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Could linear solvation energy relationships give insights into chiral recognition mechanisms? 2. Characterization of macrocyclic glycopeptide stationary phases

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Abstract

Five parameter linear solvation energy relationships (LSER) are known to have little or no shape recognition ability. However, it is proposed to use LSER studies to get insights into chiral recognition mechanisms. Since the two enantiomers have exactly the same five $A-V$ solute descriptors being still separated by chiral stationary phases (CSPs), it can be considered that they form two different transient diastereoisomers with the CSP. It is then possible to perform LSER studies on the enantioselectivity factors taken as the two enantiomer retention factor ratios. In a first step, the five $a-v$ system parameters of four CSPs of the macrocyclic glycopeptide types were determined using a set of test solutes with known $A-V$ descriptors, both in the reversed phase and the normal phase modes. In a second step, the $A-V$ descriptors of 18 enantiomeric pairs were tentatively established using five achiral columns with known $a-v$ parameters. This was successful for the five molecular enantiomers only. It was found that the predicted retention factor for the molecular enantiomers separated on a given CSP corresponded either to retention factor of the first experimentally eluted enantiomer or to the second one or to none of them. Using the enantioselectivity factors it was possible to obtain the $a-v$ parameters corresponding to the difference in CSP properties seen by the two enantiomers. For the five molecular enantiomeric pairs in the reversed phase mode with a teicoplanin CSP, it was found that there was an elevated contribution by the e coefficient that we interpret as a possible interaction between surface charges on the teicoplanin CSP and solute induced dipoles. Steric effects, seen on the v parameter, are second in magnitude followed by H-bond and polar interactions. Only one solute could be studied in the normal phase mode showing a different mechanism with polar and steric major interactions.

Keywords

Linear solvation energy relationship; Chiral stationary phase; Enantioselectivity; Chiral mechanisms; Teicoplanin; Macrocyclic glycopeptide; Reversed phase; Normal phase mode

1. Introduction

Macrocyclic glycopeptide based chiral stationary phases (CSPs) have proven to be extremely useful in enantiomeric separations and are one of the fastest growing classes of CSPs today [1–4]. They have exceptional selectivity for amino acids [5,6], and have proven to be useful for the enantiomeric separation of a wide variety of molecules [7]. Chemically, they are composed of a multiplicity of functional groups, including hydroxyls, phenols, amides, aromatic rings, various carbohydrate moieties, ionizable carboxylic acid and amine groups, as well as a basket shaped structure [8]. This plethora of functional groups is a stark contrast to the hydrocarbon chains that the majority of conventional reverse phase stationary phases are composed of. Due to these structural differences, macrocyclic glycopeptide based stationary phases are capable of participating in a greater variety of intermolecular interactions compared to a conventional brush type stationary phases. In addition to the differences observed in reversed phase HPLC, macrocyclic glycopeptide CSPs are effective in the normal phase mode and polar organic mode, and the mechanism of retention and separation is obviously very different in each mode of operation [7]. However, no quantitative physico-chemical data has been published that supports the assumed differences in retention mechanisms when the same stationary phase is used in the reversed phase mode versus the normal phase mode.

In the first article of this series, it was proposed to use linear solvation energy relationships (LSER) to get information on chiral recognition mechanisms [9]. As described by Abraham [10] and recently reviewed by Vitha and Carr [11], the LSER model is expressed by

$$\log k = c + eE + sS + aA + bB + vV \quad (1)$$

in which k is the solute retention factor ($(t_R - t_0)/t_0$). The capital letters, E , S , A , B , and V are the solute descriptors independent of the mobile and/or the stationary phase used. The lowercase letters, c , e , s , a , b and v are the system (or column) parameters reflecting the difference in solute interactions between the mobile and stationary phase. Eq. (1) clearly differentiates five contributions to the solute retention. The eE term is mainly related to polarizability interactions (Debye forces, induced dipoles), sS represents also a part of the polarizability interactions and it includes the dipolar interactions (Keesom forces) [11]. The aA and bB terms represent the H-bond interactions, a is the system parameter for H-bond basicity interacting with acidic A solutes and vice versa for the bB term. The vV term represents the cavity formation and hydrophobic or dispersive interactions [10,11].

