Talanta 81 (2010) 1281–1287

Contents lists available at ScienceDirect

Talanta

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

Interest of a chemometric approach in understanding the retention behaviour of three columns in hydrophilic interaction liquid chromatography: Application to the separation of glycerol carbonate, glycerol and urea

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ARTICLE INFO

Article history:
Received 19 October 2009
Received in revised form 26 January 2010
Accepted 7 February 2010
Available online 13 February 2010

Keywords:
Hydrophilic interaction liquid chromatography
Retention behaviour
Resolution
Mixture design
Desirability function
Glycerol
Urea

ABSTRACT

A chemometric approach was used to study the retention behaviour of glycerol, urea and glycerol carbonate in hydrophilic interaction liquid chromatography (HILIC). First, a simplex method was developed to optimize the sensitivity of an evaporative light scattering detector. A mixture design was then applied to model retention factors as a function of the mobile phase content in acetonitrile, water and methanol on three columns: Atlantis HILIC Silica, ZIC-HILIC and Monochrom diol. Atlantis HILIC Silica exhibits predominantly hydrophobic interactions, while retention on the other two columns is mainly ruled by hydrophilic interactions. Finally, a desirability function is applied on the resolution factors. The use of this function enables the compositions of eluent phases to be determined in order to achieve separation between the three chemicals. Monochrom diol proved to be the most efficient column.

1. Introduction

The mechanism of hydrophilic interaction liquid chromatography (HILIC), first proposed by Alpert in 1990 [1], is a variant of normal phase chromatography which differs however from other variants in that it does not include the adsorption of analytes directly in stationary phase [2]. Rather, the mobile phase forms a water-rich layer on the surface of the highly polar stationary phase, creating a liquid–liquid partitioning [3]. The analytes are distributed between the water-rich stationary layer with high-aqueous contents and the mobile phase with mostly organic contents. HILIC mobile phase therefore forms an aqueous–organic mixture such as water–methanol (MeOH) and water–acetonitrile (MeCN) with a majority of organic solvent [4]. Generally, the stationary phases used in HILIC are silica-based and can roughly be divided into bare silica, polar neutral, diol-bounded, amide-bonded, positively charged amine-bonded or anion-exchange, negatively charged cation-exchange and zwitterionic phases, i.e., phases able to create aqueous phase by solvation [5]. The aim of this study is to compare the HILIC behaviour of three major stationary phases used with Atlantis HILIC Silica column (bare silica) [6], ZIC-HILIC column (zwitterionic) [7] and Monochrom diol column (diol-bonded) [8]. In order to study the behaviour of each stationary phase, an experimental design approach was applied comprising a three components mixture design with constraints and the D-optimality criterion. This allows the evolution of the retention and resolution factors of polar compounds with respect to changes in mobile phase composition to be determined. A multi-response optimization was applied with a desirability function on resolution in order to determine the mobile phase composition capable of separating the analytes [9]. Polar compounds were selected as model compounds: glycerol carbonate (GC), glycerol (Gly) and urea. GC is a molecule with high added value, used mainly in organic chemistry to develop more complex chemicals. One way of producing this compound is carbonylation of Gly by urea to obtain GC and ammonia [10]. Moreover no analytical method to quantify simultaneously these three compounds exists in the literature. As they do not contain any chromophores, they can be studied by using an evaporative light scattering detector (ELSD). Optimization of the detection method was accomplished by using a simplex method.

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doi:10.1016/j.talanta.2010.02.021

2. Experimental

2.1. Chemicals

Glycerol (99.5% ACS), GC and urea (99.0%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC gradient grade), bidistilled water and methanol (HPLC gradient grade) were purchased from Carlo-Erba (Milano, Italy).

2.2. Apparatus

Separations were performed by using a Varian chromatography system consisting in a Prostar ternary pump operating at 1.0 mL min^{-1} with Monochrom diol and ZIC-HILIC columns and 0.2 mL min^{-1} with Atlantis HILIC Silica column. The mobile phase was firstly degassed in a membrane degasser (Thermo separation products, Thermo Fisher Scientific, Waltham, MA, USA) and injected by an auto sampler (Spectra AS 3000 from Thermo Separation Products) with a 5 μL injection loop. The column was placed in a Croco-cil® oven (Cil Cluzean Info Labo, Courbevoie, France) set at 25 °C. The detector was an ELSD Varian 385-LC Ice (Varian, San Fernando, CA, USA). Acquisition of chromatograms was performed by Star software (version 6.41, Varian) via an analog to digital converter Star 800 (Varian). The three columns used have the following characteristics: Atlantis HILIC Silica column (Waters, Milford, MA, USA) (particle size 3 μm, 150 mm × 2.1 mm I.D.), ZIC-HILIC column (Merck, Darmstadt, Germany) (particle size 5 μm, 150 mm × 4.6 mm I.D.) and Monochrom diol column (Varian) (particle size 5 μm, 250 mm × 4.6 mm I.D.).

