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Porous graphitized carbon and octadecyl-silica columns in the separation of some alkylglycoside detergents

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Abstract

The chromatographic behavior of several alkylglycosides on Hypercarb, a graphitized carbon column, was investigated under isocratic and gradient elution modes and compared to that on an ODS column. Higher retention and better selectivities were observed on Hypercarb S. Using acetonitrile as organic modifier reinforces alkylglycoside separation depending on the alkyl chain length, whereas methanol favours the separation of alkylglycosides which differ by their polar head. Excellent separation of five closely related octylglycosides was obtained with methanol–water (95:5) as eluent. Detection of these non-UV absorbing compounds was by an evaporative light-scattering detector. © 1997 Elsevier Science B.V.

Keywords: Stationary phases, LC; Porous graphitic coarbon; Alkylglycosides; Detergents; Glycosides

1. Introduction

Alkylglycosides are biological non-ionic surfactants widely used in membrane biochemistry for solubilization, purification and reconstitution of intrinsic membrane proteins [1–5]. Owing to their properties such as strong foaminess, good skin tolerance and improvement of skin tolerance of some anionic surfactants, they have many applications in cosmetology and as domestic detergents [6]. Recently, the behaviour of these detergents added to an electrophoretic buffer was evaluated as a chiral micellar agent in micellar electrokinetic capillary chromatography [7,8].

Alkylglycosides composed of a hydrophilic sugar contains preferentially eight to sixteen carbon atoms, the nature of their polar head, their anomeric form (α or β) and also by the nature of the bond between the sugar moiety and the alkyl chain. The surfactant properties of these compounds depend on each of these parameters.

To control the purity of these non-ionic detergents it was thus of interest to develop a selective chromatographic method which resolves a mixture of surfactants according to their non-polar moieties, polar heads and anomeric forms. The analysis of some *O*-alkylglycosides has already been investigated by supercritical fluid chromatography (SFC) with different packed columns (cyano- and diol-bonded silicas). In these chromatographic

tography (RPLC) by using different octyl- or octadecyl-silica columns and polymeric Asahipak ODP-50 [10]. In RPLC, the separation of these surfactants depends not only on the length of the alkyl chain but also on the nature of the glycoside and on the nature of the bond between the sugar and alkyl chain.

Graphitized carbon is one of the extremes as a stationary phase for RPLC, possessing rigid, planar surfaces and sites capable of dispersion and charge-transfer interactions. It is now well established that the retention increase caused by one methylene group is always greater on the carbon phase than on alkyl- or aryl-bonded silica phases [11]. Moreover, packing has been reported to be useful for the separation of solutes with closely related structures, including stereoisomers [12–16]. A recent review published on the chromatographic behaviour of monosaccharides, disaccharides, cyclodextrins (CDs), branched CDs, oligosaccharide alditols, chitooligosaccharides, N-linked oligosaccharides and glycopeptides on graphitized carbon (PGC) columns has made it clear that the elution patterns are based on the size and the planarity of the molecule (position and configuration of linkage) [17].

In this work, a porous graphitized carbon (PGC) column was evaluated under isocratic and gradient elution conditions and compared to a Zorbax ODS column for the LC analysis of alkylglycoside detergents.

2. Experimental

2.1. Apparatus

The liquid chromatographic apparatus consisted of a Varian (Palo Alto, CA, USA) Model 9010 gradient pump, a Rheodyne (Berkeley, CA, USA) Model 7125 injector with a 20-μl sample loop and an evaporative light-scattering detection (ELSD) system (Sedere, Alfortville, France) Model Sedex 45. The usual ELSD detector settings were as follows: photomultiplier, 9; evaporative temperature, 30°C; air pressure, 2.2 bar. The porous graphitized carbon column was Hypercarb S (100×4.6 mm I.D., particle size 7 μm) from Shandon Scientific (Runcorn, UK) and the octadecyl-silica column was a Zorbax ODS (150×4.6 mm) from DuPont (Wilmington, DE, USA). Data were processed using a Shimadzu (Kyoto, Japan) Model CR 5A integrator.

