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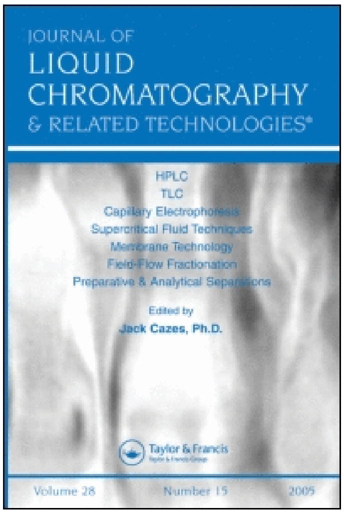

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Comparative Study of Retention of Glucosinolates and Inorganic Ions on Selected Silica-Based and Polymer-Based Ion Exchange Columns

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Comparative Study of Retention of Glucosinolates and Inorganic Ions on Selected Silica-Based and Polymer-Based Ion Exchange Columns

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ABSTRACT

The retention of two glucosinolates [Sinigrin (SIN), Glucotropaeolin (GTL)] and oxalate as organic anions and inorganic anions (chloride, nitrate, sulfate) were studied on three anion exchangers, e.g., Vydac 302 IC (250 × 4.6 mm I.D.), Dionex Ion Pac (250 × 4 mm I.D.), and Waters IC Pac HR (75 × 4.6 mm I.D.) under isocratic elution conditions. The two glucosinolates carry the same functional group (sminus;OSO_3^-),

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however, do not have the same retention in anion exchange chromatography. The plots of capacity factors *k* of organic and inorganic anions vs. the reciprocal of eluent ion concentration show good linearity. From the slope and y-intercept data, the major retention mechanisms are interpreted as ion exchange and some other interactions. The influence of organic modifier addition to the aqueous buffered mobile phase is also investigated. An Evaporative Light Scattering Detector (ELSD) was used.

Key Words: Ion exchange chromatography; LC; Hydrophobic interaction; Mechanism retention; Glucosinolates; Organic anions; Inorganic anions; ELSD.

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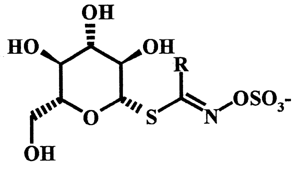
INTRODUCTION

Ion exchange chromatography is the obvious method of choice for the analysis of hydrophilic organic and inorganic anions in aqueous media. Glucosinolates are a group of plant thioglucosides found principally among members of the Cruciferae,[1,2] which are responsible for the flavor and physiological activity. Presently, glucosinolates can be analysed intact or as their desulfoanalogues by liquid chromatography (LC) methods. The general structure of glucosinolates consists of a thioglucose group liked, via a sulfonated oxime group, to an R-group (Fig. 1). A range of R-groups, approaching a hundred in number, have already been described.[2] The R-group can be alkyl, alkenyl, arylalkyl, or indolylalkyl. The great diversity of the R-group leads to wide variations in the polarity and the hydrophilic and lipophilic characters of these natural compounds.

Tertraheptylammonium bromide was used as an ion-pairing agent and octadecyl-bonded silica, using aqueous ammonium acetate/acetonitrile as mobiles phases for LC analytical systems for intact glucosinolates;[3,4] the



R-group	Systematic name of R-group	Trivial name	Symbol
CH_2=CH-CH_2-	Allyl	Sinigrin	SIN
C_6H_5-CH_2-	Benzyl	Glucotropaeolin	GTL

Figure 1. General structure of two natural glucosinolates studied (SIN, GTL).

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gradient elution is necessary to elute the more hydrophobic compounds like Glucotropaeolin. Elfakir et al.[5] have shown that the separation of intact glucosinolates on porous graphitic carbon is dominated by reversed phase interactions and a complementary electronic interaction with the electron density of support due to the anionic charge (OSO_3^-). Recently, analysis of glucosinolates from Cruciferae was made up by hydrophilic interaction LC.[6] Evaporative Light Scattering Detector (ELSD) is generally considered to be a very convenient and universal LC Detector for analytes without chromophore groups and which are less volatile than the chromatographic eluent. Furthermore, this detection mode has been proven useful for inorganic anions[7,8] and inorganic cations[9,10] determination. Evaporative Light Scattering Detector needs a volatile mobile phase when LC–ELSD coupling is investigated. In addition, to increase the field of use of ELSD, we applied it for analysis of glucosinolates.