2.3. Working solutions

Working solutions were prepared from a stock solution containing 1563 ppm of Gly, 1390 ppm of urea and 1795 ppm of GC in MeCN. This stock solution was diluted 3.6 times in a mixture of MeCN, water and MeOH according to the proportion of mobile phase studied.

2.4. Simplex method

The simplex method is a direct method used to determine an optimum of a response by varying the level of variables in such a way that the operating conditions, for a given experiment, are deduced from the results of the preceding experiments [11,12]. The evolution is based on an initial simplex composed of $k+1$ experiments where k is the number of controlled variables. In our case, three variables were chosen as the three available parameters of the ELSD: nebulization and evaporation temperatures and gas flow. A three dimensional simplex has four vertices represented by a tetrahedron. The experiments which constitute the first simplex were voluntarily chosen within the boundary of the experimental domain in order to progress inside the domain. After the initial experiments which compose the initial simplex its evolution occurs in a sequential order with the addition of a new experiment, performed in the opposite direction to the worst point (w). Calculation of the reduced coordinates of the new point was based on the following equation:

$$X_r = X_g + \alpha(X_g - X_w) \tag{1}$$

where X_r is the reduced coordinates of the new point; X_g is the centroid of the remaining trials; X_w is the ejected trial; and α is the coefficient of expansion ($\alpha > 0$) or contraction ($\alpha < 0$) [13]. The optimization process ends when the objective is reached or the responses cannot be further improved. In this case, the response must be represented by the same best point of $k+1$ simplices. This point is repeated to confirm this result.

Table 1
Experiment matrix for mixture design (volume content in %).

Experiment number	Water (%)	MeCN (%)	Methanol (%)
1	5	95	0
2	30	70	0
3	30	42	28
4	20	42	38
5	05	57	38
6	18	61	21
7	18	82	0
8	5	76	19
9	30	56	14

2.5. Design of experiments

In order to study the behaviour of compounds on the three columns, the evolutions of resolution and retention factors were modeled by a Design of Experiment (DOE), applying a response surface methodology and desirability approach [14]. To achieve this purpose, a three components mixture design with constraints and D-optimality criterion was performed for each column [15].

The three typical HILIC solvents were chosen for the mobile phase: water, MeCN and MeOH. Due to the constraints of HILIC, the volume content of water was between 5% and 30%. Moreover, MeOH was used to increase the polarity of the mobile phase without increasing the aqueous part and to improve solubility of the analytes. However MeOH is considered as a strong elution solvent and its rate was weaker than that of MeCN. The volume contents of MeOH were therefore studied between 0% and 38% and MeCN between 42% and 95%.

The models were compared using ANOVA (Analysis of Variance) and by calculating standard errors for each model coefficient and the corrected correlation coefficient. Nine experiments were carried out so as to obtain all the coefficients for the special cubic model. The experimental matrix of these trials was determined by Statgraphics® centurion XV software (version 15.2.06, Sigma Plus, Toulouse, France) with the D-optimality criterion (Table 1). With a simplex lattice design, all the experiments were at the boundaries of the experimental domain, hence the D-optimality criterion was used because it needs one experiment less and experiment 6 was inside the experimental domain. Each experiment was carried out 3 times and the 27 experiments were all used to determine the models.

Several responses were studied to assess the best experimental conditions: the retention factors of GC (k_{GC}), Gly (k_{Gly}) and urea (k_{urea}) and the resolution factors between GC and Gly ($R_{S\,GC/Gly}$), GC and urea ($R_{S\,GC/urea}$) and Gly and urea ($R_{S\,Gly/urea}$).

The three compounds were considered separated when the three R_S were at least 1.5. In order to combine the three R_S into a single function which can be maximized, the desirability function approach was used for the determination of the composition of mobile phase to optimize the separation of the three compounds. The method consists in converting each measured response to a dimensionless desirability scale d_i defined by a partial desirability function [9]. For each resolution factor, d_i is defined by:

$$d_i = 0 \quad \text{for}\ \hat{y} < l; \qquad d_i = \left(\frac{\hat{y}-l}{h-l}\right)^s \quad \text{for}\ l \le \hat{y} \le h; \qquad d_i = 1 \quad \text{for}\ \hat{y} > h \tag{2}$$

where $\hat{y}$ is the predicted value of the model; l is the lowest limit under which the response is unacceptable; h is the value from which the response is correct; and s is a coefficient which depends on the shape of the function.

In our case, a low limit of the resolution factor, l, is chosen at 1.2 for which peaks begin to be separated and a high limit, h, at 1.5

Table 2
Optimization of ELSD sensitivity by the simplex method.