In the LSER theory, two enantiomers have identical sets of descriptors, which correctly indicates that enantiomers are not separated by any isotropic stationary phase. They can however be separated by a CSP. This allows the relative Dolan–Snyder approach with a reference solute to be used for this special case [12–16]. In this work, the reference solute

will not be ethylbenzene, as recommended by Snyder, but it will be the less retained enantiomer of the enantiomeric pair. It is considered that one enantiomer sees a different CSP domain than the other enantiomer. Both enantiomers form two different transient diastereoisomeric complexes with the same CSP. Thus, the enantioselectivity factor, α , will be modeled as

$$\begin{aligned}\log k_2 - \log k_1 &= \log \alpha \\ &= \Delta eE + \Delta sS + \Delta aA + \Delta bB + \Delta vV \quad (2)\end{aligned}$$

in which k_1 and k_2 are the retention factors of the first and last eluted enantiomer and all the terms correspond to energy changes responsible for the observed enantioselectivity. This work focuses on the determination of the system parameters, e , s , a , b and v of the commercially available macrocyclic glycopeptide CSPs using achiral solutes. Next, the A – V descriptors of a set of enantiomers will be evaluated using the system parameters previously obtained for classical, achiral columns. When these enantiomers with known A – V descriptors are separated by a CSP with known a – v system parameters, it is possible to evaluate the a to v parameters and relative a/a to v/v values corresponding to the enantioselective interactions both in the reversed phase and normal phase mode.

2. Experimental

2.1. Materials

2.1.1. Chemicals—HPLC grade acetonitrile, trifluoroacetic acid, and triethylamine were purchased from Fisher (Fairlawn, NJ, USA). Triethylamine and acetic acid were obtained from Sigma (St. Louis, MO). HPLC grade water was in house reverse osmosis water filtered through an ion exchange resin. The 63 LSER probe molecules were purchased from Aldrich (St. Louis, MO) in high purity grade. The probe solutes were already listed in Table 1 of the first article of this series with their solute descriptors [9]. Fifty-one and 38 compounds were respectively used for the normal and reversed phase LSER studies (26 compounds were used in both sets of studies).

Thirteen enantiomers were selected because of their very high chiral recognition by the Chirobiotic® columns. They were the native and zwitterionic amino-acids arginine, methionine, tyrosine, *m*-tyrosine, phenyl glycine and tryptophan; the *N*-substituted *N*-acetyl-*m*-fluorophenylalanine and *N*-benzoyl phenylalanine; the molecular compounds: 5-methyl-5-phenyl-hydantoin, bromacil, dihydrofurocoumarin, 1,1-dimethyl-3-phenyl-propyl toluyll sulfoxide and α -naphthalenyl methyl sulfoxide; all obtained from Sigma–Aldrich.

2.1.2. Columns—Table 1 lists the characteristics of the columns used [8]. The C_{18} and Chirobiotic® columns were all obtained from Astec (Supelco-Sigma–Aldrich group, Whippany, NJ).

2.1.3. Chromatography—The HPLC system used consisted of a quaternary pump, an auto sampler, a UV 1050 VWD detector (Hewlett–Packard–Agilent, Palo Alto, CA), and a HP 3395 integrator (Hewlett–Packard–Agilent). Mobile phases were prepared as volumetric ratios and degassed by helium bubbling. The aqueous part of the mobile phases contained

0.1% (v/v) triethylamine and was buffered at pH 4.1 by acetic acid. UV detection was carried out at 210 nm. All separations were carried out at room temperature (22 °C) unless otherwise indicated.

2.2. Calculations

Retention factors (k) were calculated using the equation $k = (t_R - t_0)/t_0$. The hold-up time, t_0 , was taken as the first deviation of the baseline after D₂O injection in the reversed phase mode or as the peak start of tri-*t*-butyl benzene in the normal phase mode. Multiple linear regression analysis and statistical calculations were performed using the program Analyse-it, an add-in program for Microsoft Excel (Analyse-it Software, Leeds, UK).

3. Results and discussion

3.1. Test compounds

The probe solute set contains aldehydes, ketones, amides, halogenated phenols, nitro-substituted benzenes, and nitro substituted alkanes, alkyl benzenes, and polyaromatic hydrocarbons with molecular descriptors spanning a wide range. The five A–V solute descriptors were listed in Table 1 of the first part of this work [9]. Care was taken to exclude solutes that interact with the chiral selectors in a manner inconsistent with the majority of the probe solutes (i.e. strongly silanophilic compounds). Table 2 shows that parameter intercorrelation is minimized in both mobile phase modes.

3.2. System parameters for chiral stationary phase in reversed phase mode

Table 3 presents the system parameters obtained with the methanol/water and acetonitrile/water mobile phases for the Chirobiotic T column (teicoplanin selector). The results obtained for the Chirobiotic TAG column were presented in Part 1 [9]. The full results for the two other CSPs, Chirobiotic R and V, can be requested by a simple e-mail to the authors.