2.2. Reagents

Acetonitrile (RS for LC) was purchased from Carlo Erba (Milan, Italy); methanol (Hipersolv grade, BDH) was from Prolabo (Paris, France); and water from the Elgastat UHQ II System from Elga (Antony, France). 1-*O*-Octyl-β-D-galactopyranoside (C_8-Oβgal) and 1-C-octyl-1-deoxy-β-D-glucopyranoside (C_8-Cβglu) were synthesized in our laboratory. 1-*O*-Octyl-α-D-glucopyranoside (C_8-Oαglu), 1-*O*-octyl-β-D-glucopyranoside (C_8-Oβglu), 1-*S*-octyl-β-D-thioglucopyranoside (C_8-Sβglu), 1-*O*-decyl-β-D-glucopyranoside (C_{10}-Oβglu) and 1-*O*-decyl-β-D-maltoside (C_{10}-Oβmal) were obtained from Fluka (St. Quentin-Fallavier, France) and were used as received. D-Glucose and maltose were purchased from Merck (Darmstadt, Germany). The concentration of each standard in the different injected samples was about 100–500 mg l^{-1} (the differences result in varying peak areas in the figures).

3. Results and discussion

Fig. 1 reports the structure of the seven studied alkylglycosides which were selected on the basis of their closely related structure.

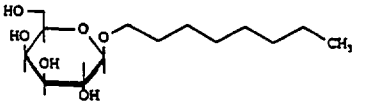
Due to the lack of UV chromophore group in their structure, the alkylglycoside analyses were performed using ELSD which is a universal detection method, more sensitive than refractive index detection and compatible with the elution gradient. The ELSD response factors for the studied alkylglycosides are highly similar.

3.1. Contribution of alkyl chain and sugar moiety to retention

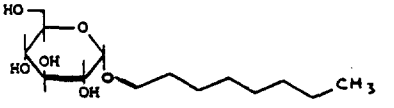
3.1.1. Influence on capacity factor

Fig. 2 and Table 1 depict the difference in retention and selectivity between a Hypercarb S column and a Zorbax ODS column for the three alkylglycosides: C_8-Oβglu, C_{10}-Oβglu and C_{10}-Oβmal. Whatever the column and the mobile phase composition, for a given hydrophilic sugar moiety

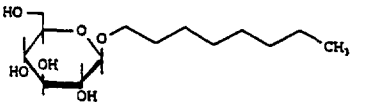
1-O-octyl-β-D-galactopyranoside
C_8-Oβgal



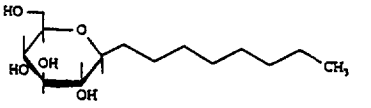
1-O-octyl-α-D-glucopyranoside
C_8-Oαglu



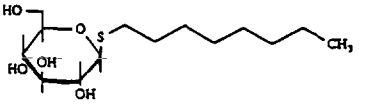
1-O-octyl-β-D-glucopyranoside
C_8-Oβglu



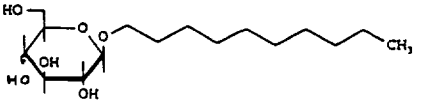
1-C-octyl-1-deoxy-β-D-glucopyranoside
C_8-Cβglu



1-S-octyl-β-D-thioglucopyranoside
C_8-Sβglu



1-O-decyl-β-D-glucopyranoside
C_{10}-Oβglu



1-O-decyl-β-D-maltoside
C_{10}-Oβmal

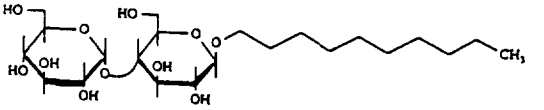


Fig. 1. Structures of the studied alkylglycosides.