Experiment number	Reduced coordinates (real coordinates)			Response			Simplex number				
	T_{Evap} (°C)	T_{Neb} (°C)	F_{Gas} (SLM)	A_{GC}	A_{Gly}	A_{urea}	1	2	3	4	5
A	1.00 (80)	1.00 (90)	0.00 (0.90)	8320	np	2730	1 (b)	2	2	2	2
B	1.00 (80)	0.00 (30)	0.00 (0.90)	675	np	2583	2	3	3	4 (w)	
C	0.00 (10)	1.00 (90)	0.00 (0.90)		Overrange		4 (w)				
D	0.66 (57)	0.66 (70)	1.00 (3.00)	np	np	3132	3	4 (w)			
E_1	0.44 (41)	0.78 (77)	0.17 (1.25)	455,166	294,678	1,362,853		1 (b)	1 (b)	1 (b)	1 (b)
F	0.74 (62)	0.63 (68)	0.53 (2.01)	1152	np	42,646			4 (w)		
G	0.78 (64)	0.61 (67)	0.29 (1.51)	6192	np	355,261				3	3
H	0.87 (71)	0.40 (54)	0.08 (1.06)	5373	np	32,048					4 (w)
E_2	0.44 (41)	0.78 (77)	0.17 (1.25)	442,730	274,993	1,228,974					

np, no peak; T_{Evap}, evaporation temperature; T_{Neb}, nebulization temperature; F_{Gas}, gas flow; SLM, standard litre/minute; A_{GC}, A_{Gly} and A_{urea}, area respectively of glycerol carbonate, glycerol and urea; b, best experiment; w, worst experiment.

which ensures the separation of peaks. The value of s was chosen at 0.2 so as to rapidly reach a plateau. The global desirability function, D, is calculated by determining the geometric mean of individual desirabilities using Eq. (4) and its value is set between 0 (when one or more R_S are weaker than 1.2) and 1 (when the three R_S are higher than 1.5).

$$D = (d_1 d_2 d_3)^{1/3} \tag{3}$$

3. Results and discussion

3.1. Optimization of detector parameters with simplex method

The sensitivity of the detector depends on the levels set for the ELSD parameters. With the ELSD used, three parameters can be selected: nebulization temperature, T_{Neb}; evaporation temperature, T_{Evap}; and gas flow, F_{Gas}. Their ranges are respectively:

$$10\ {}^\circ\mathrm{C} \leq T_{Evap} \leq 80\,{}^\circ\mathrm{C} \tag{4}$$

$$30\,{}^\circ\mathrm{C} \leq T_{Neb} \leq 90\,{}^\circ\mathrm{C} \tag{5}$$

$$0.90\,\mathrm{SLM} \leq F_{Gas} \leq 3.00\,\mathrm{SLM} \quad (\mathrm{SLM} = \text{standard litre/minute}) \tag{6}$$

Sensitivity was represented by the areas of peaks for a specific concentration of GC, Gly and urea, respectively A_{GC}, A_{Gly} and A_{urea}. The aim of the simplex was to obtain the highest area for each peak. With three parameters, the first simplex comprised of four experiments (A, B, C and D) which were placed at the extreme limits of temperatures and gas flow. For experiment D, reduced coordinates of T_{Evap} and T_{Neb} were the average of A, B and C and the upper level of gas flow was chosen. Evolutions of the simplex were calculated with reduced coordinates which are shown in Table 2; real coordinates are given in brackets.

To remain inside the domain, contractions with $\alpha = -1/2$ were performed. Evolutions of the simplex are given in Table 2; each response is the average of three results.

For similar concentrations of each compound, A_{Gly} is the lowest area, therefore it was the limiting response and was selected as critical for optimization. In cases where A_{Gly} remained the same over several experiments, A_{GC}, the second lowest area, was used to determine the simplex evolution. In each simplex, responses were classified between 1 and 4, experiment 1 being the best (b) and 4 the worst (w). Experiment E_1 was the best during four $(k+1)$ successive simplices (2, 3, 4 and 5), and can therefore be considered as an optimum. This experiment was repeated to confirm this result, E_2. The experimental values of E are $T_{Evap} = 41\,{}^\circ\mathrm{C}$, $T_{Neb} = 77\,{}^\circ\mathrm{C}$ and $F_{Gas} = 1.25$ SLM.

Using the simplex method, one optimum can be determined in the experimental domain. As the latter may however contain several optima, to ensure that another optimum with a better response does not exist, a second simplex was applied with a new strategy by beginning from point E and moving away to determine the evolution of the response. A new initial area was thus defined centred on point E and with smaller dimensions than the previous experimental domain, with a view to evolving outside this initial area. The scale for this new area was about half of the experimental domain with limits of this experimental domain. Reduced and real coordinates are shown in Table 3. In order to move away from point E, projections of α above or equal to 1 were required. When projections of the worst point were outside the experimental domain, a projection of the second worst point was done. Results are given in Table 3.

