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# Liquid chromatographic enantiomer separations of novel quinazolone derivatives on quinine carbamate based chiral stationary phases using hydro-organic mobile phases

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**Abstract**

Quinine carbamate-type weak chiral anion-exchange selectors (SOs) and the respective chiral stationary phases (CSPs) have been used for the direct liquid chromatographic enantiomer separation of a wide range of chiral acids. In the present work, we demonstrate that these CSPs can also be extended to chiral discrimination of a set of neutral polar potential NMDA (*N*-methyl-D-aspartic acid) and/or AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) antagonist imidazo-quinazoline-dione derivatives (selectands, SAs) using acetonitrile and methanol containing hydro-organic and buffered mobile phases. The influence of mobile phase composition, column temperature and structure variation of the SAs and SOs on retention and enantioselectivity was systematically investigated to gain insight into the overall chiral recognition mechanism. As was expected for the reversed-phase mode, acetonitrile has a stronger eluotropic effect compared to methanol. Except for two analytes, the acetonitrile containing mobile phases provided baseline resolution ($R_S$) of the enantiomers with $R_S$ values ranging between 1.68 and 2.76. Using methanol as the organic modifier enhanced the enantioselectivity. The enthalpic and entropic terms for the SO–SA association were calculated from the linear van't Hoff plots. Data reveal that the enantiomer separations are predominantly enthalpically driven.


*Keywords:* Enantiomer separation; Chiral stationary phases, LC; Mobile phase composition; Quinazolone derivatives; Quinine carbamate

## 1. Introduction

Cinchona alkaloid carbamate type chiral selectors (SOs) and thereof derived chiral stationary phases (CSPs) have been previously developed for HPLC enantiomer separation of chiral acidic analytes (selectands, SAs), utilizing their anionic exchange properties [1]. These CSPs (see Fig. 1) offer several sites for stereoselective intermolecular interactions in the course of SA–SO complex formation, i.e. the aliphatic basic nitrogen of the protonated quinuclidine group for ion-pairing, the planar π-basic quinoline skeleton for π–π interactions, the hydrogen acceptor-donor site of carbamate functionality for dipole-dipole interaction and hydrogen-bond formation and the bulky substituents of the carbamate group and the large quinuclidine ring for hydrophobic as well as steric interactions. These SOs provide excellent chiral discrimination capability for a broad spectrum of chiral acids, as it has been reported earlier [2–8]. Especially of interest was the separation of the very weakly acidic binaphthols [2] with cinchona-based CSPs in a normal phase mode, using non-ion exchange type mobile phase conditions [9–11]. These presumably involved molecular interaction mechanisms associated with the Pirkle concept [12] relying predominantly on π–π and hydrogen bonding interactions. To date, however, not much emphasis has been given towards the enantiomer separation of

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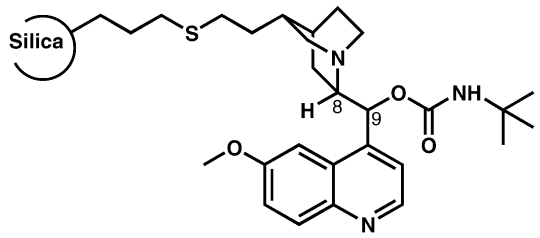
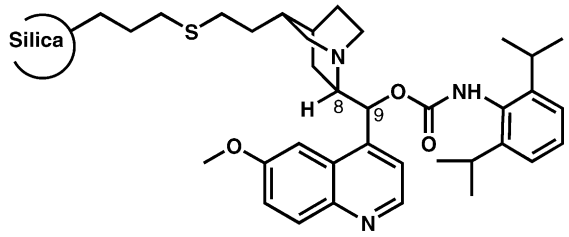



**CSP 1:** ProntoSIL Chiral AX QN-1 (8S,9R)
**CSP 2:** ProntoSIL Chiral AX QD-1 (8R,9S)




**CSP 3:** ProntoSIL Chiral AX QN-2 (8S,9R)

Fig. 1. Structures of chiral stationary phases: CSP 1 with *tert*-butyl carbamoylated quinine; CSP 2 with *tert*-butyl carbamoylated quinidine and CSP 3 with diisopropyl phenyl carbamoylated quinine as selector.

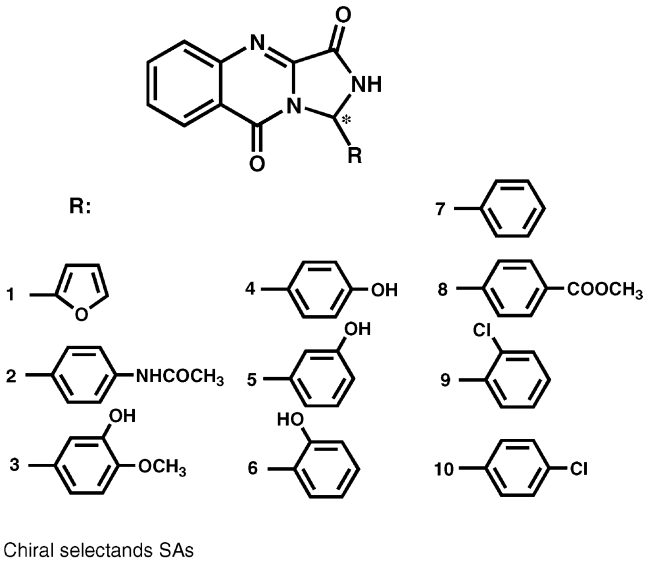



Fig. 2. Chemical structures of the investigated racemic imidazo-quinazoline-dione derivatives.

neutral analytes using the chinchona carbamate-based CSPs in reversed-phase mode, even though the columns work exceptionally well in polar organic and reversed-phase mode for enantiomer separation of chiral acids [13]. This flexibility, however, opens up many possibilities for adjusting retention and enantioselectivity of the given analytes to a large extent by changing pH, ionic strength and organic modifier content of the mobile phase. Other advantages of these CSPs are their exceptionally high chemical stability and loadability for acidic analytes, allowing for preparative separation of the individual stereoisomers in a straightforward fashion [14]. It is well known that the interest in chromatographic separations of enantiomers on preparative and even process scale keeps strongly increasing, mostly due to the advantages with regards to speed, costs, flexibility and efficiency compared to alternative synthesis methods [15].