(glucose), the order of retention is C_8-Oβglu<C_{10}-Oβglu. The retention of homologues increases with increasing carbon number n_c of the substituted alkyl chain as follows: $\log k' = \log \beta + n_c \log \alpha$ [10,18], where $\log \alpha$ is the non-specific contribution of the methylene group (α is the ratio of the capacity factors of two consecutive homologous solutes and characterizes the solvophobic effect). As expected in reversed-phase chromatography, the retention time of alkylglycosides on a PGC column tends to decrease with increasing organic modifier concentration and the elution of alkylglycosides requires a higher content of methanol than acetonitrile in the mobile phase. To elute with a similar retention time C_8-

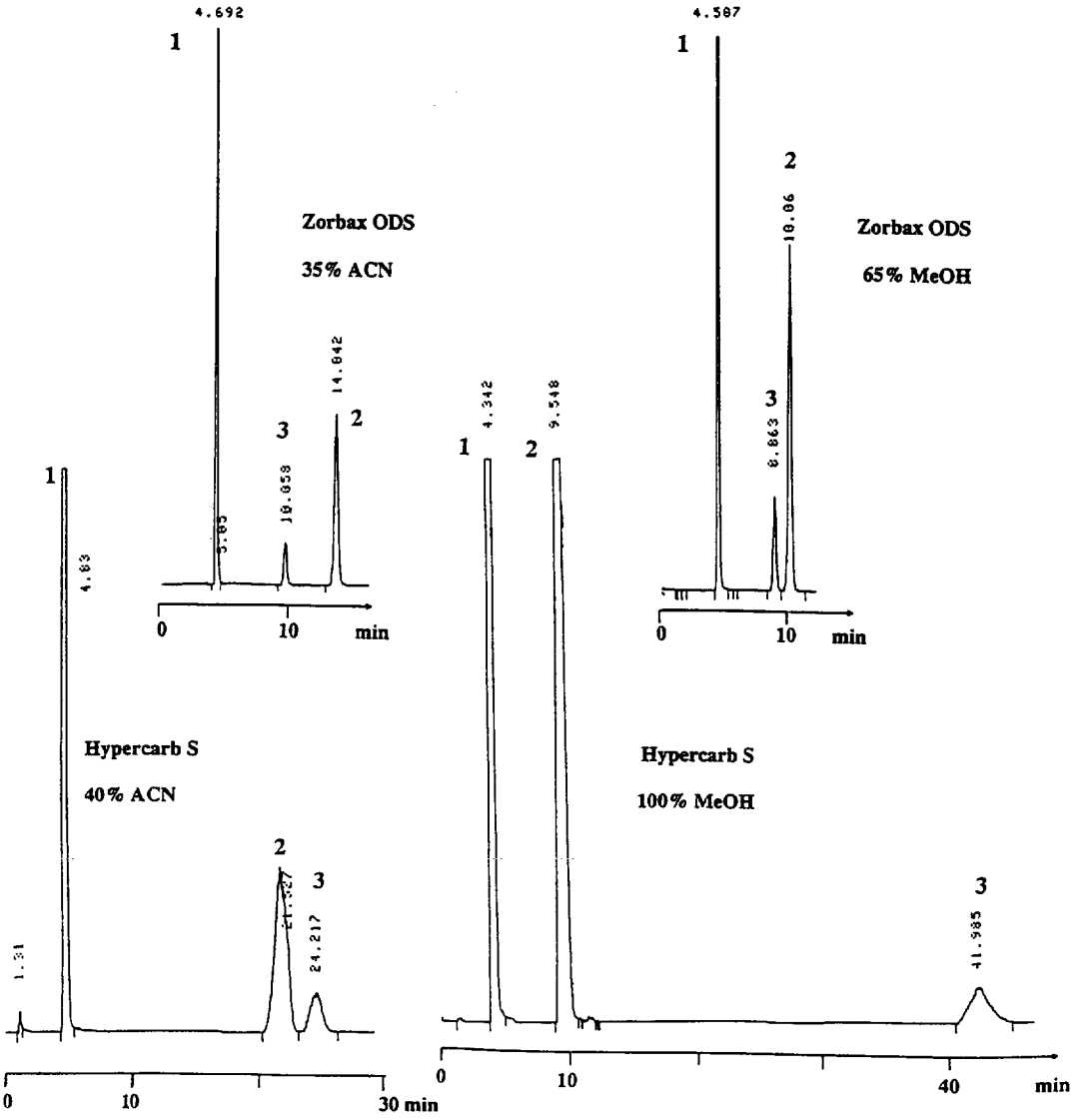


Fig. 2. Chromatograms illustrating the difference in selectivity between Hypercarb S (100×4.6 mm I.D.) and Zorbax ODS (150×4.6 mm I.D.) columns for the alkylglycoside analysis. Eluents: acetonitrile–water or methanol–water. Isocratic elution at 1 ml min^{-1}. Evaporative light scattering detection. Elution order: 1, C_8-Oβglu; 2, C_{10}-Oβglu; 3, C_{10}-Oβmal.

Oβglu, a greater percentage of organic modifier in the eluent is required on a porous graphitized column thus showing that the hydrophobicity of PGC is greater than that of other reversed-phase materials.

For a given (C_{10}) alkyl chain, as expected for a disaccharide C_{10}-Oβmal compared with a monosaccharide C_{10}-Oβglu [10], the order of retention on a Zorbax ODS column is C_{10}-Oβmal<C_{10}-Oβglu but the opposite is obtained on a Hypercarb S column C_{10}-Oβglu<C_{10}-Oβmal. This elution order on Hypercarb is similar to that observed in SFC on CN- or diol-bonded silica by an adsorption mechanism [9]. Moreover, these results are in good agreement with those observed for a series of saccharides having the