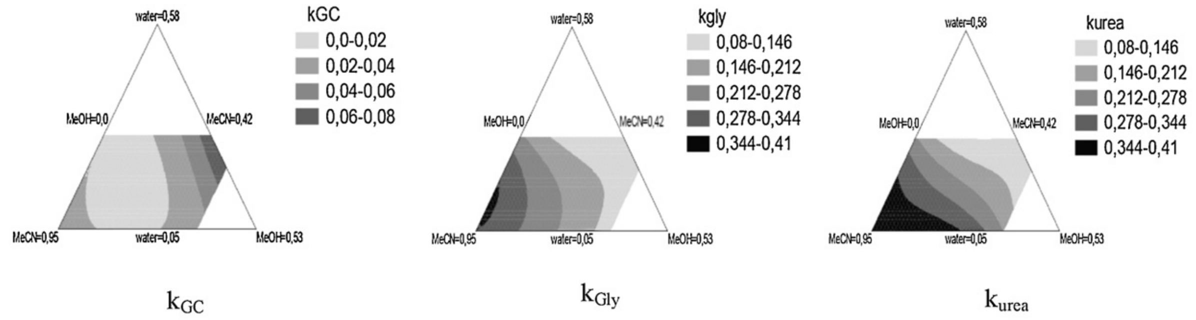
With this second simplex, another optimum was found: experiment E'_1 was the best point of four $(k+1)$ successive simplices (2′, 3′, 4′ and 5′) and was successfully repeated, E'_2. In these conditions, $T_{Evap} = 27\,{}^\circ\mathrm{C}$, $T_{Neb} = 65\,{}^\circ\mathrm{C}$ and $F_{Gas} = 1.19$ SLM, the sensitivity of each compound was significantly increased by comparison with the results of experiment E_1 by a factor of 2.4, 3.5, and 1.1 for GC, Gly and urea respectively. All the experimental area was explored; the point E' therefore represents the best conditions to obtain the highest sensitivity of the ELSD. These conditions were applied to all further experiments.

Table 3
Second optimization of ELSD sensitivity.

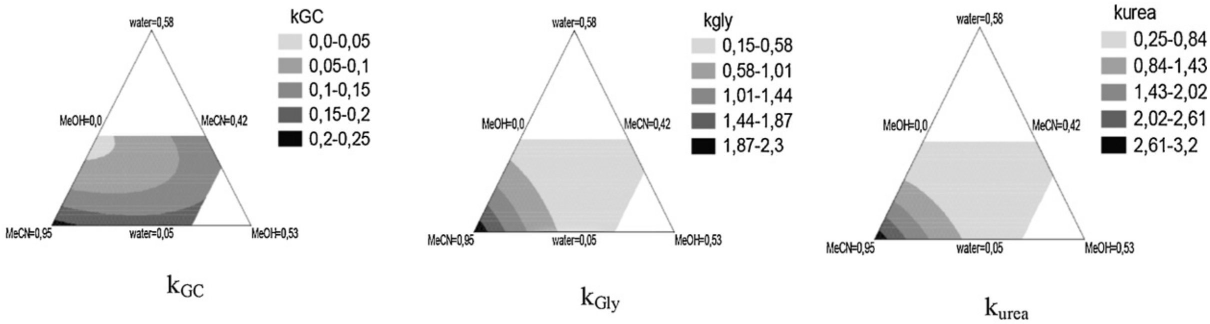
Experiment number	Reduced coordinates (real coordinates)			Response			Simplex number				
	T_{Evap} (°C)	T_{Neb} (°C)	F_{Gas} (SLM)	A_{GC}	A_{Gly}	A_{urea}	1′	2′	3′	4′	5′
A′	1.00 (59)	1.00 (90)	1.00 (1.78)	24,624	1741	221,628	3 (p)				
B′	1.00 (59)	0.00 (62)	1.00 (1.78)	21,368	1078	194,007	4 (w)	4 (w)	4 (w)	4 (w)	
C′	0.00 (23)	1.00 (90)	1.00 (1.78)	710,061	689,643	878,169	1 (b)	2	2	2	2
D′	0.66 (47)	0.66 (81)	0.00 (0.90)	151,266	55,054	843,628	2	3 (p)			
E'_1	0.11 (27)	0.11 (65)	0.33 (1.19)	1,091,535	1,070,477	1,522,239		1 (b)	1 (b)	1 (b)	1 (b)
F′	0.07 (26)	0.07 (64)	1.55 (2.27)	29,199	25,301	44,475			3 (p)		
G′	0.53 (42)	0.20 (68)	0.98 (1.76)	228,401	129,855	625,320				3	4 (w)
H′	0.35 (36)	0.80 (84)	−0.01 (0.89)	1,120,319	469,032	1,177,204					3
E'_2	0.11 (27)	0.11 (65)	0.33 (1.19)	1,050,460	936,192	1,403,943					

np, no peak; T_{Evap}, evaporation temperature; T_{Neb}, nebulization temperature; F_{Gas}, gas flow; SLM, standard litre/minute; A_{GC}, A_{Gly} and A_{urea}, area respectively of glycerol carbonate, glycerol and urea; b, best experiment; p, projected experiment; w, worst experiment.