The main goal of this contribution is to demonstrate the applicability of the chinchona carbamate SO and CSPs also for the enantiomer separation of neutral analytes; thus extending their applicability to direct chiral separation of neutral polar compounds of pharmaceutical and pharmacological interest.

The target compounds of this contribution, the quinazolones are considered to be important heterocyclic substances with diverse biological and pharmacological activities depending on the position and properties of the ring substituents [16–20]. Recently, a set of novel imidazo[1,5-*b*]-quinazoline-1,5-diones (see Fig. 2), as potential NMDA and/or AMPA antagonists has been synthesized in our laboratory.

In this study, the stereodiscriminating capability of three quinine/quinidine carbamate-type CSPs as outlined in Fig. 1 will be investigated for the enantiomer separation of a set of neutral polar imidazo-quinazoline-dione derivatives using hydro-organic mobile phases. In this context, detailed focus will be given also to structure–resolution relationships along with investigating mechanistic and methodological aspects, such as temperature effects on the overall enantioselectivity.

## 2. Experimental

### 2.1. Materials

The investigated racemic imidazo[1,5-*b*]-quinazoline-1,5-diones, summarized in Fig. 2, were synthesized according to a described method [21]. The chemical purity of the samples was controlled by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) in normal and reversed-phase mode.

For the chromatographic experiments, acetonitrile (ACN) and methanol (MeOH) were of HPLC grade and purchased from Merck (Darmstadt, Germany). Ammonium acetate and acetic acid were of analytical grade obtained from Sigma–Aldrich (Budapest, Hungary). Double distilled water was used for all measurements.

### 2.2. Instrumentation

The chromatographic system consisted of an ISCO M 2350 (Lincoln, NE, USA) pump, equipped with a Rheodyne M 7125 (Cotati, CA, USA) injector unit with a 10 μl

loop, a JASCO UV-975 (Tokyo, Japan) variable wavelength UV–VIS detector and JASCO Borwin data software for data evaluation.

The chiral stationary phases and respective columns, ProntoSIL Chiral AX QN-1; ProntoSIL Chiral AX QN-2 and ProntoSIL Chiral AX QD-1 (150 mm × 4.0 mm i.d., 5 μm particle size, respectively) (Fig. 1) were from Bischoff Chromatography (Leonberg, Germany). Unless otherwise stated, the HPLC columns were operated at a constant temperature of 25 °C using a Jones Chromatography M 7955 (Hengoed, UK) column thermostat. For the temperature dependence study, the column was immersed into an Ultraterm 6000383 (Selecta, Barcelona, Spain) water-bath thermostat with isothermal conditions (±0.1 °C precision). To obtain stable baseline signal and reproducible retention times for the consecutive injections, the chromatographic system was equilibrated by passing ∼50 column volumes of mobile phase through the column prior to analysis. Retention times were determined as mean values of duplicate injections.

The apparent pH ($pH_a$) of the mobile phase was measured with a Radiometer PHM 93 pH meter (Lyon, France) in the aqueous/organic mixture.

### 2.3. Chromatographic conditions

The mobile phases were prepared by mixing the indicated volume of ammonium acetate buffer and solvents, i.e. MeOH or ACN with a total buffer concentration of 0.1 M, unless otherwise stated. The apparent $pH_a$ was adjusted to 6.0 by addition of appropriate amounts of glacial acetic acid. The analytes were dissolved in MeOH giving a sample concentration of 0.5 mg/ml. Mobile phases were filtered through a 0.45 μm Nylon membrane filter and degassed by sonication prior to use. A flow rate of 1 ml/min and a UV detection wavelength of 250 nm were used throughout the study.

### 2.4. Modeling and calculations

Semiempirical (PM3) MO calculations were carried out using the HYPERCHEM program package. Tripos force field and the SYBYL gridsearch routine on Silicon Graphics Indigo Solid Impact workstation were used for molecular mechanics studies. ACD Labs/log *P* DB 7.0 software version was applied for log *P* calculations.

## 3. Results and discussion

The employed chinchona alkaloid-based CSPs belong to the brush type phases considered as weak chiral anion-exchangers showing strong enantioselectivity for chiral acids. In the present study the expansion of the CSPs towards the enantiomer separation of a set of neutral polar analytes was investigated using hydro-organic buffered and non-buffered mobile phase conditions.