Table 1
Comparison between capacity factors (k') and relative retentions (α) of three alkylglycosides on a Hypercarb S column (100×4.6 mm I.D.) and on a Zorbax ODS column (150×4.6 mm I.D.) with different eluents

	k'			α	
	C_8-Oβglu	C_{10}-Oβglu	C_{10}-Oβmal	$k'_{C10\text{-}O\beta glu}/k'_{C8\text{-}O\beta glu}$	$k'_{C10\text{-}O\beta mal}/k'_{C10\text{-}O\beta glu}$
Hypercarb					
100% MeOH	2.31	6.29	31.05	2.72	4.94
ACN–water (40:60)	2.69	15.43	17.49	5.74	1.13
Zorbax ODS					
MeOH–water (65:35)	3.02	7.98	6.91	2.64	0.86 ($1/\alpha$=1.15)
ACN–water (35:65)	3.19	11.54	7.98	3.62	0.69 ($1/\alpha$=1.45)

same molecular configuration (such as cyclodextrins (CDs): αCD, βCD, γCD), for which the retention on a porous graphitized column increases with increasing molecular size [19].

3.1.2. Influence on selectivity

When the different mobile phase compositions were adjusted to elute with a capacity factor similar to that of the least retained compound, C_8-Oβglu, the selectivities in terms of the relative retention α (calculated from the corresponding capacity factor (k') values reported in Table 1) show that the two organic solvents led to differences. While selectivities between C_8-Oβglu and C_{10}-Oβglu are similar on Zorbax ODS and PGC columns with methanol in the eluent, they are very different with acetonitrile (ACN) in the eluent. This effect has not been observed previously with glucosinolate series for which MeOH and ACN allow a similar methylene selectivity on a PGC column [16]. A PGC column with ACN–water mixture as mobile phase offers a similar selectivity between C_{10}-Oβmal and C_{10}-Oβglu as that of a Zorbax ODS column, whereas the use of MeOH as mobile phase quadruples this selectivity on the PGC column compared to the ODS column.