For each solute, the k_w retention factor in 100% water mobile phase was obtained extrapolating the solute retention factor to 0% organic modifier (both ACN or MeOH were used). The linear Snyder solvent strength model was used [17]. A quadratic model did give correlation coefficients closer to unity than the linear model, but at the cost of an extra variable [18]. Since the quadratically fitted k'_w retention factors were not statistically different from the linearly fitted factors, the later were used (Table 3). The LSER regression analysis was then performed using the solute log k_w and the resulting system parameters are listed in Table 3 (bottom) for all CSPs and the classical C₁₈ column for comparison. There are minor differences between the k_w values extrapolated from ACN and MeOH mobile phases. The nature of the organic modifier has some influence on the k_w extrapolated value as discussed by several authors [17–20]. However, for the same column, the observed trend is the same, as previously observed [9].

3.2.1. c coefficient—The c coefficient is actually the intercept of Eq. (1). All CSP c coefficients are negative compared to the positive value of the C₁₈ c coefficient. Since the c coefficient is not related to the solute descriptors, it indicates that, for any solute, the C₁₈

stationary phase is much more retentive for all RP mobile phases than the CSP stationary phases in this study. It will not be considered further.

3.2.2. ν coefficient—Table 3 shows that two coefficients, the b and ν coefficients, have significantly more weight on solute retention than the three other coefficients. The ν coefficient, related to the cavity formation energy between the mobile and stationary phase, has a high positive value due to the selected reference mobile phase (100% water) used to compare the stationary phases. In RPLC, solutes are highly retained with a 100% water mobile phase. The hydrophobic C₁₈ stationary phase has a significantly higher ν coefficient ($\nu = 2.86$) than the four CSPs whose ν coefficient is around 1.5 (Table 3).

3.2.3. a and b coefficients—The a and b coefficients are related to H-bond solute-stationary phase and solute-mobile phase interactions. The b coefficient, related to the difference in stationary phase and mobile phase acidity, has a high negative value of -2.4 for the C₁₈ column compared to ~ -1.4 for the R, TAG and V CSPs and -0.66 for the T-CSP. Basic compounds will show a very different retention behavior when interacting with the C₁₈ stationary phase or the four CSPs with pure water as the mobile phase. All a coefficients have the same order of magnitude being negative between -0.88 for the R-CSP and -0.42 for the TAG CSP with -0.84 for the C₁₈ stationary phase (Table 3). However, when the relative weight of the a coefficient is considered, significant differences are apparent (Table 3). The weight of the a coefficient is similar to that of the b coefficient for the T-CSP and it is about one third of that of the b coefficient for the TAG, V and C₁₈ stationary phases.

3.2.4. e and s coefficients—These coefficients are related to polarizability and dipolar interaction differences between pure water and the considered stationary phases. The magnitude of these two coefficients is lower than that of the other system parameters (Table 3). However, it is in these two coefficients that the most significant differences are observed between the hydrophobic C₁₈ stationary phase and the four more polar CSPs. The positive e coefficients of the CSPs have about twice the weight in solute retention as compared to that of the C₁₈ stationary phase (Table 3). The positive sign of the e coefficients indicates that polar interactions increase solute retention favoring solute stationary phase interactions. As already suggested [9], the relatively high values of the CSP e coefficients may be due to charge-induced dipole effects. Indeed, the chiral selectors contain carboxylic acid groups (Table 1) that bear a negative charge at pH 7 of the buffered mobile phase. These charges may increase the retention of polarizable solutes by inducing dipoles through Keesom forces. The s coefficients of the CSPs are all positive with great variability, between 0.234 for the T-CSP and 0.716 for the R-CSP indicating solute stationary phase interactions with a 100% water mobile phase. The s coefficient is negative ($s = -0.651$) for the C₁₈ stationary phase indicating that all dipole-dipole interactions occur in the aqueous mobile phase with an ODS stationary phase.

3.2.5. CSP system parameters—Table 3 shows that the four CSPs show significant differences between their system parameters. The R- and V-CSPs have a , e and s parameters that are well balanced. However, the R-CSP a , e and s values are around ± 0.8 which is twice smaller than the corresponding $b = -1.45$ and $\nu = 1.5$ values. For comparison, the V-CSP a ,

e and s values are even smaller around ± 0.4 three times smaller than the corresponding b ($= -1.34$) and v ($= 1.53$) parameters (Table 3, bottom).

The T- and TAG-CSPs show significant differences in their system parameters (Table 3). Their s parameters are the smallest showing minimized dipolar interaction in the stationary phase with a purely aqueous mobile phase compared to the other two counterparts (R- and V-CSPs). The difference between the TAG- and the T-CSPs is the removal of the teicoplanin carbohydrate units in the TAG selector [8]. This change significantly increases the b , e and v system parameters and simultaneously decreases the a and s system parameters of the TAG-CSP compared to the T-CSP (Table 3). All parameters are affected because the three carbohydrate units removed contained nine polar hydroxyl groups, two dipolar amide groups and a nine-carbon apolar alkyl chain [8]. On a normalized scale, the TAG and T v coefficient (cavity formation and hydrophobic interactions) and e coefficient (polarizability) both have a similar weight on overall solute retention.

