilylimidazole (TMSI), hexamethyldisilazane (HMDS) or trimethylchlorosilane (TMCS), and an aprotic solvent at different temperatures for different reaction times [9]. The good solubility of the carbohydrates and their derivatives in pyridine makes this solvent the most frequently used for sugar analysis. However, ILs could be considered a greener and more safety alternative replacement for pyridine, which is toxic and flammable.

Although not orientated to analytical purposes, silylation of cellulose dissolved in ILs has been widely studied [10,11]. As an example, HMDS has been proposed for the silylation of cellulose dissolved in different 1,3-dialkylimidazolium-based ILs, the solubility of the derivatization reagent in the IL was a key parameter determining the derivatization efficiency [12]. However, to the best of our knowledge, the effectiveness of silylation of LMWC dissolved in ILs, previous to their GC analysis, has not been evaluated before. It is important to consider that the previously reported procedures for derivatization of LMWC dissolved in common solvents are not directly valid in ILs because the efficiency of the silylation process markedly depends on solubility, viscosity and other physicochemical properties of ILs (they can act as catalyst, as retardant, or do not act). Moreover, the most common silylation reagents are usually very effective silyl-donors in pyridine, but when carbohydrates are dissolved in other solvents, these reagents could behave in a different manner: the high number of hydroxyls in the molecule, the presence of different tautomeric forms in solution and the steric hindrance must be taken into account when a different solvent is chosen [9]. There are only a limited number of references regarding the use of ILs for silylation of low molecular weight compounds containing hydroxyl groups [13]. 1-Butyl-3-methylimidazolium hexafluorophosphate has been proved to be useful as a green recyclable alternative to conventional solvents for the silylation of alcohols and phenols with *t*-butyldimethylchlorosilane (i.e., the derivatization yields ranged from 90 to 99% for all alcohols tested) [14].

In this study, the efficiency of a silylation method for mono-, di- and trisaccharides dissolved in ILs before their GC analysis was evaluated and applied to the analysis of carbohydrates present in real samples (fruit juices). The experimental parameters evaluated for method optimization included the time required for carbohydrate equilibration, the nature and amount of the silylation reagent used, the time and temperature of reaction and the stirring conditions.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents used in this work were analytical or research grade. Cellobiose was acquired from Difco Laboratories (Detroit, MI, USA). Glucose, fructose, tagatose, galactose, mannose, xylitol, mannitol, lactose, lactulose, gentiobiose, raffinose, melezitose, phenyl- β -D-glucoside, TMSI, HMDS and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% TMCS were obtained from Sigma Chemical Co. (St. Louis, USA). Heptane, TMCS and *N,O*-bis(trimethylsilyl)acetamide (BSA) were purchased from

Merck (Darmstadt, Germany). BSTFA was acquired from ACROS organics (Geel, Belgium) and *n*-tetracosane from PolyScience Corporation (Illinois, USA).

1-Ethyl-3-methylimidazolium dicyanamide ([EMIM][DCA]), 1-hexyl-3-methylimidazolium chloride ([HMIM][Cl]), 1-butyl-3-methylimidazolium methyl sulfate ([BMIM][MeSO₄]) were obtained from Sigma Chemical Co. and 1,3-dimethylimidazolium dimethyl phosphate ([MMIM][Me₂PO₄]) from Alfa Aesar (Massachusetts, USA). These ILs were selected on the base of LMWC solubility data available in the literature [3,15,16]. Other criteria such as diversity of anion and cation, availability, and low cost were also considered.

2.2. Dissolution of carbohydrates in ILs

ILs were dried at 60 °C under vacuum for 3 days before use. Carbohydrates (1 mg), previously indicated in Section 2.1, were individually dissolved in 50 μ L of the tested IL and the resulting mixtures were equilibrated by stirring using a Thermomixer (Eppendorf, Hamburg, Germany). Different times and temperatures (see Table 1) were assayed using glucose to optimize equilibration conditions.