Thus, with a PGC column, the choice of ACN as organic modifier is more interesting to separate two alkylglycosides which differ only by one methylene unit in their alkyl chain, while the choice of MeOH is more judicious to separate two alkylglycosides which differ in their polar sugar moiety; however, the elution power of methanol was too weak to elute the derivatized disaccharides under satisfactory conditions.

3.2. Different gradient elution modes

To combine the complementary advantages of each organic modifier, a gradient mode analysis has been investigated. Fig. 3 depicts a good separation of the three alkylglycosides with a MeOH–ACN–water gradient. The mobile phase gradient was obtained as following: MeOH during 10 min, then to 100% mixture ACN–water (70:30) for 10 min, then this composition was maintained. A satisfactory analysis time for the most retained compound, i.e. the disaccharide with the longest alkyl chain (about 24 min at a flow-rate of 1 ml min^{-1}), and an efficiency for all the compounds better than that obtained in isocratic elution mode with 100% MeOH or 40% ACN, were noticed. No problem was observed during the gradient elution mode. After modifying the eluent composition, returning to the initial conditions was easy. The re-equilibration time needed between gradient runs was about 30 min.

With a view to follow the progress of an alkylglycoside synthesis or to confirm in an alkylglycoside the absence of traces of initial carbohydrate which has no detergent properties, it was interesting to compare the retention of these two classes of compounds on a PGC support. Previously, Koizumi et al. [19] have shown that monosaccharides were weakly retained on a PGC column and were eluted with water, whereas disaccharides were adequately eluted with methanol–water (15:85) or acetonitrile–water (4:96). The PGC support appears

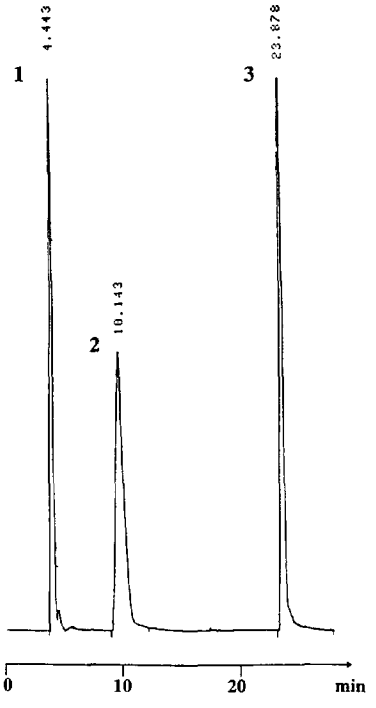


Fig. 3. Chromatogram of three alkylglycosides under gradient elution conditions. Hypercarb S column (100×4.6 mm I.D.): flow-rate, 1 ml min^{-1}; evaporative light scattering detector. Gradient elution: methanol during 10 min, then to 100% acetonitrile–water (70:30) for 10 min then this composition was maintained. Elution order: 1, C_8-Oβglu; 2, C_{10}-Oβglu; 3, C_{10}-Oβmal.

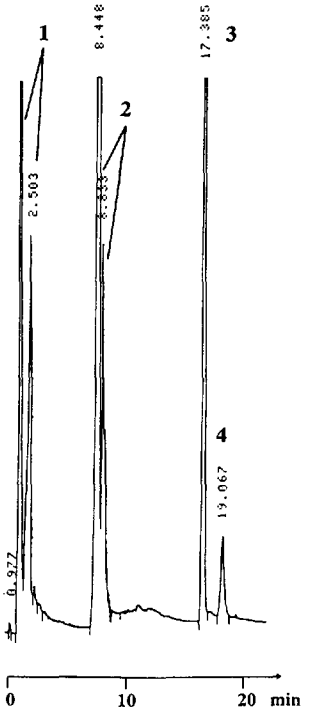


Fig. 4. Simultaneous analysis of 1-*O*-decyl-β-D-glucopyranoside and 1-*O*-decyl-β-D-maltoside with D-glucose and maltose. Column: Hypercarb S (100×4.6 mm I.D.); flow-rate 1 ml min^{-1}; evaporative light scattering detector; gradient elution, water during 4 min then to water-acetonitrile (20:80) for 11 min then this composition was maintained. Elution order: 1, D-glucose; 2, maltose; 3, C_{10}-Oβglu; 4, C_{10}-Oβmal.