3.3. System parameters for chiral stationary phases in the normal phase mode

Table 4 presents the LSER system parameters obtained in the normal phase mode for the Chirobiotic T-CSP. The results obtained for the TAG CSP were recently presented [9] and the results obtained with the R- and V-CSPs can be requested. It was not possible to perform LSER studies with the 100% heptane mobile phase since not enough solutes were eluted with an acceptable retention time (less than 1 h). Then the k_{heptane} retention factors of the solutes were obtained by extrapolating their heptane/ethanol retention factors to 0% ethanol. The extrapolated k_{heptane} retention factors were used to obtain the LSER results listed in Table 4 (bottom).

Comparing Tables 3 and 4 clearly shows the differences in the system parameters for the RPLC and NPLC modes. The a , b and v coefficients have opposite signs in the two modes. The s coefficients are three to four times higher than in RPLC keeping the same positive sign (except for the R-CSP whose s coefficient was high in RPLC, Table 3) and there is no e coefficient in the normal phase mode ($e = 0$). It was observed in NPLC chiral separations that π - π interactions could play a significant role in solute retention and enantiomer discrimination [21]. If the LSER analysis returns a nil value for the polarizability e coefficient in normal phase mode, it means that the π - π interaction contribution to solute retention is not encoded in this coefficient or that these interactions are insignificant in this apolar mode or that these interactions between the solute and the stationary phase and between the solute and the heptane mobile phase are exactly the same. These observations are supported by the results obtained in Part 1 of this series which examined π -rich stationary phases [9]. The π - π interaction contribution is spread among the three a , b and s coefficients.

3.4. Enantiomer descriptors

Thirteen enantiomers were selected because they were well separated on Chirobiotic[®] columns. The set contained five molecular solutes and eight ionic or zwitterionic amino-acids or amino-acid derivatives. The set of enantiomers was separated on the five columns whose system parameters were fully established in Part 1. Five different ethanol/water

mobile phases were used with each column producing 25 different experimental conditions. Very low retention factors were eliminated from the regression analysis.

The V parameters were calculated using the McGowan additivity scheme as described by Abraham et al. [22]. The E coefficients can be obtained using n , the solute refractive index, and the equation [23]:

$$E = 10 V \left(\frac{n^2 - 1}{n^2 + 2} \right) - 2.83 V + 0.525 \quad (3)$$

Since the solutes were solids at room temperature, their n refractive indexes were calculated using the SPARC software (<http://ibmlc2.chem.uga.edu/sparc>). Table 5 lists the A – V solute descriptors obtained for the five molecular solutes only. Indeed, it was not possible to obtain sound A , B and S descriptors for the amino-acid enantiomers: the regression calculation returned negative descriptors with no chemical or physical meaning. If one descriptor is incorrect, they all are. So, it is not possible to list A – V solute descriptors for the charged enantiomers. It is known that the LSER theory was developed for molecular solutes [10,11]. The LSER theory could be extended to charged species provided that two new parameters accounting for cations and anions are added to the five molecular parameters [11,24,25].

3.5. LSER study of the enantiomer-CSP interactions

The molecular enantiomers listed in Table 5 were separated on the Chirobiotic T[®] column with five different ethanol/buffer mobile phases (75/25 to 95/5% (v/v) in 5% increments) at 10 and 25 °C. Fig. 1 presents the results for four typical cases, three in the reversed phase mode (Fig. 1A–C) and one in the normal phase mode (Fig. 1D). The experimental $\log k'_1$ and $\log k'_2$, respectively obtained for the first and last eluting enantiomers, are compared to the LSER $\log k$ values calculated using Eq. (1) with the system parameters listed in Tables 3 and 4 and the solute descriptors listed in Table 5.

Three situations were observed: (i) the retention factors predicted by LSER corresponded to the first eluting enantiomer (e.g. dihydrofurocoumarin, Fig. 1C); (ii) the LSER predicted retention factors corresponded to the last eluting enantiomer (e.g. 5-methyl-5-phenyl hydantoin in the NP mode, Fig. 1D); (iii) the LSER predicted retention factors did not correspond to a particular enantiomer for all mobile phase compositions (e.g. 5-methyl-5-phenyl hydantoin in the RPLC mode and bromacil, Figs. 1A and B). From a mechanistic point of view, it can be speculated that in Case (i), the chiral selector has overall attractive enantioselective interactions with the second enantiomer; in Case (ii), the chiral selector has overall repulsive enantioselective interactions with the first enantiomer; in Case (iii), the chiral selector has enantioselective interactions with both enantiomers.