All glucosinolates are strongly hydrophilic, owing to the salt ($-OSO_3^-$) and thioglucoside structure. They are strongly acidic compounds, relatively unstable in alkaline solutions.[11] In ion exchange chromatography, the coulombic interaction competition between the eluite ion and developer ion for the charged functional group in the stationary phase is the primary retention mechanism. Other researchers have investigated some types of secondary non-ionic retention mechanism on silica or polymeric anion exchanger supports.[12,13] All glucosinolates contain the same functional ionic radical ($-OSO_3^-$), but a due diversity of these compounds is according to their apolar aglycone side chain. We have chosen Sinigrin (SIN) and Glucotropaeolin (GTL) for our study for the high difference of hydrophobicity. The two glucosinolates studied (SIN and GTL), represent the allyl and benzyl type of group, respectively, and they are known for their use as internal standards for rapeseed glucosinolate LC analysis. Also, the methodology used for our study is applicable for others glucosinolates.

In this paper, a systematic investigation of the retention mechanism of glucosinolates anions and retention of inorganic anions (chloride, nitrate, and sulfate) on silica or polymeric anions exchangers' supports is described. The concentration of anion eluents have been studied, as well as the influence of an organic modifier in the mobile phase.

EXPERIMENTAL

The pump of the chromatographic system used was a P200 from ThermoQuest (Les Ulis, France). It was connected to the A 100 autosampler from TSQ. An ELSD Sedex 75 from Sederee (Alfortville, France) was used

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for detection. Data were performed with EZChromElite software from Scientific Software Inc. (Pleasanton, USA) running under windows NT 4.0.

All solutions were prepared from analytical reagent grade chemicals in 18 MΩ obtained from an Elgast UHQ II system from ELGA (Antony, France). SIN and GTL were obtained from Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany), respectively. Solutes were purchased as follows: sodium chloride, sodium nitrate, sodium sulfate, oxalic acid from Prolabo (Paris, France). For glucosinolates, the concentration of the studied samples was in the order of 200 ppm and 100 ppm for chloride, nitrate, oxalate, and sulfate in aqueous solution.

The eluent constituent ammonium format was purchased from Fluka (St Quentin Fallavier, France). Acetonitrile was HPLC grade obtained from Fisons (Loygh-borough, UK).

The following chromatographic conditions were used: mobile phase flow rates were generally 3.0, 2.0, and 1.0 mL/mn with Vydac, Dionex, and Waters column, respectively. The usual ELSD settings were as follows: drift tube evaporator 60°C, nebulizer gas pressure 3.6 bar, photomultiplier 9. To determine column void volume (V_0), 20 µL of distilled water was injected. The void volume of vydac (250 × 4.6 mm I.D), dionex (250 × 4 mm I.D), and waters (75 × 4.6 mm I.D) were, respectively, 3.5 mL, 1.35 mL, and 0.84 mL.

In a typical procedure, the necessary amount of salt to produce the desired eluent anion concentration was weighed and dissolved in distilled water. For those experiments, in which the effect of acetonitrile was studied, mobile phases were prepared by mixing various volume ratios of organic solvent and distilled water. The concentration of the eluent anion was adjusted for each solvent mixture.

RESULTS AND DISCUSSION

In order to achieve the retention of two glucosinolates (SIN, GTL) and oxalate as organic anions and some inorganic anions such chloride, nitrate, and sulfate by ion exchange chromatography with evaporative light scattering detection, we have used three different stationary phases. First one is Vydac 302 IC silica bonded by an ammonium quaternary [$-N(CH_3)_3{}^+$] group. Second one is Dionex Ion Pac, a polymeric based with highly cross-linked polyethylvinylbenzene/divinylbenzene substrate, agglomerated with anion exchange latex that has been completely aminated. The latest is Waters IC Pac HR, based on a polymethacrylate gel coated with an ammonium quaternary group. These columns were chosen owing to its availability in our laboratory.

For the first study, two natural glucosinolates were selected on the basis of the large diversity of their aglycone R part: alkenylglucosinolate SIN and

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arylalkylglucosinolate GTL. Evaluating the influence of an allyl and benzyl group on the aglycone side chain R would be possible by comparing the behavior of SIN and GTL in different stationary phases. The SIN and GTL are the two major representatives of glucosinolates and also chosen for our study for their interests as internal standards for rapeseed glucosinolates LC analysis.