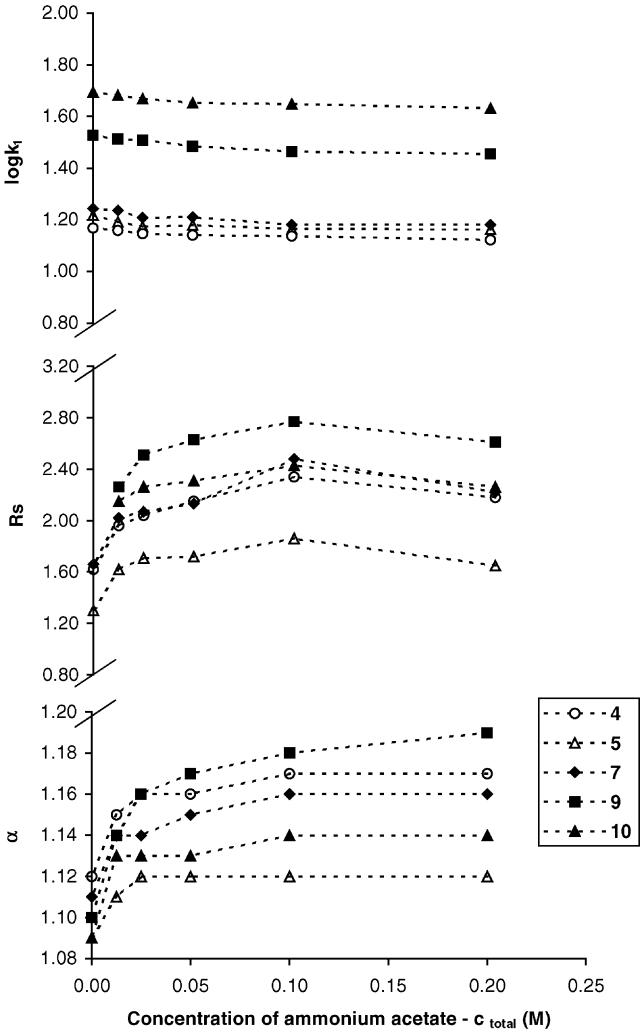



Fig. 3. Influence of buffer concentration of the mobile phase on retention factor ($k_1$), resolution ($R_S$) and enantioselectivity factor ($\alpha$) of selected analytes on CSP 1. $R_S$ values are calculated from $R_s = 1.18(t_{R_2} - t_{R_1})/(w_{h1} + w_{h2})$, where $w_{h_1}$ and $w_{h_2}$ are peak widths at half height of the first and second eluted peaks expressed in the retention time. Chromatographic conditions: mobile phase: ammonium acetate ($pH_a$ 6.0):acetonitrile 85:15 (v/v); indicated values of buffer concentration refer to the total buffer concentration in the mobile phase. Other conditions given in Section 2.

### 3.1. Influence of the buffer concentration of the mobile phase

As first aspect, the influence of buffer concentration, in particular of ammonium acetate, in the mobile phase on retention factor ($k$), enantioselectivity ($\alpha$) and resolution ($R_S$) of the SAs was investigated on the *tert*-butylcarbamoyl quinine-based CSP 1. The results obtained are summarized in Fig. 3. As expected, for the separation of neutral analytes, the buffer concentration has little effect on the retention factors of the samples at non-overloaded conditions. However, it is worth noting that both resolution and enantioselectivity are somewhat effected by ionic strength of the mobile phase, which indicates the participation of weak SO–SA hydrogen bonding

and dipole–dipole type interactions. For further investigation 0.1 M total buffer concentration was chosen as it turned out to be a reasonable compromise.

### 3.2. Effect of type and content of organic modifier

Under reversed-phase conditions, the van der Waals type SO–SA interactions are assumed to be affected most by the type and concentration of organic solvent in the aqueous mobile phase which can be related also to the solvation and desolvation effects of SO–SA binding events. One of the main goals of this investigation was to elucidate the possible contribution of the most frequently used modifiers, ACN and MeOH, to the overall chromatographic performance. In Tables 1 and 2 the chromatographic data obtained on CSP 1 are summarized as function of ACN and MeOH concentrations, respectively. The data reveal that the retention of the most analytes on this CSP strongly depends in the first glance on the overall "hydrophobicity" (see the calculated log *P* values in Table 1) and on the bulkiness and aromatic character of the substituent in the C-3 position of the imidazolone moiety (see Fig. 2). However, the retention factor does not change parallel with log *P* for analyte **7** and **9** in the presence of acetonitrile and methanol containing mobile phases. These observations emphasize that besides hydrophobic effect, additional simultaneous interactions between SO–SA complexes must play a crucial role in the binding mechanism. The lowest retention factor *k* was obtained for the most hydrophilic derivative (**1**), containing a furyl substituent in the stereogenic center, with exception for mobile phases of ammonium acetate: ACN 75:25 and 70:30 (v/v). Higher retention factors were achieved for analytes bearing more hydrophobic character; determined by the *R* substituent. Reasonably high *k* values and resolution $R_S$ factors were gained for the chloro-phenyl substituted analytes (**9**, **10**); having π acidic character. These are believed to interact favorably with the π basic quinoline moiety of the SO. A similar result was obtained for compound **8**; bearing methoxy-carbonyl functionality. These observations support the crucial importance of electron donor–acceptor and π–π type interactions between SA–SO complexes in retention and chiral recognition mechanism. It is worth noting that for analyte **9**, **10** in all cases **10** has higher $k_1$ and lower $R_S$ values than **9**, with exception at a mobile phase of ammonium acetate (0.1 M): acetonitrile 95:5 (v/v).

By increasing the organic modifier content in the mobile phase, the retention factors decreased in all cases as a general trend. These results indicate that the sum total of interactions between the SAs and the CSP are weakened in accordance with a reversed-phase type retention mechanism; dominated by hydrophobicity increments. Therefore, using a constant ionic strength of 0.1 M ammonium acetate, the enantiomers of imidazo-quinazoline-dione derivatives were found to follow linear relationships between the logarithm of the retention factor *k* and the volume fraction *Φ* of the organic modifier, according to the general equation characteristic for reversed-phase systems:

$$\log k = \log k_0 - S\Phi; \quad (1)$$

where $k_0$ is the retention factor of the given enantiomer in the absence of organic modifier and *S* is the solvent strength parameter. Fig. 4a and b depict the experimental log $k_1$ versus *Φ* plots for acetonitrile and methanol, respectively, which are linear in the concentration range used with a correlation coefficient higher than 0.9855. The slopes of these plots are