(a) Atlantis Hilic Silica column, f = 0.2 mL.min^{-1}.



(b) ZIC-HILIC column, f = 1.0 mL.min^{-1}.



(c) Monochrom diol column, f = 1.0 mL.min^{-1}.

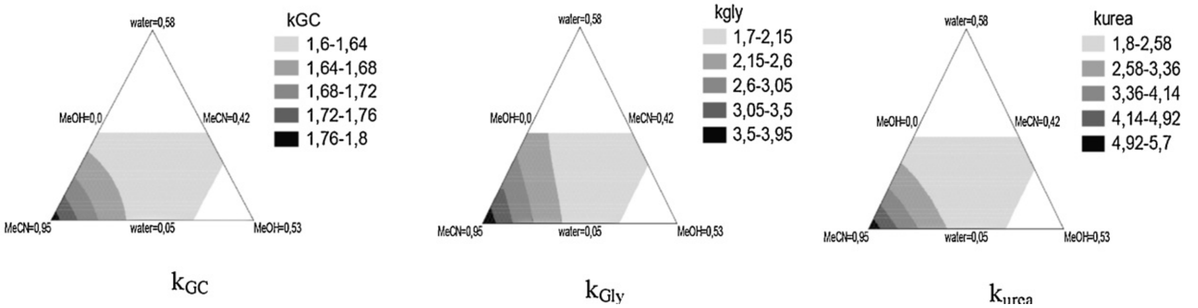


Fig. 1. Evolution of retention factors of glycerol carbonate, glycerol and urea as a function of eluent composition on three stationary phases, detected by ELSD (T_{Evap} = 27 °C, T_{Neb} = 65 °C and F_{Gas} = 1.19 SLM).

3.2. Behaviour of three columns with mixture design

The behaviour of the three columns (Atlantis HILIC Silica, ZIC-HILIC and Monochrom diol) was studied by using a design of experiment (mixture design), to determine the evolution of the three retention factors and the three resolution factors corresponding to the separation of GC, Gly and urea.

3.2.1. Retention behaviour

By using the results of the previously described experimental matrix, models for all the parameters were determined and the best corrected correlation coefficients were obtained in all cases for special cubic models. Their values were above 0.95 except for the model describing the retention factors on the Atlantis HILIC Silica column, coefficient which was above 0.90. The corresponding response surfaces, obtained from model parameters, were used to study retention behaviour (Fig. 1) [16].

For the three columns, the elution order is GC, Gly and finally urea. Typically in HILIC, the retention factor is directly correlated to the polarity of the compounds [1]. In agreement with the polarity of the analytes, GC is the first eluted compound. The result is in accordance with its value of log P: −0.11 against −1.30 for Urea and −1.98 for Gly. Therefore GC which should be the most retained analyte, is in fact eluted before urea. This inversion in retention orders suggests that retention is not only governed by the classical partition phenomenon usually observed in HILIC, but that other retention mechanisms sometimes described in HILIC separations such as adsorption, hydrogen bonding and hydrophilic interactions probably occur [17].

The response surfaces reveal different kinds of behaviour. A classical HILIC behaviour presents a retention directly correlated to the MeCN content of the mobile phase. This describes a partition mechanism between a water-rich layer on the stationary phase and the mobile phase. It can be seen that this retention mechanism is predominant when retention factors are higher than 1, indicating that this mechanism implies good retention. When retention factors are lower than 1 other retention mechanisms can be involved. This can be observed mainly for the Atlantis HILIC Silica column. For example, the retention of GC on this column versus MeCN content clearly describes a typical 'U-shape' curve. This implies that with a low