Organic and Inorganic Anions Analysis Under Isocratic Elution Mode

Figure 2 reports the retention factor (*k*) for inorganic anions (chloride, nitrate, and sulfate) and organic anions (SIN, GTL, and oxalate) under isocratic elution conditions using formate as competitor ion, compatible with ELSD requirements. This study was carried out on three stationary phases, like Vydac, Dionex Ion Pac, and Waters IC Pac HR with formate ammonium 50 mM at pH 4. The elution order of inorganic anion is chloride, nitrate, and sulfate. This order is very adequate and awaits a mechanism of ion exchange chromatography for organic anions (oxalate, SIN, and GTL). We have shown that Waters is more retentive than Dionex and Vydac. Retention of SIN and GTL on the Waters and Dionex columns is analogous and differs significantly from that on the Vydac column. It's worthy to note, that retention of SIN on the Waters and Dionex columns is about four times higher than retention on the Vydac, and retention of GTL on the Waters and Dionex columns is 6–7 fold higher than retention on the Vydac column.

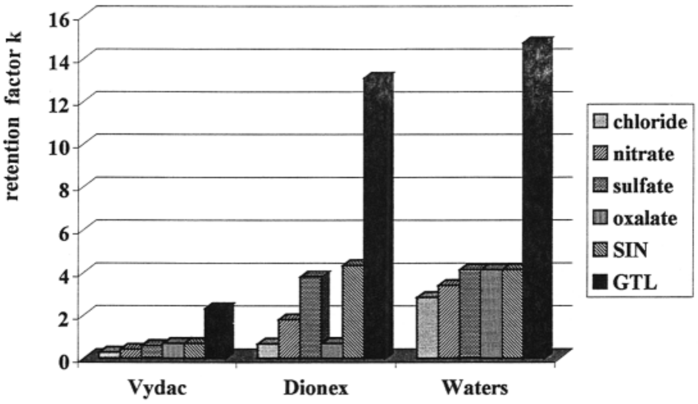

Figure 2. Variation of retention factor (*k*) of organic anions (SIN, GTL, and oxalate) and inorganic anions (chloride, nitrate, and sulfate) with three stationary phases with format ammonium 50 mM at pH 4 as mobile phase.

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Determination of Retention Mechanism

Table 1 summarizes the results of linear regression analysis of the plots of $\log k$ vs. the log of the eluent anion concentration. Four different eluent anion concentrations have been studied: 50, 75, 100, 150 mM. The theoretical slope of this linear curve should be (-1) for the monovalent eluite and (-2) for the divalent eluite.[14] The results were close to (-1) and (-2) and, in this way, supports the idea that normal ion exchange was occurring predominantly between the organic eluent and the organic or inorganic eluted ion.

Table 2 shows the results of the linear regression analysis of the plot of k against the reciprocal of eluent ion concentration (studied concentrations: 50, 75, 100, 150 mM) on three stationary phases. Good linearity of all curves of organic anions was observed.[15] At infinite eluent ion concentration, no analyte ion exchange should have occurred, so the curve of capacity factor

Table 1. Linear regression analysis of $\log k = -(a/b)\log [X^-] + c$ for eluite ion with different columns.

Column	Eluite ion	$-a/b$[a]	Correlation coefficient
Vydac 250 × 4.6 mm I.D	Cl^-	−1.01	0.9938
	NO_3^-	−0.97	0.9944
	SO_4^{2-}	−1.97	0.9909
	$C_2O_4^{2-}$	−1.94	0.9999
	SIN	−0.92	0.9999
	GTL	−0.94	0.9977
Dionex 250 × 4 mm I.D	Cl^-	−0.96	0.9985
	NO_3^-	−0.96	0.9975
	SO_4^{2-}	−2.00	0.9991
	$C_2O_4^{2-}$	−1.65	0.9985
	SIN	−0.85	0.9985
	GTL	−0.78	0.9987
Waters IC Pac HR 75 × 4.6 mm I.D	Cl^-	−0.96	0.9964
	NO_3^-	−0.96	0.9973
	SO_4^{2-}	−1.98	0.9995
	$C_2O_4^{2-}$	−1.83	0.9952
	SIN	−0.91	0.9952
	GTL	−0.88	0.9997

[a] Where a is the charge of the eluite ion, b is the charge of the eluent ion, and $[X^-]$ is the molar concentration of the eluent ion.

