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Simultaneous analysis of intact and desulfated glucosinolates with a porous graphitized carbon column

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Abstract

A porous graphitized column was evaluated for the qualitative analysis of glucosinolates and desulfoglucosinolates. Successful separations were achieved using 0.1% aqueous trifluoroacetic acid–acetonitrile (85:15, v/v) as eluent. The selectivity obtained by LC on Hypercarb S allows compounds with a slight hydrophobic structure difference (e.g., a difference of one CH_2 unit between two glucosinolates) to be satisfactorily resolved. Excellent separation of the two diastereoisomers progoitrin and epiprogoitrin in anionic or neutral form was observed.

Keywords: Stationary phases, LC; Porous graphitized carbon; Mobile-phase composition; Glucosinolates; Desulfoglucosinolates

1. Introduction

Glucosinolates are thioglucosides, an important and unique class of secondary plant compounds found in the seeds, roots, stems and leaves of plants. They are mainly responsible for the flavour and physiological activity of the Cruciferae family. Well over 100 glucosinolates have now been described [1,2]. Purified and non-derivatized glucosinolates are needed as pure reference analytical standards but also in relatively large amounts as materials for plant, food and nutrition investigations.

All natural glucosinolates are anions containing β-D-thioglucose as the sugar component and generally show a Z configuration around the carbon–nitrogen double bond between the R and sulfate groups (Fig. 1a).

Some procedures, including mainly column chromatographic techniques with alumina acidic and gel ion-exchange columns [3–7], have been described for the separation and isolation of intact glucosinolates from plant materials, espe-

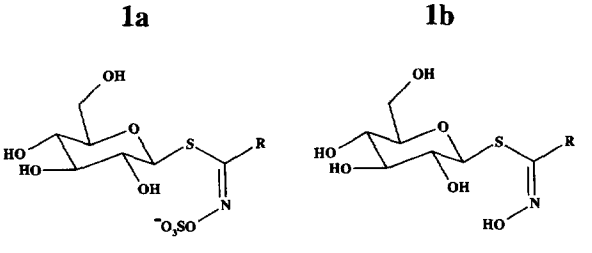

Fig. 1. General structure of glucosinolates. (a) Intact glucosinolate; (b) desulfated glucosinolate.

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cially seeds. It is difficult with these methods to obtain 100% pure individual glucosinolates even though seed materials containing predominantly the required glucosinolate are selected [8]. Hence additional purification of these extracts is needed.

For minor amounts (mg or g levels), separation of the compounds can be performed by liquid chromatography (LC) on a semi-preparative scale. Owing to the wide range of polarities of glucosinolates to be analysed and/or purified, there is a real need for several simple, reliable, complementary and alternative LC methods. Moreover, the selectivity of these LC systems must be carefully optimized with a view to their transposition to the preparative scale.

Few LC analytical systems have been investigated. Intact glucosinolates are usually separated using a reversed-phase ion-pair liquid chromatographic method with UV detection [9–11], but the presence of the ion-pairing agent, e.g., tetraheptylammonium bromide, in the eluent limits the use of this system to obtain pure individual glucosinolates. Separation on octadecyl-bonded silica using aqueous ammonium acetate–acetonitrile mixtures as the mobile phase has also been described [12]. Under these conditions, the indolylglucosinolates are highly retained and gradient elution is necessary to elute the more hydrophobic compounds.

Recently we have demonstrated that glucosinolates can be analysed by anion-exchange chromatography [13,14]. More retention and better selectivities were observed on a polymeric anion exchanger than on a silica anion exchanger [14]. The isocratic analysis of glucosinolates on a polymeric anion exchanger is a suitable and a convenient method that should be used as a complement to the current ion-pair chromatographic method.

Porous graphite is a novel high-performance packing material, initially developed for use in reversed-phase chromatography [15,16]. The uniqueness of porous graphitized carbon (PGC) compared with conventional reversed-phase materials is due to its delocalized band of electrons available for electronic interactions, especially donor–acceptor (charge-transfer) interactions and direct π-electron overlap [17]. The planar surfaces of the PGC Hypercarb S allows special stereoselectivity and specific separations of diastereoisomers have been observed on this type of support [16].