Table 1
Retention factor ($k_1$), enantioselectivity (α) and resolution ($R_S$) data for the enantiomers of analyte **1**–**10** as a function of ACN concentration

| Analyte | Calculated log *P*[a] | 0.1 M ammonium acetate ($pH_a$ 6.0):ACN (v/v) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 95:5 | 90:10 | 85:15 | 80:20 | 75:25 | 70:30 |
| **1** | 1.10 ± 0.69 | | | | | | |
| $k_1$ | | 27.6 | 15.8 | 9.16 | 4.43 | 2.59 | 1.56 |
| α | | 1.16 | 1.14 | 1.13 | 1.11 | 1.08 | 1.00 |
| $R_S$ | | 1.19 | 2.08 | 1.87 | 1.21 | 1.04 | – |
| **2** | 1.21 ± 0.69 | | | | | | |
| $k_1$ | | 48.9 | 23.1 | 11.6 | 4.89 | 2.45 | 1.46 |
| α | | 1.19 | 1.16 | 1.15 | 1.13 | 1.11 | 1.09 |
| $R_S$ | | 1.71 | 2.00 | 1.89 | 1.39 | 1.15 | 1.18 |
| **3** | 1.24 ± 0.69 | | | | | | |
| $k_1$ | | 46.2 | 21.5 | 11.7 | 5.37 | 2.69 | 1.71 |
| α | | 1.24 | 1.20 | 1.18 | 1.16 | 1.12 | 1.11 |
| $R_S$ | | 2.10 | 2.56 | 2.29 | 1.59 | 1.21 | 1.10 |
| **4** | 1.20 ± 0.69 | | | | | | |
| $k_1$ | | 40.3 | 23.9 | 13.5 | 6.04 | 3.02 | 2.06 |
| α | | 1.22 | 1.19 | 1.18 | 1.16 | 1.14 | 1.12 |
| $R_S$ | | 1.49 | 2.60 | 2.46 | 1.74 | 1.57 | 0.80 |
| **5** | 1.20 ± 0.69 | | | | | | |
| $k_1$ | | 39.2 | 27.9 | 13.7 | 5.74 | 3.59 | 2.24 |
| α | | 1.16 | 1.14 | 1.12 | 1.11 | 1.09 | 1.07 |
| $R_S$ | | 1.19 | 2.15 | 1.86 | 1.27 | 1.27 | 0.57 |
| **6** | 1.20 ± 0.69 | | | | | | |
| $k_1$ | | 42.6 | 31.2 | 15.6 | 7.21 | 4.32 | 2.86 |
| α | | 1.08 | 1.08 | 1.07 | 1.07 | 1.05 | 1.00 |
| $R_S$ | | 1.08 | 1.25 | 1.19 | 0.95 | 0.88 | 0.80 |
| **7** | 1.94 ± 0.69 | | | | | | |
| $k_1$ | | 37.0 | 26.4 | 15.1 | 7.39 | 3.99 | 2.32 |
| α | | 1.20 | 1.17 | 1.16 | 1.14 | 1.12 | 1.11 |
| $R_S$ | | 1.40 | 2.56 | 2.48 | 1.79 | 1.61 | 1.10 |
| **8** | 1.92 ± 0.69 | | | | | | |
| $k_1$ | | 97.3 | 57.5 | 25.8 | 10.6 | 4.73 | 2.84 |
| α | | 1.15 | 1.12 | 1.11 | 1.10 | 1.08 | 1.06 |
| $R_S$ | | 1.28 | 1.68 | 1.65 | 1.29 | 1.11 | 0.56 |
| **9** | 2.54 ± 0.69 | | | | | | |
| $k_1$ | | 74.2 | 48.6 | 26.2 | 10.9 | 6.22 | 3.65 |
| α | | 1.23 | 1.19 | 1.18 | 1.16 | 1.14 | 1.14 |
| $R_S$ | | 1.41 | 2.76 | 2.73 | 2.24 | 2.01 | 1.13 |
| **10** | 2.54 ± 0.69 | | | | | | |
| $k_1$ | | 139.5 | 78.9 | 37.0 | 15.4 | 8.08 | 4.58 |
| α | | 1.19 | 1.15 | 1.14 | 1.12 | 1.10 | 1.09 |
| $R_S$ | | 1.92 | 2.45 | 2.43 | 1.78 | 1.59 | 0.89 |

CSP: CSP 1; flow rate: 1 ml/min; temperature: 25 °C; UV detection 250 nm; other conditions given in Section 2.
[a] See Section 2.