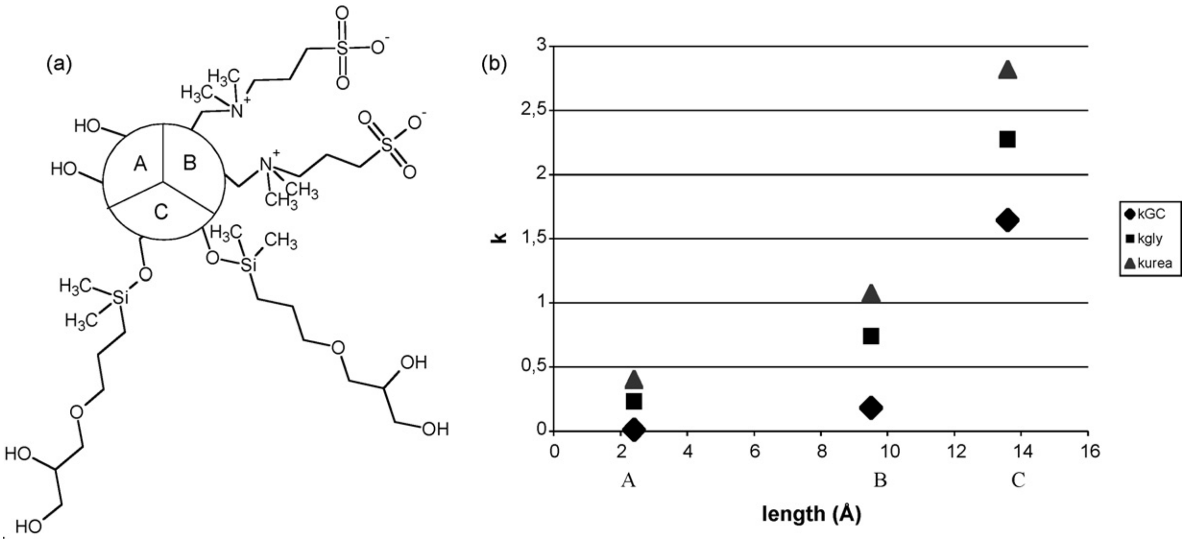


Fig. 2. (a) Nature of functional groups grafted on stationary phase of Atlantis HILIC Silica column (A), ZIC-HILIC column (B) and Monochrom diol column (C). (b) Evolution of retention factor versus the chain length of stationary phase with acetonitrile–water–methanol (v/v/v: 76/5/19; $f = 0.2\,\mathrm{mL\,min^{-1}}$ for A and $f = 1.0\,\mathrm{mL\,min^{-1}}$ for B and C) detected by ELSD ($T_{\mathrm{Evap}} = 27\,^{\circ}\mathrm{C}$, $T_{\mathrm{Neb}} = 65\,^{\circ}\mathrm{C}$ and $F_{\mathrm{Gas}} = 1.19$ SLM).

MeCN content in mobile phase, GC is retained (to a small extent) on the stationary phase by hydrophobic interactions [4]. On the contrary, with a higher MeCN content, retention is increased by the partition mechanism. To a lesser extent this behaviour is also observed for GC on the ZIC-HILIC column. In this case the partition mechanism is predominant, again implying higher retention factors.

A classical partition interaction depends on two equilibria. The first is between the mobile phase and the water layer created on the column surface, which implies hydrophilic interactions. These interactions include predominantly hydrogen bonding depending on the acidity or the basicity of the solutes, electrostatic interactions, and dipole–dipole interactions based on the dipole moments and polarizabilities of molecules [2]. The second equilibrium is between the water layer and the stationary phase support, which implies an adsorption phenomenon. The water layer is induced by the solvation of polar groups grafted onto the stationary phase. The three columns are based on silica bonded with different chains as shown in Fig. 2a. The Atlantis HILIC Silica column is composed of bare silica, the ZIC-HILIC column presents a grafted chain composed of an anion and a cation, and the Monochrom diol column by a carbonated chain containing two hydroxyl groups. Considering that the thickness of the water layer corresponds to the length of each column from the silica base, the values determined by Chemsketch software are 2.4 Å (Atlantis HILIC Silica), 9.5 Å (ZIC-HILIC) and 13.6 Å (Monochrom diol). For the GC molecule, the polarity is weak, so hydrophilic interactions are weaker than for Gly and urea. Considering that the analytes interact preponderantly at the interface between the water layer and the mobile phase, for GC on the Atlantis column, the molecule is so large (maximum length: 6.7 Å) that it can interact with the silica base. Therefore the retention of GC on Atlantis HILIC Silica column seems to be regulated by a mixed mode of partition and adsorption as shown by Fig. 1. The lowering of the partition part can be related to a thinner water layer, which also implies less diffusion in this layer. The higher polarity of the other two compounds and their sizes mean that hydrophilic interactions predominate. For the ZIC-HILIC and Monochrom diol columns, the thickness of the water layer is too great and hence the partition phenomenon is mainly preponderant.

For each compound, the values of k are the highest for the Monochrom diol column, followed by the ZIC-HILIC column and finally the Atlantis HILIC Silica column, whatever the mobile phase composition. As explained above, retention on the three columns is predominantly ruled by hydrophilic interactions with the water layer. In this case, Fig. 2b, the longer the chain is, the greater the retention and the thicker the water layer. This confirms that the thickness of the water layer depends on the length of the grafted chain.