In this work, a porous graphitized carbon stationary phase was investigated as a possible additional column material providing an alternative separation potential to reversed-phase and anion-exchange packings for the LC analysis of glucosinolates. The retention behaviour of some typical intact glucosinolates and of their uncharged desulfo analogues, obtained by an enzymatic degradation, was investigated. The influence of different organic and electronic modifiers in the aqueous mobile phase was studied.

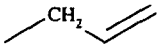
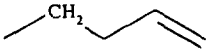
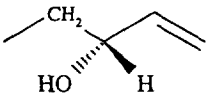
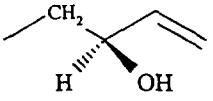
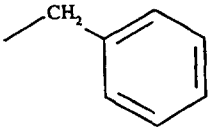
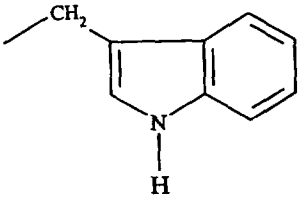
2. Experimental

Table 1 lists the glucosinolates studied. Sinigrin and glucotropaeolin were obtained from Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany), respectively. Gluconapin was purchased from Dr. A. Quinsac (Centre Technique Interprofessionnel des Oléagineux Métropolitains, Ardon, France). Progoitrin was extracted and isolated from rapeseed in our laboratory. Glucobrassicin and non-sulfated glucobrassicin were synthesized in our laboratory [18]. Epiprogoitrin was kindly provided by Dr. S. Palmieri (Istituto Sperimentale per le Colture Industriali, Bologna, Italy). The desulfoglucosinolates were prepared by enzymatic desulfation according to the method described for the seed extracts [19].

The liquid chromatographic apparatus consisted of a Varian (Palo Alto, CA, USA) Model 2010 solvent-delivery pump, a Rheodyne (Berkeley, CA, USA) Model 7125 injector with a 20-μl sample loop and a Varian Model 2550 UV spectrophotometric detector. The porous graphitized carbon column was Hypercarb S (100×4.6 mm I.D., particle size 7 μm) from Shandon Scientific (Runcorn, UK). Data were processed with a Shimadzu (Kyoto, Japan) CR 6A integrator/recorder.

The flow-rate was generally 1 ml/min, experiments were carried out at room temperature and the detection wavelength was 229 nm. To de-

Table 1
Natural glucosinolates studied

R group	Systematic name of R group	Trivial name of compound	Abbreviation
	2-Propenyl	Sinigrin	SIN
	3-Butenyl	Gluconapin	GNA
	(2*R*)-2-Hydroxy-3-butenyl	Progoitrin	PRO
	(2*S*)-2-Hydroxy-3-butenyl	Epiprogoitrin	EPIPRO
	Benzyl	Glucotropaeolin	GTL
	Indol-3-ylmethyl	Glucobrassicin	GBS

termine the column void volume, V_0, 20 μl of acetonitrile or methanol were injected in each system. Each glucosinolate was injected alone and in admixture in the chromatographic mixtures. The retention time of each compound was determined in each eluent by three consecutive determinations. The concentration of the standards were ca. 100 ppm in aqueous solution. Eluent constituents were purchased as follows: distilled water from the Cooperative Pharmaceutique Française (Melun, France); methanol (MeOH), Hipersolv for HPLC, from BDH (Poole, UK); acetonitrile (ACN), RS for preparative HPLC, from Carlo Erba (Milan, Italy); tetrahydrofuran (THF), Uvasol, trifluoroacetic acid (TFA) and perchloric acid ($HClO_4$) from Merck (Darmstadt, Germany). $NaClO_4$ and KH_2PO_4 were purchased from Aldrich (Steinheim, Germany).