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Table 2. Linear regression analysis of $k = A[X^{-}]^{-1} + B$ for eluite organic anion with different columns.

Column	Eluite ion	A^{a}	B^{a}	Correlation coefficient
Vydac	$C_2O_7^{2-}$	0.0419	−0.065	0.9995
250 × 4.6 mm I.D	SIN	0.0522	−0.089	0.9991
	GTL	0.0762	0.022	0.9998
Dionex	$C_2O_7^{2-}$	0.0321	0.885	0.9979
250 × 4 mm I.D	SIN	0.128	0.965	0.9993
	GTL	0.263	1.802	0.9995
Waters IC Pac	$C_2O_7^{2-}$	0.042	0.563	0.9968
HR 75 × 4.6 mm I.D	SIN	0.191	0.796	0.9991
	GTL	0.229	1.523	0.9989

[a] Where A is the slope, B is the y-intercept, and $[X^{-}]$ is the molar concentration of the eluent ion.

(k) vs. the reciprocal of the eluent anion should pass through the origin. However, in Table 2, the y-intercepts are not zero, so this value should be the result of some mechanism other than the interionic electrostatic attraction. The intercept precision was very sensitive to small errors in the individual points, especially the (k) values producing the curve. The retention mechanism of glucosinolates and oxalate on a silica anion exchanger, Vydac 302 IC has interpreted the combination of two phenomena: the main one is ion exchange chromatography, and hydrophobic interaction assists the ion exchange and improves separation, but this effect is very minor. We have investigated the chromatographic behavior of SIN, GTL, and oxalate on a polyethyvinylbenzene (Dionex) and polymetacrylate (Waters) copolymeric anion exchangers. The y-intercept of SIN, GTL, and oxalate are not zero but are higher than the values of the slope, indicating a stronger adsorption of the organic analyte ions onto the polymeric stationary phase than for silica stationary phase. This adsorption was stronger as the compound includes a much more apolar aglycone component. The increase of the slope for SIN and GTL has been interpreted as a solvophobic interaction, helping electrostatic attraction at the ion exchange site.[16,17] With this type of support, it has been proven that some interactions in the reverse phase make a considerable contribution to the retention of the ionised species, particularly those which have both an anionic and a hydrophobic center. It very logical to test the effect of organic modifier on reduction on these hydrophobic interactions.

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Influence of Organic Modifier

The influence of the addition of organic solvent to the aqueous mobile phase on SIN, GTL, and oxalate retention was also studied. It is well known that an organic modifier will have an effect on the retention of ions on an ion exchange column.[18,19] In general, those organic analytes that are retained predominantly by anion exchange interactions increase in retention as the concentration of organic modifier increases, whereas analytes that are retained predominantly by adsorption decrease in retention.[20] Figure 3(a)–(c) show the variation of retention factors (*k*) of SIN, GTL, and oxalate vs. acetonitrile percentage in aqueous mobile phase with three stationary phases: Vydac (250 × 4.6 mm I.D.), Dionex Ion Pac (250 × 4 mm I.D.), and Waters IC Pac HR (75 × 4.6 mm I.D.), under isocratic elution conditions with formate ammonium 150 mM at pH 4 as constituent of mobile phase. The retention factors (*k*) of SIN, GTL, and oxalate decreases when the percentage of acetonitrile increases for the three stationary phases. These results agree well with those that we have demonstrated by the retention mechanism. For inorganic anions (chloride, nitrate, and sulfate)there is no variation of retention factor (*k*) with increasing percentage of acetonitrile.

Figures 3(a)–(c) show that the support silica is not usable for the inorganic and organic anions, whereas both polymeric supports can potentially be