The enantioselectivity factors were obtained as the ratio k'_2/k'_1 . From a LSER point of view, it appears that the enantiomers were effectively seeing two different stationary phases. The a_1 – v_1 and a_2 – v_2 system parameters correspond to these two hypothetical stationary phases. The parameters were determined for the two enantiomers using respectively the $\log k'_1$ and

$\log k'_2$ experimental results. Actually, in Case (i) only the $\log k'_2$ were used to obtain a_2-v_2 and in Case (ii), only the $\log \log k'_1$ were used to obtain a_1-v_1 system parameters. The Table 4 $a-v$ parameters were used as the other parameters. Next the five xX terms of Eq. (2) were obtained as $(x_2 - x_1)X$, x being an a to v parameter and X is the corresponding A to V solute descriptor. Since the solute A coefficients are all nil, the aA terms of Eq. (2) also have a zero value. Table 5 (bottom) lists the enantioselective terms obtained with excellent regression coefficients ($r^2 > 0.990$). It should be noted that only five solutes were used for the LSER study in the reversed phase mode and, in the normal phase mode, only one solute, 5-phenyl-5-methyl hydantoin, could be separated in enough different mobile phases to perform a statistically significant LSER regression.

Before commenting on the results, it is worthwhile to point out that this LSER study done on the enantioselectivity factors does not respect the Vitha and Carr recommendation recently set out [11]. First, the solute set is not large enough since we had to drop the eight amino-acid derivatives and all five studied solutes have the same nil A descriptor. Second, the 10 mobile phases tested are somewhat intercorrelated but it is not possible to use any mobile phase composition since the separation of the two enantiomers is required. Last, the regression is very good because it is done with a too low data set. We still propose this study because we follow exactly the Vitha and Carr statement saying that one of the main reason for performing LSER analyses is to obtain a better understanding of the system being studied [11]. It will be important to validate our results with a larger set of both enantiomeric pairs and CSPs.

For the five enantiomers studied in the reversed phase mode, two terms dominated: the eE and the vV terms, the first being positive and the second being negative (Table 5, bottom). They almost cancel each other. The eE term encodes interactions through polarizable n and π electrons. The data in Table 3 shows that the e coefficient has a minor importance in solute retention. It has a major effect on enantioselectivity. In Part 1 of this work it was suggested that the elevated value of this coefficient could be due to the ability of the surface charge of the teicoplanin CSP to induce dipoles in the five polarizable test molecules [9]. The negative enantioselective contribution of the vV term is likely an indication of steric repulsion. Since these two terms almost cancel each other for our five test solutes, it means that the dipolar, sS and especially H-bonding, bB , terms will be mainly responsible for the experimentally observed enantioselectivity factor.

Knowing the $A-V$ solute descriptors (Table 5, top), the $a-v$ terms are easily obtained. Radar plots were used to visualize these values corresponding to the CSP effect on solute enantioselectivity. The five terms of Eq. (2) form a branch of a star. The branch is symmetrically graduated so that zero is the middle. This representation clearly show the strong negative contribution of the v term, the positive contribution of the e term and also, for the dihydrofurocoumarin solute and the two chiral sulfoxides only (Fig. 2), the significant contribution of the b term. Using the $a-v$ coefficients obtained for the teicoplanin CSP (Table 3), it is possible to prepare the radar plots of the relative contribution x/x (Fig. 2, bottom). These last plots show the significant effect of the e coefficient on the

enantio recognition of the five solutes in the reversed phase mode. The steric and H-bond influences are similar in this relative representation.

The enantioselective LSER study in the normal phase mode was possible with 5-methyl-5-phenyl hydantoin only. As expected, the results show a normal phase enantio recognition mechanism completely different from that observed in the reversed phase mode. It will not be commented much because the results may be peculiar since a value of 0.12 was obtained for eE when $e = 0$ was obtained in normal phase mode with achiral test solutes for all CSPs (Table 4). The corresponding radar plots are shown in a smaller size in Fig. 4. The relative x/x values show that the dipolar s/s terms has the most weight when the peculiar e/e term was set to zero.

4. Summary

LSER studies can give insights into the chiral recognition mechanism of CSPs assuming that the enantiomers see different stationary phases when forming their transient diastereomeric solute-CSP complexes that are responsible for the enantioseparation. Macrocyclic glycopeptide chiral stationary phases can work in reversed phase and normal phase modes. It was found that the enantio recognition mechanism in the two modes was completely different for the same enantiomer separated on the same CSP. The macrocyclic glycopeptide CSPs bear electrical charges and the LSER theory was not developed for charged solute or charged stationary phases. The LSER study on the enantioselectivity factors obtained with molecular enantiomers and glycopeptide CSPs returned an elevated contribution of the e coefficient (polarizability and excess molar refraction) that is likely due to dipoles induced by charges on the CSP. This result is important in that it shows that a particular interaction that may have a minor effect on the solute retention may be essential to solute enantio recognition and vice versa. Steric effects are also important in enantiomer separation with a significant and negative contribution of the v coefficient to enantioselectivity. H-bond and polar interaction (b and s coefficients) cannot be ignored and vary with the nature of the solute and the mobile phase.