to have a considerable retention capacity for alkylglycosides in comparison with their underivatized corresponding sugar moiety so, to retain the mono- or disaccharides and to elute the corresponding alkylglycosides in satisfactory conditions (time and efficiency), it was necessary to investigate a mobile phase gradient. Fig. 4 shows that a simultaneous separation of D-glucose, maltose, C_{10}-Oβglu and C_{10}-Oβmal was successfully obtained by gradient elution at ambient temperature on a Hypercarb S column. The mobile phase gradient was obtained as follows: water during 4 min then to water–acetonitrile (20:80) for 11 min, then this composition was maintained. As has been previously observed [19], each peak of D-glucose and maltose was split into two anomer (α or β) peaks under these temperature conditions.

3.3. Separation of closely related octylglycosides

Fig. 5 depicts the separation of five octylglycosides with closely related structures: C_8-Oβgal, C_8-Oαglu, C_8-Oβglu, C_8-Cβglu and C_8-Sβglu on a Zorbax ODS column with acetonitrile–water (35:65) as mobile phase and on a Hypercarb S column with acetonitrile–water (40:60) or methanol–water (95:5) as mobile phases. Fig. 5 shows clearly that under similar conditions of retention, only the PGC support was able to separate all the compounds of the mixture. The elution order of these octylglycosides on Hypercarb varied with the organic modifier (Table 2). With ACN, the elution order was the same as that on octadecyl-bonded silica, whereas it was different with MeOH.

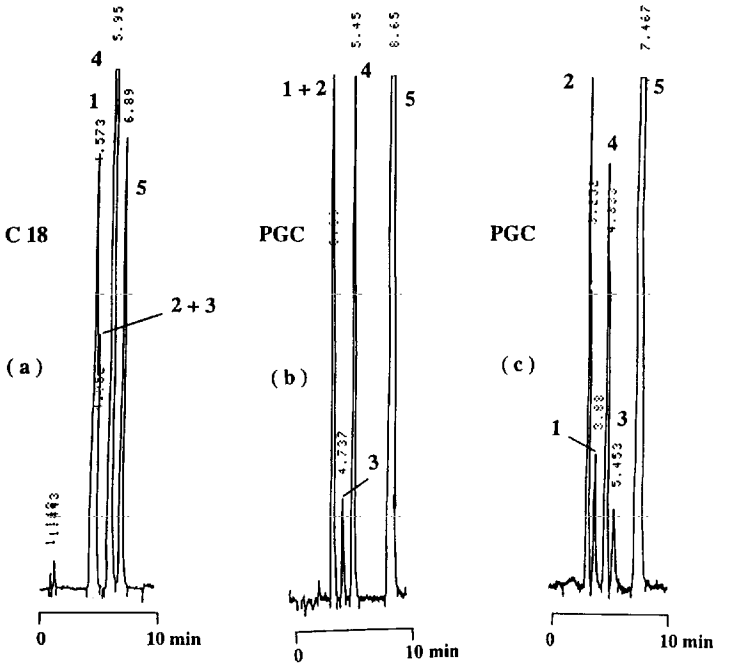


Fig. 5. Separation of five octylglycosides. Column, mobile phase: (a) Zorbax ODS (150×4.6 I.D. mm), ACN–water (35: 65); (b) Hypercarb S (100×4.6 I.D. mm), ACN–water (40: 60); (c) Hypercarb S (100×4.6 I.D. mm), MeOH–water (95: 5). Flow-rate: 1 ml min^{-1}; evaporative light scattering detection. Elution order: 1, C_8-Oβgal; 2, C_8-Oαglu; 3, C_8-Oβglu; 4, C_8-Cβglu; 5, C_8-Sβglu.