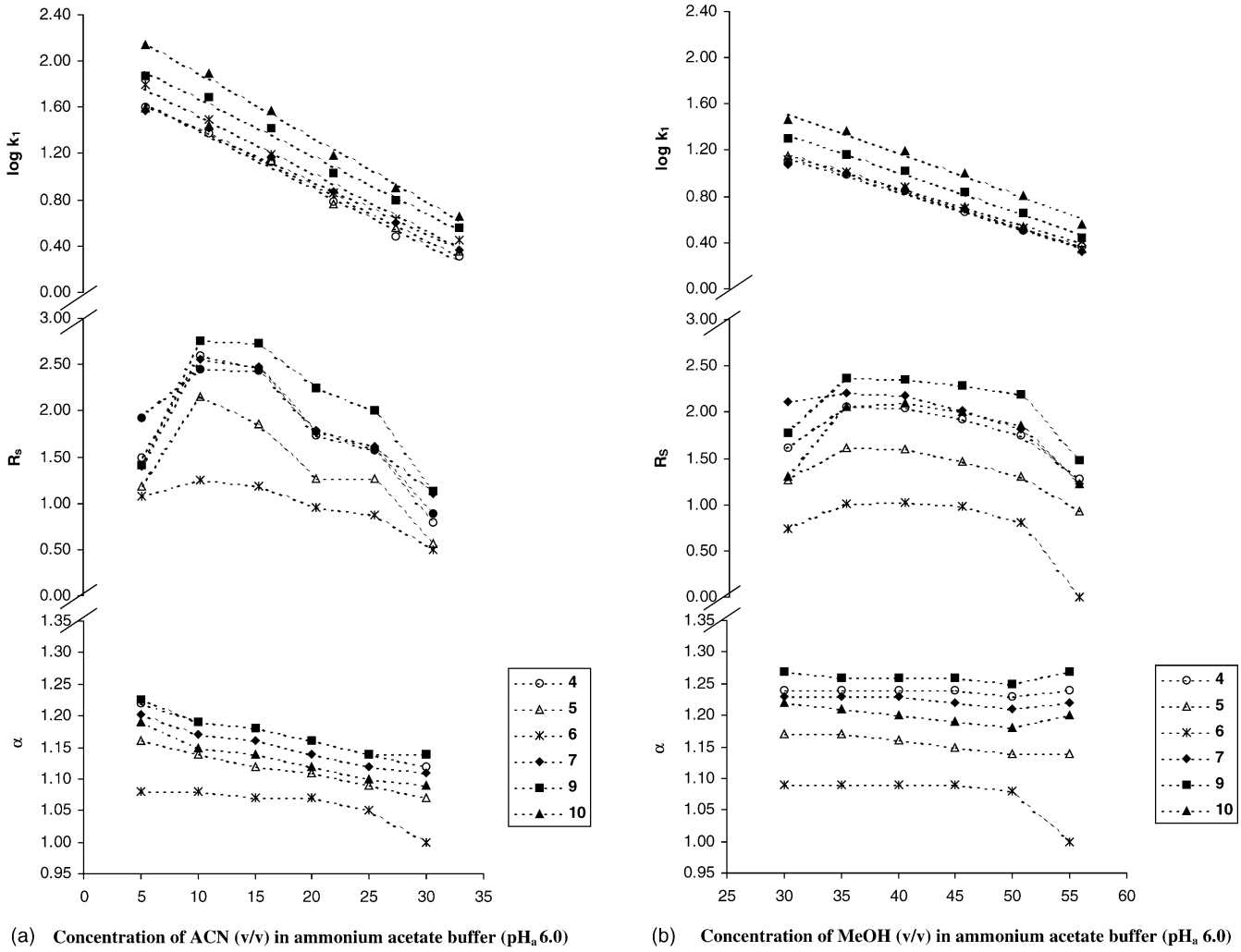



Fig. 4. Influence of acetonitrile (a) and methanol (b) content of the mobile phase on retention factor ($\log k_1$), resolution ($R_S$) and enantioselectivity factor ($\alpha$) of selected analytes on CSP 1. Chromatographic conditions given in Section 2.

dependent on the type of modifier, indicating that each modifier has a different influence on the overall retention in this chromatographic system. Similarly, they have distinct effect on the resolution and enantioselectivity as well. According to Fig. 4a and b, the resolution $R_S$ shows maximum for both ACN and MeOH in the concentration range studied, whereas the enantioselectivity is slightly decreased in the presence of ACN compared to MeOH. Under more or less isoeluotropic conditions (i.e. 20% (v/v) of ACN and 40% (v/v) of MeOH in 0.1 M ammonium acetate ($pH_a$ 6.0)), methanol provides slightly enhanced resolution and enantioselectivity for all compounds. In the presence of 10% (v/v) of ACN, except for analyte **6**, the enantiomers of imidazo-quinazolinediones could be baseline separated with $R_S$ ranging between 1.68 and 2.76. This clearly reveals that methanol and acetonitrile differ in selectivity, as well as with regards to the kinetics of the adsorption/desorption processes.

Using a constant total buffer concentration in the mobile phase, acetonitrile has stronger eluotropic effect than methanol in the reversed-phase mode; e.g. $S \approx 5$ for acetonitrile and $S \approx 3$ for methanol as solvent. Thus, the $S$ value obtained for ACN is much higher than described in the literature for reversed-phase systems (e.g. $S \approx 3$). Data obtained for MeOH are in good accordance with the previous findings [22]. Acetonitrile is a weak hydrogen-bond acceptor, is polarizable and interacts mainly via electron donor–acceptor (π–π) and van der Waals interactions with the SA and CSP, respectively. The solvent strength values $S$ reflect that ACN should have a strong effect on diminishing the intermolecular associations between SAs and *tert*-butyl carbamoyl quinine complex by weakening the π–π interactions between the aromatic system of the corresponding enantiomers and the quinoline ring of the SO. It can be expected that the slopes of $\log k_1$ versus $\Phi$ plots are somewhat higher for analytes bearing larger hydrophobic fragments in the presence of both modifiers. Accordingly, the largest $S$ value of 6.15 and 3.64 for ACN and MeOH, respectively, was obtained for compound **10**, which contains a *para*-chloro substituted aromatic ring close to the stereogenic center. For the corresponding *ortho*-substituted analyte **9**, the $S$ values are decreased,