2.3. Samples

Fresh fruits (strawberry, grape, orange and tangerine) were purchased at a local market in Madrid (Spain) and juices were produced from them in the laboratory, after removing skins and seeds when necessary. They were then centrifuged at 10,000 rpm for 20 min at 5 °C and the supernatant was filtered to remove any suspended solid material. Juices (0.5 mL) were diluted to 25 mL with 70% methanol, as previously done by Sanz et al. [17]. After that, 0.5 mL of the solution was evaporated under vacuum in a Speed Vac (Genevac, Ipswich, UK).

2.4. General procedure for the silylation reaction

Silylation of the assayed carbohydrates dissolved in the corresponding IL was carried out with 100 μ L of different silylation reagents assayed [i.e., TMSI, TMSI:TMCS (1:1, v/v), HMDS, HMDS:TMCS (1:1, v/v), BSTFA, BSTFA + 1% TMCS and BSA] for different times (0, 30, 60 and 120 min), at different temperatures (25, 45 and 60 °C) and under different agitation conditions (i.e., ultrasonic agitation, conventional stirring and static incubation). 200 μ L of ultrapure water were added to finish the reaction. The derivatized carbohydrates were then recovered by liquid–liquid extraction (LLE) with 100 μ L of heptane. After optimization of the derivatization procedure, two more successive LLEs with heptane were performed to ensure quantitative recovery of the derivatized analyte. In this case, extracts were jointly collected and directly analyzed by GC. Otherwise specified, all assays were carried out in triplicate.

Carbohydrates present in fruit juices were derivatized by adding to the dried extracts (i) pyridine (100 μ L) and TMSI (100 μ L) as reference method [9], and (ii) [EMIM][DCA] (100 μ L) and TMSI (100 μ L) under optimized conditions. Reaction was stopped by adding water and the derivatized carbohydrates were recovered as indicated above for their further GC analysis.

2.5. GC analysis

GC analysis of the derivatized carbohydrates was carried out using an HP 7890A gas chromatograph equipped with a flame ionization detector (FID) from Agilent Technologies (Palo Alto, CA, USA) using nitrogen as carrier gas (flow rate, 0.7 mL min⁻¹). Derivatized glucose tautomers were analyzed using a 7890A

Table 1

Percentage of isomeric forms of TMS glucose dissolved in [EMIM][DCA] and equilibrated under different conditions and derivatized using TMSI at 25 °C for 30 min. Standard deviation (S.D.) in parenthesis ($n = 3$).

Equilibration conditions		% glucose tautomers (S.D.)			
Time (h)	Temperature (°C)	α -Pyranose	β -Pyranose	α -Furanose	β -Furanose
0.5	60	67.6 (0.3)	26.8 (0.1)	3.1 (0.2)	2.5 (0.1)
0.5	25	84 (5)	14 (6)	0.6 (0.1)	0.7 (0.3)
21	25	47.9 (0.2)	50.8 (0.3)	0.5 (0.1)	0.8 (0.1)
27	25	45.2 (0.3)	51 (1)	1.2 (0.6)	2.3 (1.0)
45	25	40.6 (0.1)	53.7 (0.1)	1.9 (0.1)	3.8 (0.2)
70	25	39.2 (0.3)	52 (3)	3.0 (1.0)	6 (2)

Table 2

Silylation yields (%) of glucose dissolved in [EMIM][DCA] using TMSI under different derivatization conditions. Standard deviation (S.D.) in parenthesis ($n = 3$).

Temperature (°C)	Yield (S.D.)					
	25		45		60	
Time (min)	0*	30	60	120	60	60
Yield (S.D.)	80 (1) ^a	96 (1) ^b	103 (2) ^{c,A}	101 (1) ^c	105 (4) ^A	102 (2) ^A

Different letters indicate significant differences ($p < 0.05$) for each time at the same temperature (lower case letters), and for each temperature at the same time (capital letters).

* Immediately after reagent addition.

Table 3

Silylation yields (%) of carbohydrates dissolved in [EMIM][DCA] using different reagents at 60 °C for 1 h under ultrasound agitation. Standard deviation (S.D.) in parenthesis ($n = 3$).