3. Results and discussion

For this preliminary study, six natural glucosinolates were selected on the basis of the large diversity of their aglycone part, which can be alkenyl [sinigrin (SIN); gluconapin (GNA)], hydroxyalkenyl [progoitrin (PRO); epiprogoitrin

(EPIPRO)], arylalkyl [glucotropaeolin (GTL)] or indolylalkyl [glucobrassicin (GBS)]. The great diversity of the R groups leads to wide variations in the polarity and the hydrophilic and lipophilic characters of these natural compounds. The chromatographic study of these compounds should display the possible separation of glucosinolates in accordance with the three important subclasses, alkenyl, arylalkyl or indolylalkyl. Moreover, a resolution study of PRO and EPIPRO should be of particular interest because these compounds are two natural diastereoisomers often present together in variable proportions in plant material and they cannot be sufficiently separated by current analytical methods.

With a view to developing, as far as possible, simple and ordinary conditions for glucosinolate analysis, we first tested the elution strength of water and of different water–organic modifiers mixtures.

The PGC support appears to have a considerable retention capacity for glucosinolates. PRO, EPIPRO and SIN, the more hydrophilic anions, cannot be retained and separated on conventional silica-based reversed-phase materials or on polystyrene–divinylbenzene polymeric supports with water, aqueous acid solution or salt solution as eluent [20,21]; however, they were totally retained, like all the glucosinolates studied, on a PGC column with distilled water as mobile phase. Although there are no ion-exhange sites on PGC, total retention of glucosinolates with water as eluent can be due to specific electronic interactions between solutes and delocalized electrons on PGC, as reported for small organic anions [22]. Hence addition in the aqueous mobile phase of TFA, a common competing agent for electronic interaction chromatography [22,23], was investigated, but the mixture was not sufficient to elute glucosinolates. Addition of an organic modifier (ACN, MeOH or THF) in variable amounts in the aqueous eluent allows the elution of the glucosinolates but the elution peaks were broad and the retention times were not reproducible and decreased as the glucosinolates were injected. The presence of both organic and electronic modifiers in the mobile phase was necessary to elute the compounds with good efficiency. It was evident that both hydrophobic and electronic interactions take part in the retention mechanism. To evaluate the contribution of each type of interaction and also to optimize the chromatographic conditions for a good separation, it was of interest to compare the behaviour of ionic glucosinolates with that of their uncharged desulfo analogues. The desulfoglucosinolates (Fig. 1b) were obtained by enzymatic degradation of glucosinolates, which replaced the anionic sulfate group by a neutral hydroxyl group. As ionic glucosinolates, the desulfoglucosinolates were totally retained on the PGC column with distilled water as mobile phase. Addition of an organic modifier was necessary to elute these compounds. The presence of an electronic modifier in the eluent had no effect on retention. The suppression of ionization should greatly increase the hydrophobicity of these solutes in comparison with intact glucosinolates but should decrease considerably the possibility of electronic interactions with the PGC column. It was of interest to investigate special chromatographic conditions that would allow the simultaneous analysis of intact and desulfated glucosinolates.

3.1. Mobile phase: water–organic modifier mixture

Only the behaviours of desulfated glucosinolates were evaluated with these mobile phases. Fig. 2a and b depict typical chromatograms obtained with ACN–H_2O (12:88, v/v) and MeOH–H_2O (45:55, v/v), respectively, as mobile phase for a standard mixture of four desulfated glucosinolates. In Table 2, the capacity factors (k') and the selectivities in terms of the relative retention (α), calculated from the corresponding k' values, are reported for five desulfated glucosinolates using THF–H_2O, ACN–H_2O and MeOH–H_2O as mobile phase. Whatever be the organic modifier in the aqueous–organic eluent, Fig. 2 and Table 2 show that on a porous graphitized carbon column, the desulfated glucosinolates were eluted roughly in order of decreasing polarity (alkenyl- then arylalkyl-