3.2.2. Desirability

The models of resolution factors are not shown individually; all have been found to correspond to the special cubic model, meaning that interactions between the three solvents in mobile phase were statistically significant with a corrected correlation coefficient above 0.95. The evolution of the desirability function, which includes the resolution factors, is given in Fig. 3. In the domain where desirability is the best, the mobile phase composition which induces the best resolution for the three compounds is determined for each column and the corresponding chromatograms are shown in Fig. 3.

For the three columns, the similar polarities of Gly and urea render their separation the most difficult and entail that $R_{\mathrm{S\ Gly/urea}}$ plays the most important part in determining the desirability function.

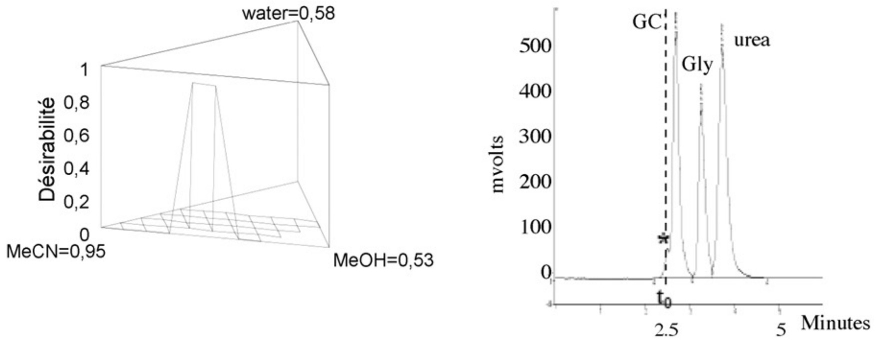
As the desirability of the Atlantis HILIC Silica column does not reach the value of 1 (Fig. 3a), the three compounds cannot be separated in the domain of mobile phase composition studied. On the ZIC-HILIC column, desirability is equal to 1 for mobile phase composed of less than 10% of water content whatever the amount of MeOH. The domain of the best desirability on the Monochrom diol column is similar to ZIC-HILIC, with a maximum between 10% and 15% of water for an MeOH amount above 8%.

The efficiencies of each compound on the three columns are presented in Table 4. The most efficient column is Monochrom diol and the least efficient is Atlantis HILIC Silica. As the Atlantis col-

Table 4
Efficiencies of the three compounds, GC, Gly and urea on the three columns (data obtained from the chromatograms shown in Fig. 3).

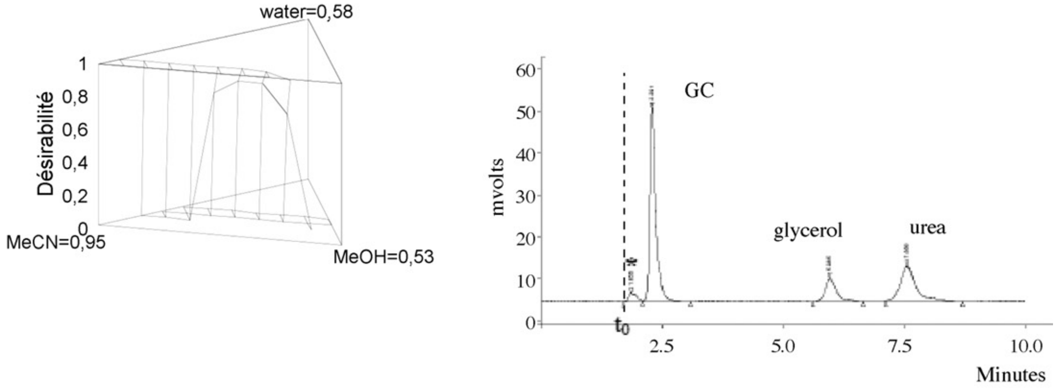
Column	Efficiency for GC (plates $\mathrm{m^{-1}}$)	Efficiency for Gly (plates $\mathrm{m^{-1}}$)	Efficiency for urea (plates $\mathrm{m^{-1}}$)
Atlantis HILIC Silica	12,517	15,053	12,755
ZIC-HILIC	22,810	28,020	31,779
Monochrom diol	37,740	64,360	60,999

(a) Atlantis Hilic Silica column, f = 0.2 mL.min^{-1}.