Reagent	Glucose	Mannose	Fructose	Lactose
HMDS	–	–	–	–
HMDS + TMCS	103 (1)	100 (2)	21 (5)	13 (6)
BSTFA + TMCS	103.2 (0.3)	104 (2)	–	97 (4)
BSTFA	87 (23)	89 (2)	100 (5)	94 (3)
TMSI	96 (10)	102 (4)	107 (1)	100 (3)
TMSI + TMCS	82 (11)	89 (14)	93 (2)	96 (19)
BSA	–	–	–	–

gas chromatograph coupled to a 5975 C quadrupole mass detector (GC–MS, both from Agilent Technologies) using helium at 1 mL min^{−1} as carrier gas. In both cases, a fused silica capillary column coated with 100% dimethylpolysiloxane (30 m × 0.25 mm i.d. × 0.25 μ m *df*) was used (Zebron, Phenomenex, CA, USA). The oven temperature was programmed as follows: 180 °C to 250 °C (10 °C min^{−1}) and held for 3 min, and then to 300 °C at 15 °C min^{−1} and held for 20 min. The injection port was heated to 300 °C and injections were made in the split mode, with a split ratio 1:20. FID temperature was 320 °C. The MS was operated in electron impact (EI) mode at 70 eV, scanning the 35–700 *m/z* range. The transfer line and ionization source were heated at 280 and 230 °C, respectively. Chromatographic peaks were measured using an HPChem acquisition system (Agilent Technologies).

2.6. Quantitative analysis

Quantitation analyses were performed in triplicate using the internal standard procedure. For this purpose, calibration curves of the derivatized carbohydrate in the 0.1–1 mg range were constructed. Response factors of glucose, *myo*-inositol, lactose and raffinose (which were used for monosaccharides, *myo*-inositol, disaccharides and trisaccharides quantitation, respectively) relative to two internal standards (*n*-tetracosane and phenyl- β -D-glucoside) were determined for quantitative analysis. The reproducibility of the method was determined on the basis of the intra-day and inter-day precision, and estimated as the relative standard deviation (RSD) of the calculated concentrations for each carbohydrate standard in five separate determinations.

2.7. Statistical analysis

Statistical analysis was performed using Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA). Univariate analysis of variance (ANOVA) and Fisher test were used to evaluate significant differences among the several derivatization treatments, and also to evaluate the efficiency of the silylation method using real samples. The differences were considered to be significant when $p < 0.05$.

3. Results and discussion

Information available in the literature about the solubility of mono-, di- and trisaccharides in ILs is rather scarce [15]. However, [EMIM][DCA] has been described as a good solvent for different carbohydrates such as glucose and sucrose, for which solubilities above 10% wt have been reported [18]. Therefore, this IL was selected for the optimization of the proposed derivatization method. The effectiveness of the derivatization procedure was initially evaluated using glucose and TMSI as silylation reagent due to the experimentally determined miscibility of this reagent in [EMIM][DCA].

3.1. Evaluation of conformational isomerism of glucose

α - and β -pyranoses have been identified as the most abundant isomers of glucose dissolved in pyridine (45 and 53%, respectively) at room temperature; meanwhile, α - and β -furanoses only appear at low levels (0.6 and 1%, respectively) [19]. However, no data regarding conformational equilibrium of glucose dissolved in ILs can be found in the literature. Therefore, the equilibrium of the

Fig. 1. Gas chromatographic profiles of strawberry (A) and grape (B) juices derivatized using [EMIM][DCA] and TMSI. Peak identification: (1) fructose, (2) α -glucopyranose, (3) β -glucopyranose, (4) *myo*-inositol, (5) phenyl- β -D-glucoside (internal standard), (6) *n*-tetracosane (internal standard), and (7) sucrose.

tautomeric forms of crystalline glucose dissolved in [EMIM][DCA] was evaluated taking into account two main experimental parameters, namely temperature and time of dissolution.

For comparative purposes, the identification of the tautomers of sugars silylated in pyridine was carried out by GC–MS according to Martínez-Castro et al. [20].