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Fig. 1. Enantiomer retention factors obtained on the Chirobiotic[®] T column (teicoplanin selector) plotted versus the organic modifier content in the mobile phase. (A–C) RPLC mode with ethanol/pH 4 buffer mobile phases; (D) normal phase mode with ethanol/heptane mobile phases. Diamonds: LSER calculated retention factors using Tables 3 and 4 system parameters and Table 5 solute descriptors; squares: experimental retention factors of the first eluting enantiomer; triangles: experimental retention factors of the last eluting enantiomer.

Fig. 2.

Radar plots showing the five components of the enantioselectivity factors of molecular enantiomers separated on a teicoplanin chiral stationary phase with an ethanol/water 75/25% (v/v) mobile phases except for the smaller radar plots corresponding to 5-me-5-phe-hydantoin and a normal phase heptane/ethanol 75/25% (v/v) mobile phase. Top plots: absolute values; bottom plots: relative values. Data listed in Table 5.

Table 1

Physicochemical characteristics of the HPLC columns used

Column code	Trade name	Description	Characteristics
C ₁₈	Astec ODS	C ₁₈ polymeric bonded Type B silica	Silica base: $d_p = 10$ nm, $v_p = 0.9$ ml/g, $S = 310$ m ² /g, %C = 16%, cov 3 μ mol/m ²
R	Chirobiotic R	Ristocetin A selector, C ₉₅ H ₁₁₀ N ₈ O ₄₄ m.w. 2066, 6 carbohydrate units, 2 amine groups	Silica base: $d_p = 10$ nm, $v_p = 0.9$ ml/g, $S = 300$ m ² /g, cov 0.7 μ mol/m ²
T	Chirobiotic T	Teicoplanin selector, C ₈₈ H ₉₅ Cl ₂ N ₉ O ₃₃ m.w. 1877, 3 carbohydrate units, 1 amine group, 1 carboxylic acid	Silica base: $d_p = 10$ nm, $v_p = 0.9$ ml/g, $S = 300$ m ² /g, cov 0.5 μ mol/m ²
TAG	Chirobiotic TAG	Teicoplanin aglycon selector, C ₅₈ H ₄₅ Cl ₂ N ₇ O ₁₈ m.w. 1197, no carbohydrate unit, 1 amine group, 1 carboxylic acid	Silica base: $d_p = 10$ nm, $v_p = 0.9$ ml/g, $S = 300$ m ² /g, cov 0.6 μ mol/m ²
V	Chirobiotic V	Vancomycin selector, C ₆₆ H ₇₅ Cl ₂ N ₉ O ₂₄ m.w. 1449, 2 carbohydrate units, 2 amine groups, 1 carboxylic acid	Silica base: $d_p = 10$ nm, $v_p = 0.9$ ml/g, $S = 300$ m ² /g, cov 0.6 μ mol/m ²

All columns are 5 cm long $\times$ 4.6 mm i.d. tubing and contain 5 μ m bonded silica particles obtained from Astec (Supelco-Sigma-Aldrich group, Whippany, NJ). d_p : pore diameter; v_p : pore volume, S : surface area, %C: carbon content, cov: surface coverage.

Table 2

Correlation coefficients, R , between the molecular descriptors for the two mobile phase modes^a

<i>R</i> values	<i>E</i>	<i>S</i>	<i>A</i>	<i>B</i>	<i>V</i>
Normal phase mode (51 solutes) ^a					
<i>E</i>	1.00				
<i>S</i>	0.46	1.00			
<i>A</i>	-0.12	0.29	1.00		
<i>B</i>	-0.11	0.36	0.24	1.00	
<i>V</i>	0.59	0.31	-0.22	0.03	1.00
Reversed phase mode (38 solutes) ^a					
<i>E</i>	1.00				
<i>S</i>	0.46	1.00			
<i>A</i>	0.07	0.31	1.00		
<i>B</i>	0.08	0.47	0.25	1.00	
<i>V</i>	0.46	0.28	-0.21	0.11	1.00

^aThe full list of the *A*–*V* solute descriptors can be found in Table 1 of Ref. [9].