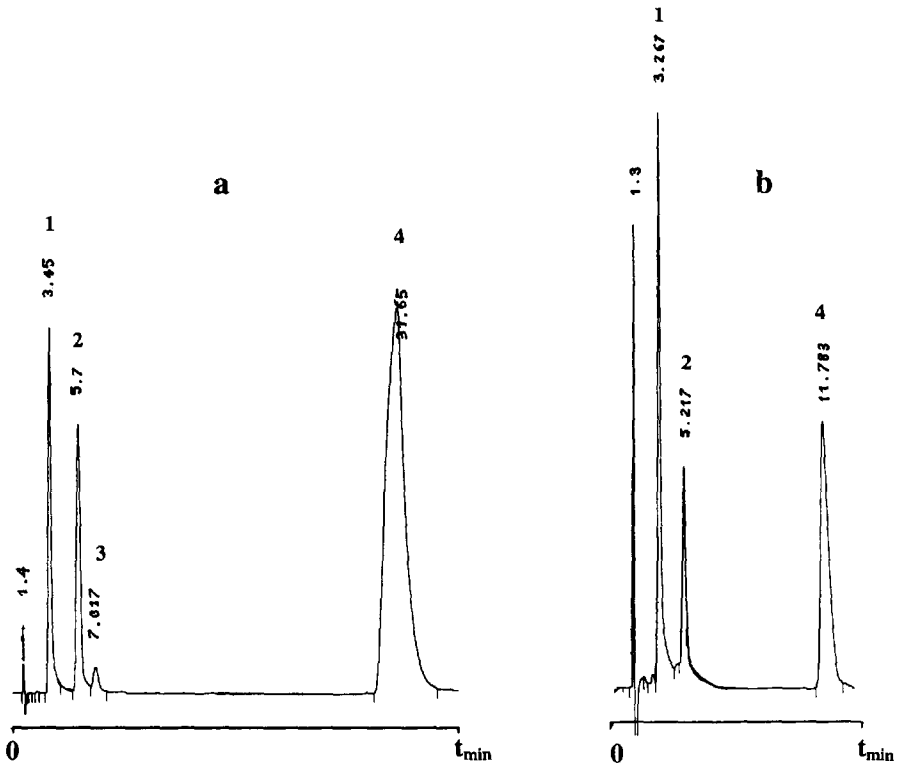


Fig. 2. Chromatograms of standard mixture of desulfated glucosinolates. Column, Hypercarb S (100 × 4.6 mm I.D.); flow-rate, 1 ml min^{-1}; detection, UV at 229 nm. (a) Eluent, ACN–H_2O (12:88); P = 100 bar. (b) Eluent: MeOH–H_2O (45:55); P = 150 bar. Elution order: 1 = desulfated progoitrin; 2 = desulfated epiprogoitrin; 3 = desulfated gluconapin; 4 = desulfated glucotropaeolin.

glucosinolates) as in reversed-phase chromatography [19,24,25]. The retention of a desulfated indolylglucosinolate, DS-GBS, was considerable in these systems. On a PGC column the percentage of organic modifier necessary to elute this compound was so high (with 75% of acetonitrile the capacity factor was only 31.5) that all alkenyl- and arylalkylglucosinolates were eluted in the void volume. Hence the chromatographic behaviour of DS-GBS cannot be studied at the same time as the other five glucosinolates; it was for this reason that the capacity factors of DS-GBS were not reported. However, from this specific behaviour, its is interesting that the simple chromatographic system consisting of a Hypercarb S column and ACN–H_2O (80:20, v/v) as eluent is suitable for isolating pure desulfated indolylglucosinolates from desulfated natural mixtures with all the alkenyl- and arylalkylglucosinolates eluted in the void volume.

High selectivity between glucosinolates was observed. Table 2 shows that the two diastereoisomers DS-PRO and DS-EPIPRO are well separated with good baseline resolution with each type of eluent. This result is interesting because these two compounds are eluted very close together in the official method for desulfated glucosinolates analysis [19] and at present the quantification of small amounts of one in the presence of a large proportion of the other is an obvious difficulty. Increasing the length of the aglycone R part by only one methylene unit in the case of the two desulfated alkenylglucosinolates DS-SIN and DS-GNA is sufficient to bring about significant retention variations between these compounds; an increase in polarity re-

Table 2
Capacity factors (k') and relative retentions (α) of five desulfated glucosinolates on a Hypercarb S column (100 × 4.6 mm I.D.) with three different aqueous–organic eluents