When crystalline glucose was dissolved in [EMIM][DCA] at 60 °C for 30 min (longer times were not assayed to avoid degradations) and then silylated with TMSI, α -pyranose was noticeably more abundant (68%) than the other tautomers. This percentage was even higher at 25 °C (84%; Table 1). However, when the glucose solution in [EMIM][DCA] was kept at 25 °C for 21 h before silylation, similar percentages of TMS α - and β -pyranose were obtained (48 and 51%, respectively). Times of equilibration longer than 27 h resulted in percentages of α - and β -furanoses higher than those previously found in pyridine solutions [19] and arose up to 3% and 6%, respectively, after 70 h of incubation. According to these results, for the following experiments, carbohydrates were dissolved in [EMIM][DCA] and kept at 25 °C for 21 h before derivatization.

3.2. Optimization of the silylation procedure in [EMIM][DCA]

To evaluate the effectiveness of the derivatization of glucose, two internal standards were used: phenyl- β -D-glucoside, as a derivatizable carbohydrate, and *n*-tetracosane, as a non-derivatizable standard. The solubility of phenyl- β -D-glucoside in [EMIM][DCA] was experimentally confirmed in the laboratory, while *n*-tetracosane was insoluble in this IL but fully soluble in heptane.

Reactions were developed at 25 °C for 1 h, recovery of TMS-glucose derivatives being carried out by single LLE with 100 μ L of heptane. Different yields were calculated for glucose depending on the internal standard used for quantitation. Whereas the recovery calculated for glucose using phenyl- β -D-glucoside as internal standard was $99 \pm 4\%$, its yield relative to *n*-tetracosane was $85 \pm 4\%$. This discrepant result could be associated to either an incomplete derivatization of both glucose and phenyl- β -D-glucoside or to an incomplete extraction of these compounds into the heptane layer. The latter possibility was evaluated by successive LLEs of the derivatized mixture with heptane. It was found that quantitative recoveries of both glucose and phenyl- β -D-glucoside from the [EMIM][DCA] was only achieved after three successive LLE with 100 μ L of heptane, yields being $87 \pm 5\%$, $11 \pm 4\%$ and $2 \pm 1\%$, respectively. Therefore, this recovery procedure was followed for further experiments and the following data are only expressed related to phenyl- β -D-glucoside.

The time and temperature of the reaction and the volume of silylation reagent were also optimized. As shown in Table 2, silylation was not immediately produced after addition of TMSI and at least 30 min were required to achieve acceptable yields of 96%. Complete derivatization was only achieved after 1 h of treatment (yield, 103%) with no significant improvement at longer reaction times (e.g., the yield after 2 h of reaction was 101%; Table 2). Therefore, 1 h was selected for following assays. Under these conditions, no significant differences existed between the different temperature assayed, 25 °C being chosen for following experiments. Different volumes (100 and 200 μ L) of TMSI were then evaluated for the silylation reaction and similar results were obtained in both cases (i.e.,

Table 4

Silylation yields (%) of different low molecular weight carbohydrates dissolved in [EMIM][DCA] and submitted to the proposed optimized method. Standard deviation (S.D.) in parenthesis ($n = 3$).

Compound	Yield (S.D.)
Xylitol	100 (6)
Mannitol	101 (6)
myo-Inositol	101 (2)
Glucose	96 (10)
Mannose	102 (4)
Galactose	102 (1)
Tagatose	99 (2)
Fructose	100 (6)
Maltose	98 (2)
Lactose	100 (3)
Lactulose	101 (5)
Cellobiose	110 (4)
Raffinose	101 (7)
Maltotriose	95 (10)
Melezitose	106 (9)

$103 \pm 2\%$ for 100 μL and $99 \pm 6\%$ for 200 μL). Therefore, 100 μL of TMSI were proved to be enough to achieve a complete derivatization.

The influence of different stirring procedures on the efficiency of the derivatization process was also investigated. No significant differences were observed when the reaction was carried out under static conditions ($101 \pm 6\%$), stirring with a vortex ($101 \pm 4\%$) or under ultrasonic agitation ($101 \pm 1\%$). Therefore, the three procedures could be considered suitable for the silylation of glucose with TMSI.