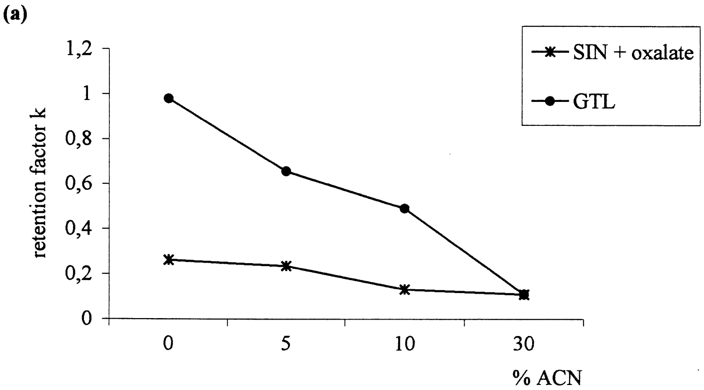

Figure 3. Influence of percentage acetonitrile on SIN, GTL, and oxalate retention factor (*k*). Eluent: ammonium format 150 mM, pH 4. Detection: ELSD. (a) Column: Vydac (250 × 4.6 mm I.D.). (b) Column: Dionex Ion Pac (250 × 4 mm I.D.). (c) Column: Waters IC Pac HR (250 × 4 mm I.D.).

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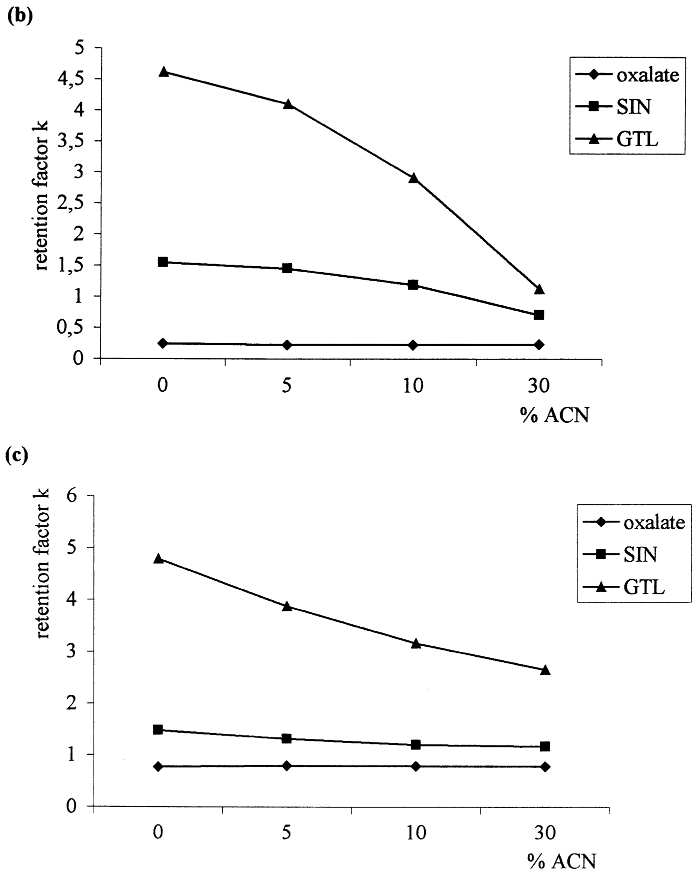

Figure 3. Continued.

employed, according to required separations. The use of acetonitrile with respect to the solubility of the solutes can appear necessary and compatible with a selectivity which does not exist any more on silica.

In Table 3, the values of selectivity (α) for the pair (SIN/GTL) are reported, vs. acetonitrile percentage on three column and an eluent constituted with ammonium formate 150 mM at pH 4. It appeared that the selectivity decreases when the acetonitrile percentage increases.

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Table 3. Influence of acetonitrile percentage on the values of selectivity (α) between the pair (SIN/GTL).

Column	0% ACN	5% ACN	10% ACN	30% ACN
Vydac 302 IC	3.74	2.79	2.23	1.00
Dionex Ion Pac	2.98	2.82	2.43	1.59
Waters IC Pac HR	3.22	2.95	2.61	2.12

CONCLUSION

This work illustrates a comparison of retention of organic anions (SIN, GTL, and oxalate) and inorganic anions (chloride, nitrate, and sulfate) using three anion exchangers. It appeared that Waters IC Pac HR is more retentive than Dionex Ion Pac and Vydac 302 IC. This study proves that glucosinolates (SIN, GTL) can be analysed by anion exchange chromatography with minor hydrophobic interactions on Vydac 302 IC. The retention mechanism of SIN and GTL on a polymeric anion exchangers (Dionex Ion Pac and Waters IC Pac HR) has been interpreted as a combination of two phenomena; ion exchange chromatography with more hydrophobic interactions. The addition of an organic modifier in the mobile phase decreases the retention, as well established by the mechanism retention study.

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