Eluent	Capacity factor, k'					Relative retention, α[a]			
	DS-SIN	DS-PRO	DS-EPIPRO	DS-GNA	DS-GTL	DS(SIN-GNA)	DS(PRO-GNA)	DS(PRO-EPIPRO)	DS(SIN-GTL)
THF–H_2O (5:95)	0.98	0.88	2.10	3.18	23.04	3.24	3.61	2.39	23.51
ACN–H_2O (12:88)	1.47	1.46	3.07	4.01	21.61	2.73	2.75	2.10	14.70
MeOH–H_2O (45:55)	1.05	1.51	3.01	3.02	8.06	2.88	2.00	2.00	7.68

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

sulting from the substitution of hydrogen by hydroxyl on the alkenyl R aglycone (DS-PRO–DS-GNA pair) brings about a great reduction in retention. The hydroxylated compound DS-PRO was eluted more rapidly than DS-GNA, as in reversed-phase chromatography [19,24,25].

The order of elution power was THF > ACN > MeOH, as is well known in reversed-phase chromatography [26]. The three organic solvents led to differences in the selectivities but not in the efficiencies: the number of theoretical plates was about 80 000/m for DS-SIN and 120 000/m for DS-GTL, which were common values observed with this PGC column. The selectivity becomes excellent when THF and ACN are used to separate DS-EPIPRO–DS-GNA ($\alpha = 1.51$ with THF and $\alpha = 1.31$ with ACN), which is impossible with MeOH. However, the opposite result occurred with the DS-SIN–DS-PRO pair, which could be separated with MeOH in the eluent ($\alpha = 1.44$) but not with THF or ACN. The highest values of α for each pair, except DS-SIN–DS-PRO, were observed with THF and the lowest values with MeOH. ACN, which offers intermediate, but sufficient, selectivities and transparency in the low-UV region, was chosen for further studies as it allowed faster analyses than with THF for the desulfated arylalkylglucosinolate DS-GTL, the last-eluted compound.

The relationship between the logarithm of the capacity factor and the concentration of acetonitrile in the eluent (Table 3) was linear in each instance: the retention of desulfated glucosinolates decreases linearly with increasing concentration of the organic component in the eluent. This finding further indicates that the desulfated glucosinolates follow the general rule on the PGC column as for apolar bonded phases; no anomalous retention behaviour was observed. The slope and the intercept values differ from each other, which means that these compounds can be separated on the PGC column using acetonitrile–water eluent. The acetonitrile content of the mobile phase can be used to control the retention and resolution of desulfated glucosinolates. The y-intercept value is the extrapolated value of capacity factor to zero organic mobile-phase concentration. For all glucosinolates, the theoretical value is high. This is in agreement with the fact that the desulfoglucosinolates were totally retained on the PGC column with distilled water as eluent, but the experimental conditions were not favourable for obtaining each corresponding experimental value of the capacity factor.

Table 3
Linear regression analysis of $\log k' = \log k'_0 + aC$[a] for desulfated glucosinolates with acetonitrile–water as eluent

Desulfated glucosinolate	$\log k'_0$	a	Correlation coefficient
DS-SIN	1.764	−0.124	0.984
DS-PRO	1.527	−0.113	0.999
DS-EPIPRO	1.798	−0.110	0.996
DS-GNA	1.705	−0.092	0.996
DS-GTL	2.400	−0.088	0.998

[a] k'_0 is the capacity factor for 100% water, a is the slope and C is the acetonitrile concentration (%, v/v). C varies between 5% and 25%.

3.2. Mobile phase: electronic modifier added to acetonitrile–water mixtures

Fig. 3 depicts the influence of TFA addition to the acetonitrile–water mobile phase for the analysis of a standard solution of four anionic glucosinolates and Fig. 4 depicts the same influence for the separation of a standard solution of their four neutral desulfo analogues. As desulfated glucosinolates, the intact glucosinolates were eluted roughly in order of decreasing polarity (alkenyl- then arylalkylglucosinolates) as in ion-pair and ion chromatography. The retention of the anionic indolylglucosinolate was considerable. The presence of a high percentage of acetonitrile in the mobile phase remained the first condition to elute this compound, although the addition of TFA was necessary. As expected, TFA functions as an electronic modifier and has a significant effect on the retention of anionic glucosinolates (Fig. 3) but a very weak effect on the retention of their neutral desulfo analogues (Fig. 4). An increase in TFA concentration decreases the retention of anionic glucosinolate