Besides TMSI, other silylation reagents (i.e., HMDS, BSTFA, BSA and their combinations with TMCS) were investigated. Except TMSI, the other evaluated reagents were not miscible in [EMIM][DCA] and they formed viscous/jelly solutions or remained as a separated layer. Nevertheless, they dissolved using ultrasounds agitation. Therefore, the efficiency of all these reagents for the silylation of carbohydrates was evaluated under sonication conditions. In addition, these experiments were not only limited to glucose, but also extended to other LMWC, i.e. mannose, fructose and lactose, as representative analytes for aldoses, ketoses and disaccharides, respectively. Table 3 summarizes the results obtained in this part of the study. Whereas TMSI was effective for derivatization of all the carbohydrates analyzed, different behaviors were observed for the other investigated reagents depending on the nature of the carbohydrate. Silylation of aldose monosaccharides was also effective using HMDS + TMCS and BSTFA + TMCS, albeit these reagents failed for fructose derivatization. Silylation of this ketose was effective using BSTFA, whereas lactose was effectively silylated using BSTFA + TMCS, BSTFA, and TMSI + TMCS. However, with the latter reagent the dispersion was high as reflects its standard deviation (19%, Table 3), and derivatization could not be considered reliable. HMDS and BSA were not efficient for the silylation of the carbohydrates evaluated and different by-products were formed after reaction. The behavior of these reagents indicates that silylation of sugars is more difficult in ILs than in the usual solvents. It seems that it is not a mere problem of solubility; probably viscosity also contributes impairing the movement of molecules in the medium; or maybe also other molecular properties of ILs could act as retardant.

Table 5

Silylation yields (%) of carbohydrates using TMSI at 60 °C for 1 h under ultrasonic agitation. Standard deviation (S.D.) in parenthesis ($n = 3$).

Reagent	Glucose	Fructose	Lactose
[HMIM][Cl]	98 (6)	103 (6)	96 (11)
[BMIM][MeSO ₄]	6 (2)	3.4 (0.1)	11 (5)
[MMIM][Me ₂ PO ₄]	96 (6)	101 (12)	93 (5)

Regarding sample handling, TMSI was also the best choice, considering that clear solutions were obtained in all cases and that the recovery of silylated carbohydrates from the miscible solution was easier. Moreover, it could be simultaneously applied for all the carbohydrates studied.

3.3. Linear response and reproducibility of the complete analysis

Good linear relationship between carbohydrate amount and peak areas was obtained for calibration curves in the evaluated range (0.1–1 mg); correlation coefficients (R^2) ranged from 0.974 to 0.999. Intra- and inter-day reproducibilities were evaluated derivatizing 1 mg of glucose as a reference compound ($n = 5$) under the optimized conditions (i.e. 100 μL of TMSI at 25 °C for 1 h). Relative standard deviation was lower than 4%.

3.4. Application to different carbohydrates

Different carbohydrates, including mono-, di- and trisaccharides, dissolved in [EMIM][DCA] were silylated following the optimized derivatization procedure. As shown in Table 4, the proposed derivatization procedure was effective for most of the assayed carbohydrates, providing yields in the range of 96–110% with SD below 10 ($n = 3$).

These yield and repeatability values are in the range of those reported in the literature for the derivatization of carbohydrates using more toxic solvents such as pyridine [21–23].

3.5. Use of different ILs

Once optimized, the straightforward silylation procedure was finally extended to other ILs, i.e. [HMIM][Cl], [BMIM][MeSO₄] and [MMIM][Me₂PO₄], for glucose and fructose taken as representatives of monosaccharides (aldose and ketose) and lactose representing a disaccharide (Table 5). Considering that [HMIM][Cl] was not miscible with TMSI and has a high viscosity (7500 cP at 25 °C), derivatization had to be performed under ultrasounds agitation at 60 °C. Silylation failed in [BMIM][MeSO₄] (mean value of $6 \pm 2\%$, $3.4 \pm 0.1\%$, and $11 \pm 5\%$; for glucose, fructose and lactose yield, respectively) and different by-products were formed. However, carbohydrates were correctly derivatized in [HMIM][Cl] and [MMIM][Me₂PO₄] (recovery values in a range of 96–98%, and 93–101%, respectively). The formation of by-products was not observed and clean chromatograms were obtained in all cases, something which make possible the accurate determination of the carbohydrates even if a non selective detector as FID was used.