Table 3

LSER system parameters for the teicoplanin CSP (Chirobiotic T) and C₁₈ stationary phase in the reversed phase mode

Mobile phase	<i>e</i>	<i>s</i>	<i>a</i>	<i>b</i>	<i>v</i>	<i>c</i>	<i>r</i> ²	S.E.	<i>F</i>	<i>n</i>
Teicoplanin chiral stationary phase										
20% ACN	0.228 0.025	0.108 0.035	-0.368 0.025	-0.794 0.062	0.909 0.036	-0.953 0.039	0.99	0.034	558	36
15% ACN	0.269 0.021	0.129 0.031	-0.388 0.022	-0.792 0.054	1.031 0.032	-1.017 0.034	0.99	0.033	704	36
10% ACN	0.320 0.022	0.148 0.031	-0.430 0.023	-0.757 0.055	1.153 0.032	-1.075 0.034	0.99	0.039	616	36
5% ACN	0.335 0.018	0.207 0.026	-0.503 0.019	-0.683 0.047	1.322 0.027	-1.184 0.029	0.99	0.038	829	35
Water, <i>k_w</i>	0.381 0.019	0.234 0.027	-0.534 0.020	-0.661 0.048	1.443 0.028	-1.253 0.030	0.99	0.044	745	36
25% MeOH	0.260 0.022	0.315 0.032	-0.523 0.023	-0.692 0.055	1.000 0.032	-1.132 0.034	0.99	0.039	577	35
20% MeOH	0.272 0.022	0.323 0.031	-0.541 0.023	-0.684 0.054	1.102 0.032	-1.154 0.034	0.99	0.039	674	35
15% MeOH	0.282 0.021	0.313 0.03	-0.568 0.022	-0.638 0.052	1.207 0.031	-1.177 0.032	0.99	0.037	847	35
10% MeOH	0.280 0.022	0.299 0.031	-0.605 0.023	-0.588 0.054	1.301 0.032	-1.177 0.034	0.99	0.039	871	35
5% MeOH	0.223 0.024	0.307 0.034	-0.685 0.025	-0.484 0.060	1.385 0.035	-1.084 0.037	0.99	0.042	834	35
Water, <i>k_w</i>	0.253 0.026	0.299 0.038	-0.701 0.028	-0.463 0.066	1.490 0.039	-1.123 0.041	0.99	0.047	755	35
Stationary phase										
	<i>e</i>	<i>s</i>	<i>a</i>	<i>b</i>	<i>v</i>	<i>c</i>	<i>r</i> ²	S.E.	<i>F</i>	<i>n</i>
Log <i>k_w</i> ; 100% water mobile phase										
R (ACN)	0.799	0.716	-0.887	-1.454	1.500	-1.796	0.99	0.045	443	30
T (ACN)	0.381	0.234	-0.534	-0.661	1.443	-1.253	0.99	0.044	745	36
TAG (ACN)	0.584	0.214	-0.419	-1.350	2.077	-1.234	0.99	0.062	475	36
V (ACN)	0.518	0.383	-0.520	-1.339	1.528	-1.607	0.99	0.029	875	32
C ₁₈ (ACN)	0.302	-0.651	-0.842	-2.402	2.859	0.596	0.98	0.101	478	40
R (MeOH)	0.783	0.646	-0.898	-0.984	1.317	-1.531	0.98	0.065	257	31
T (MeOH)	0.253	0.299	-0.701	-0.463	1.490	-1.123	0.99	0.047	755	35

Stationary phase	<i>e</i>	<i>s</i>	<i>a</i>	<i>b</i>	<i>v</i>	<i>c</i>	r^2	S.E.	<i>F</i>	<i>n</i>
TAG (MeOH)	0.533	0.356	-0.535	-1.148	1.958	-1.190	0.99	0.058	535	36
V (MeOH)	0.369	0.279	-0.564	-0.458	1.223	-1.193	0.98	0.052	437	35

e: parameter related to interaction with the solute through π - or n-electron pairs; *s*: parameter related to dipole- or induced dipole-type interactions; *a*: system parameter related to the stationary phase H-bond basicity; *b*: parameter measuring the stationary phase H-bond acidity; *v*: dispersive and cavity formation energy between mobile and the stationary phase; *c*: regression intercept; r^2 : regression coefficient; S.E.: regression standard error; *F*: Fisher statistic index; *n*: number of solutes in the regression.