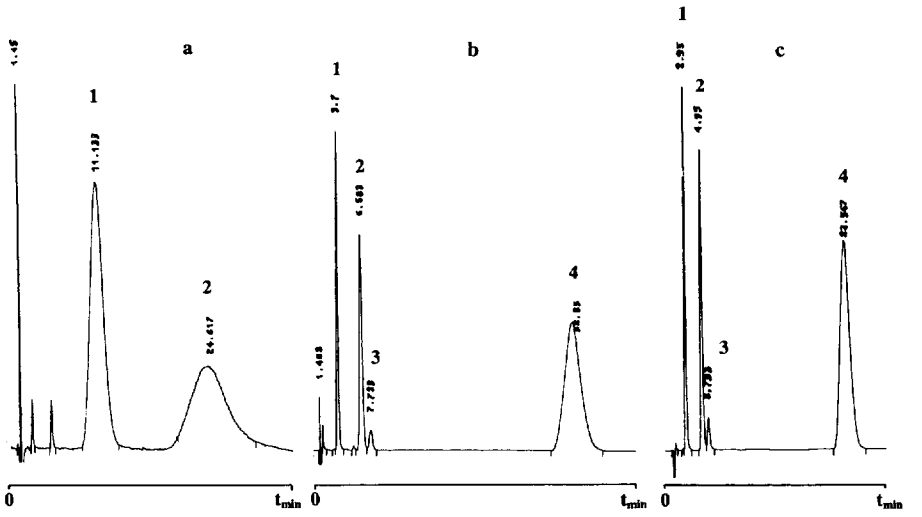


Fig. 3. Chromatograms of standard mixture of intact glucosinolates. Column, Hypercarb S (100 × 4.6 mm I.D.); eluent, acetonitrile–aqueous TFA (15:85); flow-rate, 1 ml min^{-1}; detection, UV at 229 nm. TFA concentration in eluent: (a) 0.01; (b) 0.05; (c) 0.1%. Elution order: 1 = progoitrin; 2 = epiprogoitrin; 3 = gluconapin; 4 = glucotropaeolin.

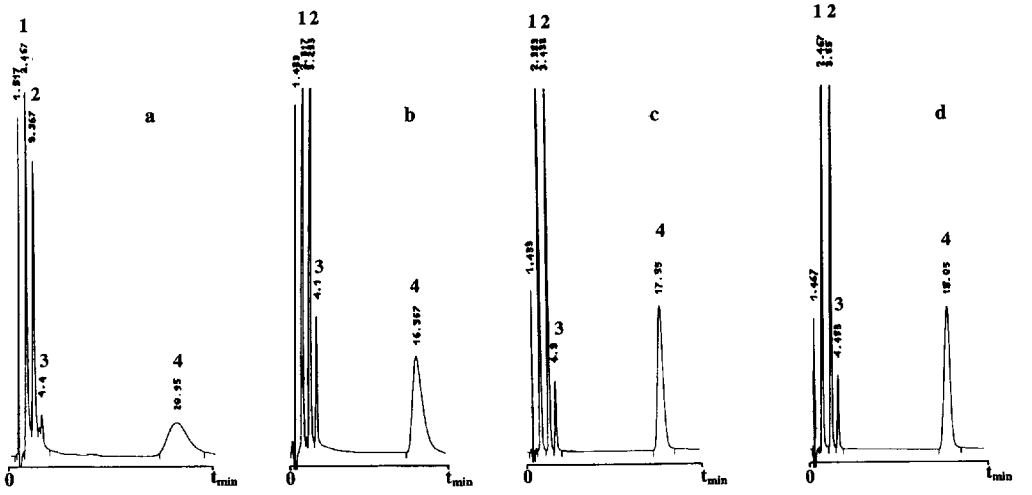


Fig. 4. Chromatograms of standard mixture of desulfated glucosinolates. Column, Hypercarb S (100 × 4.6 mm I.D.); eluent, acetonitrile–aqueous TFA (15:85); flow-rate, 1 ml min^{-1}; detection, UV at 229 nm. TFA concentration in eluent: (a) 0; (b) 0.01; (c) 0.05; (d) 0.1%. Elution order as in Fig. 2.