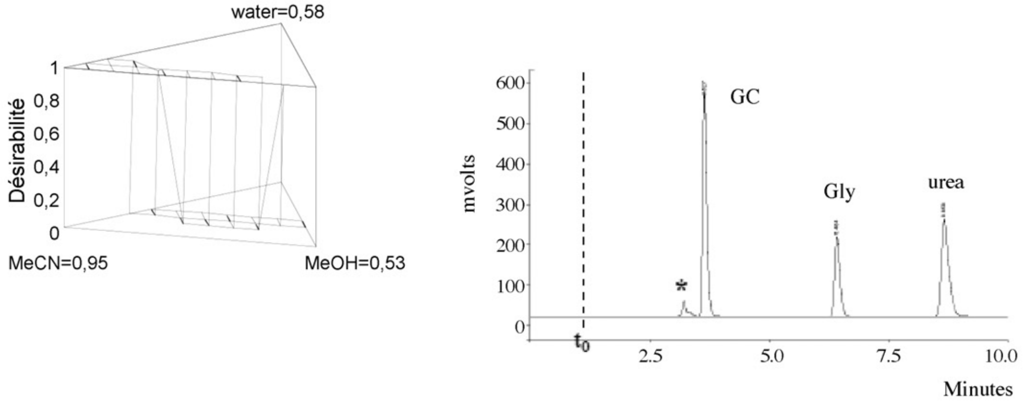
mobile phase MeCN-water-MeOH (v-v-v: 76-5-19)

(b) ZIC-HILIC column, f = 1.0 mL.min^{-1}.



mobile phase MeCN-water (v-v: 95-5)

(c) Monochrom diol column, f = 1.0 mL.min^{-1}.



mobile phase MeCN-water (v-v: 95-5)

Fig. 3. Desirability function of retention factors and chromatogram of glycerol carbonate (GC), glycerol (Gly), urea and an impurity (*) as a function of mobile phase composition, detected by ELSD (T_{Evap} = 27 °C, T_{Neb} = 65 °C and F_{Gas} = 1.19 SLM).

umn contains particles with the smallest size of 3 μm and the other two of 5 μm, it should have a better efficiency. This unexpected result can be assumed to result from the mixed mode of retention mechanism. Moreover the speed exchanges at the different equilibria can influence this efficiency, suggesting that exchanges in partition are quicker than in other mechanisms. When partition predominates, therefore, sharper peaks are obtained, as shown in the chromatograms in Fig. 3.

3.2.3. *Application to glycerol carbonate synthesis*

The results of this study can be applied to carry out the carbonylation kinetics of Gly by urea in GC. The best sensitivity of ELSD is used to determine a poor concentration of the product at the beginning of synthesis and of reagent at the end of the process. The column used is the Monochrom diol column as it provides the highest resolution factors. The peak of a synthesis intermediary was probably detected and

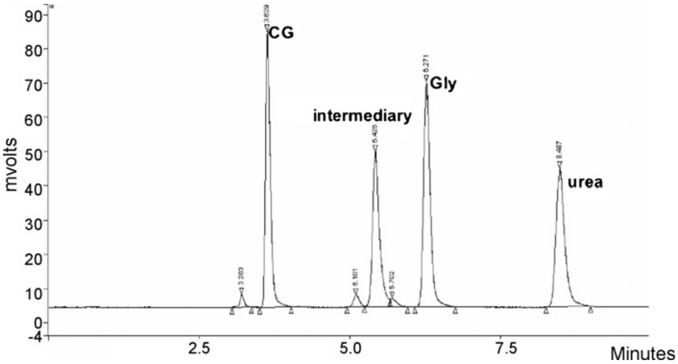


Fig. 4. Chromatogram of sample of carbonylation of Gly by urea on Monochrom diol column, mobile phase MeCN–water (v/v: 95/5; f = 1.0 mL min^{-1}), detected by ELSD (T_{Evap} = 27 °C, T_{Neb} = 65 °C and F_{Gas} = 1.19 SLM).

separated from other peaks as shown in the chromatogram in Fig. 4.

4. Conclusions

Chemometric approaches have enabled the development of an analytical method and the study of the behaviour of three columns with three polar compounds in HILIC. Firstly detection of the chemicals was optimized by a simplex method to obtain the best sensitivity of an ELSD for Gly, urea and GC. Two different kinds of evolution were applied. The first, by contraction, makes it possible to evolve rapidly inside the domain towards an optimum and the second to improve the response.

DOE, and specifically mixture design, was then applied and revealed the retention behaviour of polar compounds on the three columns. Moreover it established that retention depends on the length of the water layer which is induced by the length of the functional group grafted on silica. The Monochrom diol column, which has the greatest length, exhibited the best retention, followed by the ZIC-HILIC column and finally the Atlantis HILIC Silica column. Finally the separation of three compounds was studied by the desirability function based on three resolution factors and has highlighted the domains of mobile phase composition in which separation of the peaks of the three analytes was observed.

Resolution of these three compounds was optimal with the Monochrom diol column for a mobile phase composed of 95% MeCN and 5% water. These conditions were checked by analysing a sample of reaction mixture of glycerol carbonate synthesis and confirmed that the analytical method can be employed for the simultaneous quantification of glycerol, urea and glycerol carbonate.

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