Table 2
Retention factor ($k_1$), enantioselectivity ($\alpha$) and resolution ($R_S$) data for the enantiomers of analyte **1**–**10** as a function of MeOH concentration

| Analyte | 0.1 M ammonium acetate ($pH_a$ 6.0):MeOH (v/v) | | | | | |
|---|---|---|---|---|---|---|
| | 70:30 | 65:35 | 60:40 | 55:45 | 50:50 | 45:55 |
| **1** | | | | | | |
| $k_1$ | 7.69 | 6.21 | 4.51 | 3.11 | 2.14 | 1.51 |
| $\alpha$ | 1.16 | 1.16 | 1.15 | 1.14 | 1.13 | 1.13 |
| $R_S$ | 1.21 | 1.53 | 1.42 | 1.26 | 1.13 | 0.77 |
| **2** | | | | | | |
| $k_1$ | 10.6 | 8.59 | 5.90 | 3.98 | 2.59 | 1.78 |
| $\alpha$ | 1.22 | 1.22 | 1.22 | 1.22 | 1.20 | 1.23 |
| $R_S$ | 1.41 | 1.81 | 1.81 | 1.69 | 1.53 | 1.11 |
| **3** | | | | | | |
| $k_1$ | 10.3 | 8.97 | 6.31 | 4.23 | 2.84 | 2.03 |
| $\alpha$ | 1.25 | 1.24 | 1.24 | 1.23 | 1.21 | 1.23 |
| $R_S$ | 1.77 | 2.18 | 1.84 | 1.65 | 1.69 | 1.21 |
| **4** | | | | | | |
| $k_1$ | 12.5 | 9.78 | 7.04 | 4.69 | 3.23 | 2.30 |
| $\alpha$ | 1.24 | 1.24 | 1.24 | 1.24 | 1.22 | 1.24 |
| $R_S$ | 1.61 | 2.06 | 2.04 | 1.92 | 1.75 | 1.28 |
| **5** | | | | | | |
| $k_1$ | 14.2 | 10.2 | 7.70 | 5.13 | 3.45 | 2.50 |
| $\alpha$ | 1.17 | 1.17 | 1.16 | 1.15 | 1.14 | 1.14 |
| $R_S$ | 1.26 | 1.62 | 1.60 | 1.46 | 1.30 | 0.93 |
| **6** | | | | | | |
| $k_1$ | 13.3 | 10.4 | 7.63 | 5.02 | 3.39 | 2.20 |
| $\alpha$ | 1.09 | 1.09 | 1.09 | 1.09 | 1.08 | 1.00 |
| $R_S$ | 0.74 | 1.01 | 1.02 | 0.98 | 0.81 | – |
| **7** | | | | | | |
| $k_1$ | 11.8 | 10.1 | 7.15 | 4.89 | 3.22 | 2.11 |
| $\alpha$ | 1.23 | 1.23 | 1.23 | 1.22 | 1.20 | 1.23 |
| $R_S$ | 2.11 | 2.20 | 2.18 | 2.02 | 1.81 | 1.22 |
| **8** | | | | | | |
| $k_1$ | 20.5 | 15.9 | 11.9 | 7.09 | 3.82 | 2.65 |
| $\alpha$ | 1.17 | 1.17 | 1.16 | 1.15 | 1.15 | 1.16 |
| $R_S$ | 1.15 | 1.49 | 1.49 | 1.42 | 1.27 | 0.96 |
| **9** | | | | | | |
| $k_1$ | 19.8 | 14.7 | 10.4 | 6.87 | 4.51 | 2.73 |
| $\alpha$ | 1.27 | 1.26 | 1.26 | 1.26 | 1.24 | 1.27 |
| $R_S$ | 1.77 | 2.37 | 2.36 | 2.29 | 2.19 | 1.48 |
| **10** | | | | | | |
| $k_1$ | 29.2 | 23.1 | 15.6 | 9.99 | 6.34 | 3.67 |
| $\alpha$ | 1.22 | 1.21 | 1.20 | 1.19 | 1.18 | 1.20 |
| $R_S$ | 1.31 | 2.06 | 2.10 | 2.01 | 1.85 | 1.22 |

CSP: CSP 1; flow rate: 1 ml/min; temperature: 25 °C; UV detection 250 nm; other conditions given in Section 2.

especially in the presence of ACN ($S$ = 5.49 and 3.44 for ACN and MeOH, respectively). When introducing a hydroxyl group (analyte **4**–**6**) onto the aromatic ring substituent of compound **7**, no remarkable effect on retention could be detected using ACN content mobile phases ($S$ values are the follows 5.30, 5.43, 5.29 and 5.50 for compound **7** and **4**–**6**, respectively). Regarding the calculated log $P$ values (i.e. 1.94 ± 0.69 for analyte **7** and 1.20 ± 0.69 for SAs **4**–**6**), these findings reflect that beyond van der Waals and π–π interactions, additional hydrogen bond formations may become active in

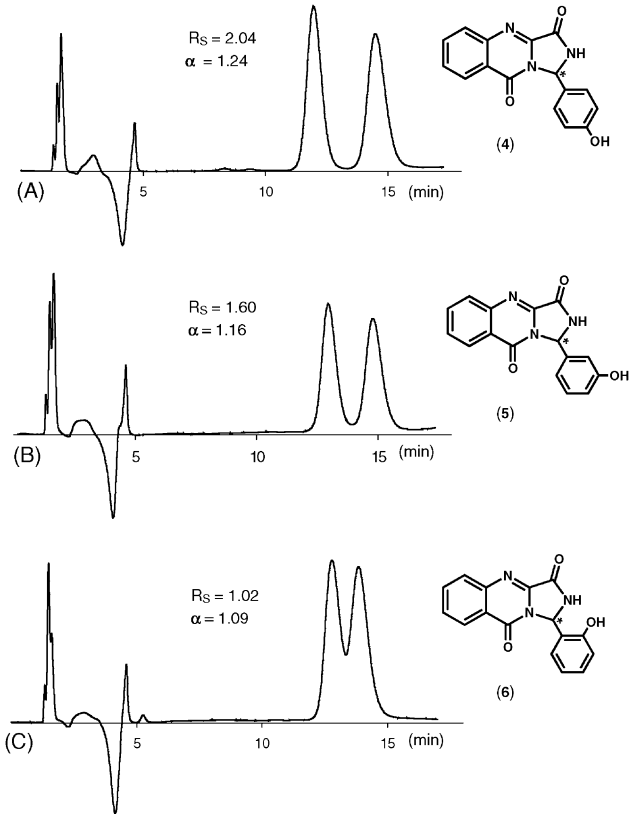



Fig. 5. Effect of the position of phenolic hydroxyl group on the enantiomer separation of the 3-(hydroxy-phenyl) substituted compounds **4**–**6** on CSP 1. Chromatographic conditions: ammonium acetate (0.1 M; $pH_a$=6.0):methanol 60:40 (v/v); other conditions given in Section 2.