Table 4

LSER system parameters for the teicoplanin CSP (Chirobiotic T) stationary phase in the normal phase mode (ethanol/heptane mobile phases) and for the four CSPs with 100% heptane mobile phases

Mobile phase	<i>e</i>	<i>s</i>	<i>a</i>	<i>b</i>	<i>v</i>	<i>c</i>	<i>r</i> ²	S.E.	<i>F</i>	<i>n</i>
Teicoplanin chiral stationary phase										
25% EtOH	0	0.56	0.144	1.455	-0.826	-0.57	0.97	0.09	432	46
		0.049	0.037	0.064	0.049	0.061				
20% EtOH	0	0.573	0.233	1.52	-0.848	-0.529	0.98	0.093	459	46
		0.05	0.038	0.066	0.05	0.063				
15% EtOH	0	0.615	0.352	1.629	-0.896	-0.529	0.98	0.104	442	46
		0.057	0.043	0.074	0.057	0.072				
10% EtOH	0	0.664	0.542	1.796	-0.979	-0.51	0.98	0.112	506	46
		0.061	0.047	0.08	0.061	0.077				
5% EtOH	0	0.743	0.911	2.107	-1.082	-0.566	0.98	0.125	515	44
		0.071	0.059	0.094	0.068	0.088				
0% EtOH ^a	0	0.831	1.302	2.450	-1.218	-0.537	0.98	0.130	530	44*
		0.069	0.049	0.091	0.062	0.078				
Stationary phase	<i>e</i>	<i>s</i>	<i>a</i>	<i>b</i>	<i>v</i>	<i>c</i>	<i>r</i> ²	S.E.	<i>F</i>	<i>n</i>
Log <i>k</i> _{heptane} : 100% heptane mobile phase										
R	0	0.803	0.980	2.568	-1.132	-0.428	0.98	0.122	559	45
T	0	0.831	1.302	2.450	-1.218	-0.537	0.98	0.130	530	44
TAG	0	0.871	0.991	2.736	-1.172	-0.495	0.99	0.109	704	42
V	0	1.135	1.280	2.029	-1.077	-0.671	0.99	0.100	775	37

e: parameter related to interaction with the solute through π - or n-electron pairs; *s*: parameter related to dipole- or induced dipole-type interactions; *a*: system parameter related to the stationary phase H-bond basicity; *b*: parameter measuring the stationary phase H-bond acidity; *v*: dispersive and cavity formation energy between mobile and the stationary phase; *c*: regression intercept; *r*²: regression coefficient; S.E.: regression standard error; *F*: Fisher statistic index; *n*: number of normal phase solutes in the regression.

^a 0% EtOH means pure heptane; the system parameters were obtained using 44 log *k*_{heptane} values extrapolated to 0% EtOH.

Table 5

LSER solute descriptors of five molecular enantiomeric pairs and the corresponding Eq. (2) enantioselectivity coefficients obtained on the teicoplanin macrocyclic glycopeptide chiral stationary phase

Compound	<i>E</i>	<i>S</i>	<i>A</i>	<i>B</i>	<i>V</i>	<i>r</i> ²	S.E.	<i>n</i>
5-Methyl-5-phenyl-hydantoin	1.416	1.59	0	1.49	1.403	0.945	0.049	22
Bromacil	1.180	1.41	0	1.66	1.631	0.953	0.025	20
Dihydrofurocoumarin	1.034	1.19	0	0.59	1.663	0.945	0.026	23
1,1-Dimethyl-3-phenyl-propyl tolyl sulfoxide	1.935	0.40	0	1.67	2.392	0.932	0.037	22
α-Naphthalenyl methyl sulfoxide	2.145	1.55	0	0.19	1.448	0.952	0.058	22
Enantioselectivity on teicoplanin ^a								
	<i>eE</i>	<i>sS</i>	<i>aA</i>	<i>bB</i>	<i>vV</i>	log <i>α</i>	<i>n</i> _{refractive index}	
5-Methyl-5-phenyl-hydantoin ^a	0.5	-0.05	0	0.23	-0.42	0.26	1.61	
Bromacil	0.21	-0.01	0	0.08	-0.22	0.06	1.56	
Dihydrofurocoumarin	0.11	0	0	0.11	-0.12	0.10	1.54	
1,1-Dimethyl-3-phenyl-propyl tolyl sulfoxide	0.21	0.01	0	0.14	-0.21	0.15	1.60	
α-Naphthalenyl methyl sulfoxide	0.45	0	0	0.36	-0.61	0.20	1.72	
5-Methyl-5-phenyl-hydantoin ^b	0.12	0.21	0	-0.10	0.24	0.47	1.61	

E: descriptor related to interaction with the stationary phase through polarizable bonds; *S*: descriptor related to dipole- or induced dipole-type interactions; *A*: descriptor related to the solute acidity; *B*: descriptor measuring the solute basicity; *V*: descriptor linked to the size of the solute (McGowan volume); *r*²: regression coefficient; S.E.: regression standard error; *n*: number of experiments in the regression/number of data points.

^a Mobile phase ethanol/buffer 75/25% (v/v) pH 4 with 10 mM triethylamine + acetic acid; *n*: solute refractive index calculated using the SPARC software (<http://ibmlc2.chem.uga.edu/sparc>).

^b Normal mobile phase heptane/ethanol 75/25% (v/v).