The only difference between C_8-Oβglu, C_8-Cβglu and C_8-Sβglu is the nature of the bond between sugar and alkyl chain. Whatever the nature of modifier (ACN or MeOH) in the mobile phase, the order of retention on a PGC support as on Zorbax ODS or other reversed-phase materials [10] is C_8-Oβglu<C_8-Sβglu. The presence of S-bonding decreases the hydrophilicity of the polar moiety and, thus, increases the retention in accordance with a reversed-phase mechanism. The highest value of relative retention α between these two compounds (Table 2) was obtained on Hypercarb with ACN–water as eluent. On Zorbax ODS, as on Hypercarb with ACN, the retention of C_8-Cβglu is greater than

Table 2
Comparison between capacity factors (k') and relative retentions (α) of five octylglycosides on a Zorbax ODS column (150×4.6 I.D. mm) and on a Hypercarb S column (100×4.6 mm I.D. mm) with different eluents

Chromatographic system	k'					α		
	C_8-Oβgal	C_8-Oαglu	C_8-Oβglu	C_8-Cβglu	C_8-Sβglu	C_8-Oαglu/C_8-Oβglu	C_8-Oβgal/C_8-Oβglu	C8-Oβglu/C8-Sβglu
Zorbax ODS								
35% ACN	3.01	3.19	3.28	4.22	5.04	1.03	1.09	1.54
Hypercarb								
40% ACN	1.90	2.02	2.62	3.16	5.60	1.30	1.38	2.14
95% MeOH	1.96	1.48	3.16	2.63	4.7	2.13	1.61	1.49

$\alpha_{1,2}=k'_2/k'_1$.

that of C_8-Oβglu, so the elimination of the O-bonding between the alkyl chain and the sugar moiety leads to increased hydrophobicity of the corresponding alkylglycoside. With MeOH–water as mobile phase, the elution order was C_8-Cβglu<C_8-Oβglu. These results further support the idea that ACN rather than MeOH favours the separation of alkylglycosides by hydrophobic interaction with the PGC support.

C_8-Oβglu and C_8-Oαglu are two anomer forms of the *n*-octyl-D-glucopyranoside. By comparing their retention, it was established that on octadecyl-bonded silica no anomeric separation was observed; however, as for the underivatized carbohydrates [19], with the PGC support two anomers could be separated. The elution order (C_8-Oαglu<C_8-Oβglu) was opposite to that observed with the underivatized carbohydrates anomers. The highest value for selectivity between these compounds was obtained with MeOH as organic modifier.

By comparing the retention of C_8-Oβgal and C_8-Oβglu (Table 2) it was proved that two compounds which differ only by the spatial configuration (axial or equatorial) of a hydroxyl group at the C-4 position of the sugar moiety were separated only on a PGC support. With ACN–water as mobile phase, C_8-Oβgal and C_8-Oαglu were co-eluted, whereas with MeOH–water as mobile phase a higher value of α(C_8-Oβgal/C_8-Oβglu) and a satisfactory separation of the five octylglycosides were observed. These results confirm that MeOH favours the separation of alkylglycosides which have closely related structures of their sugar moiety.

4. Conclusion

The PGC column has a great potential for alkylglycoside separation and purification. The elution order of these non-ionic detergents is quite different from that on reversed phases, as the retention on Hypercarb was shown to increase with increasing molecular size (either by increasing the length of the alkyl chain or by increasing the sugar moiety size). In addition, the nature of the modifier determines the predominant separation process: with methanol as organic modifier, the main parameters are the glucoside nature and the spatial configuration of the molecule, while with acetonitrile, it is the molecule hydrophobicity.

The chromatographic system consisitng of a Hypercarb S column and a mobile phase [MeOH–water (95:5) or ACN–water (40:60)] was a suitable system to separate and quantify two anomer alkylglycosides.

Acknowledgments

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