3.6. Application to real sample analysis

The optimized derivatization method was applied to four different hand-made fruit juices (grape, strawberry, orange and tangerine). Fig. 1 shows GC profile of strawberry (A) and grape (B) samples dissolved in [EMIM][DCA] and silylated using 100 μL of TMSI at 25 °C for 1 h. According to previous works [17], fructose, glucose, sucrose and myo-inositol were detected in all the juices. Table 6 shows the concentrations (mg mL^{-1}) of these low molecular weight carbohydrates found in the juices when using pyridine or [EMIM][DCA] as solvents in the derivatization procedure. Grape juice had the highest concentration of fructose and glucose and the lowest of sucrose, whereas tangerine juice showed the highest content of sucrose and myo-inositol. When both derivatization procedures were compared, no statistically significant differences were observed between determined concentrations of the four carbohydrates in all cases which confirmed the high efficiency of the optimized procedure.

Table 6
Concentrations (mg mL⁻¹) of carbohydrates dissolved in either pyridine or [EMIM][DCA] in different fruit juices and submitted to the proposed optimized method. Standard deviation (S.D.) in parenthesis (*n* = 3).

	Fructose		Glucose		Sucrose		myo-Inositol	
	Pyridine	IL	Pyridine	IL	Pyridine	IL	Pyridine	IL
Strawberry	19.2 (2.4) ^a *	19.2 (2.1) ^a	10.8 (1.8) ^a	10.4 (0.9) ^a	13.2 (0.9) ^a	9.8 (1.5) ^a	0.04 (0.02) ^a	0.05 (0.02) ^a
Grape	122.4 (11.8) ^a	128.4 (8.3) ^a	83.5 (7.2) ^a	88.0 (4.4) ^a	0.6 (0.4) ^a	0.5 (0.1) ^a	0.4 (0.2) ^a	0.6 (0.1) ^a
Orange	24.7 (3.2) ^a	28.4 (2.4) ^a	17.5 (3.4) ^a	18.8 (0.4) ^a	46.3 (1.9) ^a	48.1 (5.1) ^a	1.4 (0.04) ^a	1.5 (0.1) ^a
Tangerine	54.8 (1.8) ^a	55.1 (5.1) ^a	30.8 (1.7) ^a	31.7 (1.4) ^a	51.2 (6.2) ^a	51.5 (4.7) ^a	2.6 (0.1) ^a	2.7 (0.1) ^a

* Similar letters indicate not significant differences (*p* < 0.05) for each carbohydrate concentration in each juice.

4. Conclusions

Silylation of LMWC dissolved in selected ILs, particularly in [EMIM][DCA], has been proved to be effective under optimized conditions, so avoiding the use of toxic solvents such as pyridine. The use of TMSI under mild conditions for relatively short periods of time (1 h) resulted in good derivatization yields for most of the carbohydrates investigated. Other silylation reagents such as the mixtures of HMDS and BSTFA with TMCS can be used under ultrasonic agitation conditions but variable derivatization efficiencies have been obtained depending on the nature of the carbohydrate, especially for aldoses or ketoses. The application of this green solvent based methodology could also be extended to the analysis of other polar compounds present in real samples.

Acknowledgements

This work has been funded by Ministerio de Ciencia e Innovación (project AGL2009-11909), Ministerio de Economía y Competitividad (project CTQ2012-32957) and Comunidad Autónoma of Madrid (Spain) and European funding from FEDER program (project S2009/AGR-1464, ANALISYC-II). L. Ruiz-Aceituno is supported by CSIC by a JAE-Pre grant, co-financed by the European Social Fund (ESF).

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