Table 4
Capacity factors (k') of five glucosinolates and their desulfo analogues on a Hypercarb S column (100 × 4.6 mm I.D.) with different electronic modifiers added to ACN–H_2O (15:85, v/v) as mobile phase

Electronic modifier	Intact glucosinolate					Desulfated glucosinolate				
	SIN	PRO	EPIPRO	GNA	GTL	DS-SIN	DS-PRO	DS-EPIPRO	DS-GNA	DS-GTL
TFA, $2.5 \cdot 10^{-3}$ *M*	1.42	1.49	3.44	4.21	20.81	0.73	0.66	1.39	2.00	11.11
$HClO_4$, $2.5 \cdot 10^{-3}$ *M*		0.66	1.60	1.95	9.91		0.64	1.36	1.97	11.06
$NaClO_4$, $2.5 \cdot 10^{-3}$ *M*	0.92	0.86	1.97	2.45	11.96	0.67	0.58	1.21	1.81	10.14
KH_2PO_4, $2.5 \cdot 10^{-3}$ *M*		2.73	12.62			0.72	0.65	1.29	2.00	11.00
TFA, $5 \cdot 10^{-3}$ *M*	0.90	1.02	2.39	2.93	14.46	0.65	0.68	1.42	2.06	11.30
$HClO_4$, $5 \cdot 10^{-3}$ *M*	0.35	0.34	0.85	1.05	5.69	0.70	0.60	1.28	1.88	10.73
$NaClO_4$, $5 \cdot 10^{-3}$ *M*	0.4	0.43	1.08	1.35	6.96	0.71	0.63	1.30	1.91	10.72

but not that of the desulfated alkenylglucosinolate. There was probably also a slight electronic interaction involved in the retention of the more hydrophobic neutral desulfated glucosinolate, as increasing the TFA concentration caused a small drop in the capacity factor for the arylalkylglucosinolate DS-GTL. The aromatic ring of the aglycone R part of this glucosinolate must undergo a specific donor-acceptor interaction with the delocalized band of electrons at the graphite surface.

It is therefore possible to control on a PGC column the separation of intact and desulfated glucosinolate mixtures precisely according to the nature of the application by altering the TFA concentration or acetonitrile content in the mobile phase. Under the experimental conditions in Figs. 3a and 4b, a mixture of the four DS-GSL and two GSL can be separated in 25 min.

Table 4 reports the capacity factors of five glucosinolates obtained with different acids and salts added to ACN–H_2O (15:85, v/v) mobile phase. The electronic modifier strength decreased in the order $HClO_4 > NaClO_4 > TFA > KH_2PO_4$. KH_2PO_4 at low concentration did not provide an adequate anion concentration for competitive electronic interaction for elution of intact glucosinolate. For desulfated glucosinolates, Table 4 indicates that whatever the nature of the electronic modifier, no significant variation in the capacity factors was observed.

Table 5 shows the effect of nature and concentration of the electronic modifier on the relative retention α and the resolution factor R_s of some specific glucosinolate pairs. For the rapeseed glucosinolate analysis the resolution of the PRO–GNA pair is particularly interesting owing to the importance of the ratio of these two major glucosinolates in this Cruciferae. PRO and EPIPRO are two natural diastereoisomers which cannot be sufficiently separated using current analytical methods, and GNA is eluted close to EPIPRO on a PGC column, so the separation of this pair appears delicate. Table 5 indicates that the separation of these three pairs was related to the electronic modifier used. The relative retention between PRO and GNA is always satisfactory, so the chromatographic system consisting of a Hypercarb S column and a mobile phase containing acetonitrile and an aqueous solution of TFA is suitable for isolating pure individual progoitrin or gluconapin from natural mixtures. The use of TFA or $HClO_4$ at the same concentration bring about very minor variations in the relative retention (α) of these three pairs: no variation for the EPIPRO–GNA pair but a weak increase in $\alpha_{PRO-EPIPRO}$ and $\alpha_{PRO-GNA}$ was observed when $HClO_4$ was used. Increasing the concentration of the electronic modifier had no significant effect on the relative retention values. We can therefore conclude that nature and concentration of the electronic modifier are not determining parameters for an increase in selectivity between two glucosinolates.