the overall retention mechanisms for analyte **4**–**6**. Similar tendency could be established for mobile phases comprising methanol, as the slope obtained $S$ for the aromatic ring derivative (**7**) was 3.09, for *para*-hydroxy substituted analyte (**4**) is 3.03, for the corresponding *meta*-substituted derivative (**5**) was 3.06 and for *ortho*-hydroxy substituted compound (**6**) was 3.17. However, for enantioselectivity a marked effect could be observed for the position of phenolic OH group as depicted in Fig. 5. The SAs **4** and **5** were baseline resolved using 40% (v/v) of MeOH and also 20% (v/v) of ACN in acetate buffer (0.1 M), whereas analyte **6** could only be partially separated.

For interpretation of the chromatographic differences between *ortho*-; *meta*-; and *para*-hydroxy-phenyl analogues, molecular modeling by semiempirical MO calculations was used to predict conformational preferences of 3-(substituted-phenyl)-imidazo-quinazoline-diones.

The geometrical optimization of analyte **4** and **6** resulted in different global energy minimum conformation for the ring C (Fig. 6). The *para*-hydroxy-phenyl substituent of derivative **4** occupies a quasi-equatorial position on the nearly planar heterocyclic ring. The calculated dihedral angle ($\Phi$) between $N_2$–$C_3$–$C_{1'}$–$C_{2'}$ in the geometrically optimized structure was found to be 121.25°. As Fig. 6 shows, for *ortho*-hydroxy-phenyl derivative **6**, the nearly planar conformation of the

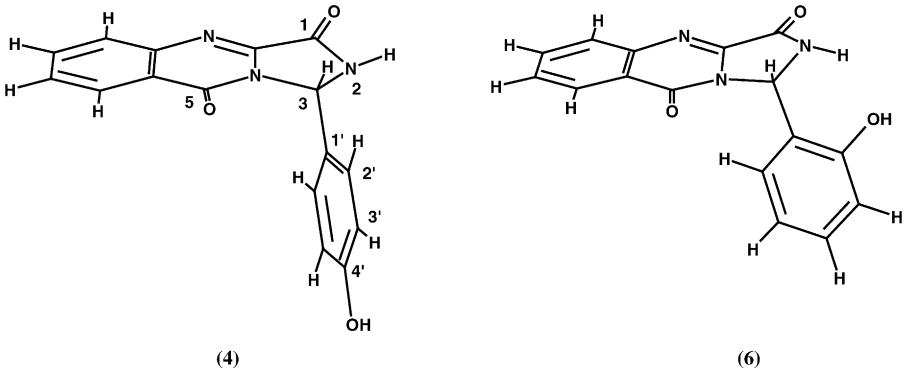



Fig. 6. Computer generated structure of *para*-hydroxy-phenyl **(4)** and *ortho*-hydroxy-phenyl substituted derivative **(6)** of imidazo-quinazoline-diones.

quite rigid C-ring has little changed as a result of steric rotation of the 3-phenyl group, offering the possibility for a hydrogen bonding interaction between the aromatic hydroxyl group and the lactam amide NH. The calculated dihedral angle ($\Phi$) between $N_2$–$C_3$–$C_{1'}$–$C_{2'}$ in the geometrically optimized structure was found to be 8.40°. The distance of the hydrogen bond between the amide NH and phenolic hydroxy group is 1.84 Å.

According to these data, for derivative **6**, a preferred intramolecular hydrogen-bonding interaction might take place, resulting in a significant decrease in the overall enantioselectivity. On the basis of the results obtained from molecular modeling study, it can be concluded that the chromatographic behavior of the hydroxy substituted SAs could be explained by different conformational structures of the analytes. Accordingly, these findings reveal again that intramolecular interactions and the resulting conformation have indispensable importance for achieving reasonable enantiomer separation of given analytes by given selectors and CSPs. This fact was also supported by testing the stereodiscriminating potential of different chinchona-derived CSPs using an ammonium acetate (0.1 M):MeOH 60:40 (v/v) mobile phase. As Table 3 shows, CSP 1 was the only selector, which provided baseline resolution of the SAs. Using the corresponding quinidine selector (CSP 2), both retention and enantioselectivity was reduced for all analytes, emphasizing a less optimal spatial arrangement around the binding cleft of the SO. As it was expected, on CSP 3, which contains the hydrophobic and bulky diisopropyl-phenyl substituent in the carbamate functionality, the retention factors were increased, especially for the easily polarizable chlorine substituted derivatives **9** and **10** and also for analyte **8**, as a consequence of strong π–π interactions. On the contrary, the enantioselectivity was strongly decreased in most cases, or was lost, for SA **1** and **6**, indicating that binding (expressed as retention factor) and chiral discrimination are entirely different events.