On comparing Figs. 3 and 4, it appears that the addition of an electronic modifier increases the

Table 5
Relative retention (α[a]) and resolution (R_s[b]) of some intact and desulfated glucosinolate pairs on a Hypercarb S column (100 × 4.6 mm I.D.) with ACN–[H_2O + x% (v/v) electronic modifier] (15:85) as eluent

Content of electronic modifier, x (%)	Intact glucosinolate					Desulfated glucosinolate				
	$\alpha_{PRO-EPIPRO}$	$\alpha_{PRO-GNA}$	$\alpha_{EPIPRO-GNA}$	$R_{sPRO-EPIPRO}$	$R_{sEPIPRO-GNA}$	$\alpha_{DS(PRO-EPIPRO)}$	$\alpha_{DS(PRO-GNA)}$	$\alpha_{DS(EPIPRO-GNA)}$	$R_{sDS(PRO-EPIPRO)}$	$R_{sDS(EPIPRO-GNA)}$
0% TFA						1.94	3.01	1.56	1.92	
0.01% TFA	2.39			1.83		2.03	3.00	1.48	2.74	
0.05% TFA	2.31	2.82	1.22	4.39	1.46	2.11	3.03	1.44	3.15	2.50
0.1% TFA	2.34	2.87	1.22	4.28	1.61	2.09	3.03	1.45	3.15	2.50
0.05% $HClO_4$	2.42	2.95	1.22	2.51	1.30	2.12	3.08	1.45	3.14	2.15
0.1% $HClO_4$	2.5	3.09	1.23	1.84	0.64	2.13	3.13	1.47	3.07	2.15

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

[b] $R_{s1,2} = \frac{1}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'_2}{k'_2 + 1} \cdot \sqrt{N_2}$.

efficiency of the separation for all anionic or desulfated glucosinolates. Moreover, increasing the percentage of electronic modifier increases the number of theoretical plates calculated from the retention time, the peak height and the peak area for each glucosinolate. Consequently, the values of the resolution factor for each pair (Table 5) were dependent on the nature and the concentration of the electronic modifier. To obtain a separation between two solutes (within 2%), the resolution value should be equal to 1 [26]. Except for the eluent containing 0.1% $HClO_4$, this condition was satisfied for all other eluents.

For the two diastereoisomers PRO and EPIPRO, the separation is better for the anionic form than for the neutral desulfated form, but for the EPIPRO–GNA pair the opposite is observed.

The best separations for all glucosinolate pairs were obtained with ACN–0.1% aqueous TFA (15:85, v/v) as the eluent (Figs. 3c and 4d).

4. Conclusion

These studies have shown that the Hypercarb column is very effective for separations of intact and desulfated glucosinolate mixtures. As expected, the retention of the desulfated glucosinolates on a PGC column is dominated by reversed-phase interactions, so these neutral compounds can be eluted with aqueous–organic eluents such as ACN–H_2O, MeOH–H_2O or THF–H_2O. The anionic charge of the intact glucosinolates involves a complementary electronic interaction with the electron density of the graphite surface. The presence of an electronic modifier such as TFA in the aqueous–organic eluent is indispensable to elute these compounds. Moreover, adding TFA allows the efficiency of the desulfated glucosinolate separation to be increased without modifying the elution strength.

The same chromatographic system consisting of a Hypercarb S column and a mobile phase containing acetonitrile and aqueous TFA is suitable for separating with excellent selectivity the two natural diastereoisomers progoitrin and epiprogoitrin in either the intact or desulfated form.

For glucosinolate analysis, the use of a PGC column offers complementary selectivities which are important and necessary for qualitative identification in the absence of an on-line identification technique such as mass spectrometry or an off-line characterization technique such as nuclear magnetic resonance spectrometry.

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