### 3.3. Effect of temperature on enantiomer separation

In general, determination of thermodynamic parameters is a useful approach for analyzing mechanistic aspects of retention and chiral discrimination on CSPs. The temperature dependence of the solute retention can be expressed in terms of retention factor with the thermodynamic equation given below:

$$\ln k = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln \Phi \; ; \tag{2}$$

where $k$ is the retention factor, $\Delta H$ and $\Delta S$ the molar enthalpy and entropy of transfer of the solute from the mobile phase to the stationary phase, respectively. $R$ the universal gas constant, $T$ the absolute temperature in Kelvin and $\Phi$ is the phase volume ratio. Combination of Eq. (2) with the Gibbs–Helmholtz Eq. (3) gives Eq. (4) as $\alpha = k_2/k_1$:

$$\Delta\Delta G = -RT \ln \alpha; \tag{3}$$

where $\Delta\Delta G$ is the difference in Gibbs free energy of association between SO and the enantiomers of a racemic compound (SAs).

$$\ln \alpha = -\frac{\Delta\Delta H}{RT} + \frac{\Delta\Delta S}{R} \tag{4}$$

On the basis of the van't Hoff Eq. (2), the plot of $\ln k$ versus $1/T$ should be a straight line with a slope of $-\Delta H/R$ and an intercept of $\Delta S/R + \ln \Phi$ when enthalpy and entropy changes are temperature independent. Fig. 7 shows the linear van't Hoff plots of compound **7** (with $r^2$ of 0.9995 for both enantiomers) using ammonium acetate (0.1 M; $pH_a$ = 6.0):

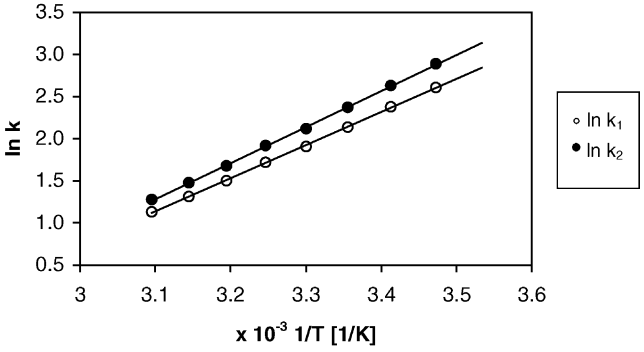



Fig. 7. van't Hoff plots of analyte **7** obtained on CSP 1. For chromatographic conditions see Table 4.

Table 3
Enantiomer separations for imidazo-quinazoline-dione derivatives on different cinchona alkaloid-type CSPs

| Analyte | CSP 1 | | | | CSP 2 | | | | CSP 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $k_1$ | $k_2$ | $\alpha$ | $R_S$ | $k_1$ | $k_2$ | $\alpha$ | $R_S$ | $k_1$ | $k_2$ | $\alpha$ | $R_S$ |
| **1** | 4.51 | 5.21 | 1.15 | 1.42 | 4.04 | 4.25 | 1.05 | 0.50 | 5.28 | – | 1.00 | – |
| **2** | 5.90 | 7.20 | 1.22 | 1.81 | 5.80 | 6.54 | 1.13 | 0.99 | 8.19 | 9.49 | 1.16 | 1.05 |
| **3** | 6.31 | 7.81 | 1.24 | 1.84 | 5.79 | 6.88 | 1.19 | 1.43 | 7.20 | 8.06 | 1.12 | 0.50 |
| **4** | 7.04 | 8.74 | 1.24 | 2.04 | 6.56 | 7.61 | 1.16 | 1.32 | 7.74 | 8.96 | 1.16 | 0.50 |
| **5** | 7.70 | 8.94 | 1.16 | 1.60 | 6.93 | 7.76 | 1.12 | 1.13 | 7.86 | 8.71 | 1.11 | 0.79 |
| **6** | 7.63 | 8.35 | 1.09 | 1.02 | 7.11 | 7.56 | 1.06 | 0.50 | 8.18 | – | 1.00 | – |
| **7** | 7.15 | 8.78 | 1.23 | 2.18 | 5.79 | 6.63 | 1.14 | 1.22 | 8.20 | 9.14 | 1.11 | 0.50 |
| **8** | 11.92 | 13.84 | 1.16 | 1.49 | 9.57 | 10.62 | 1.11 | 1.04 | 14.63 | 15.65 | 1.07 | 0.50 |
| **9** | 10.42 | 13.15 | 1.26 | 2.36 | 8.49 | 9.84 | 1.16 | 1.52 | 12.63 | 14.22 | 1.13 | 0.97 |
| **10** | 15.57 | 18.72 | 1.20 | 2.10 | 12.45 | 14.04 | 1.13 | 1.35 | 19.59 | 21.74 | 1.11 | 0.93 |

Mobile phase: ammonium acetate (0.1 M; $pH_a$ 6.0):MeOH 60:40 (v/v); other conditions given in Section 2.

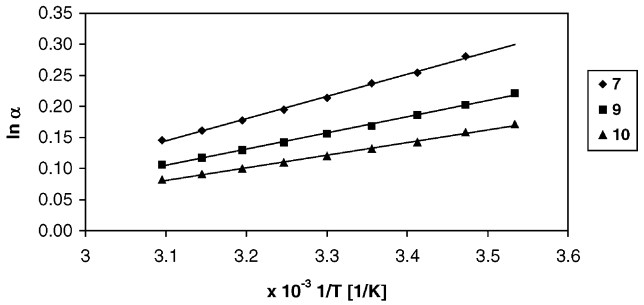



Fig. 8. van't Hoff plots of enantioselectivity ($\alpha$) obtained on CSP 1. For chromatographic conditions see Table 4.

MeOH 60:40 (v/v) as mobile phase. The corresponding thermodynamic parameters: $\Delta\Delta G$, $\Delta\Delta H$ and $\Delta\Delta S$ values could be calculated from the enantioselectivity according to Eqs. (3) and (4). The analysis of $\ln\alpha$ versus 1/T should also give linear plots with a slope of $-\Delta\Delta H/R$ and an intercept of $\Delta\Delta S/R$ provided that the molar differential enthalpy ($\Delta\Delta H$) and entropy ($\Delta\Delta S$) of the enantioselective adsorption are constant within the temperature range studied.

In the present study, the retention factors of compound **7**, **9**, and **10** were determined between 10 and 50 °C or 15 and 50 °C by 5 °C steps on CSP 1. In all cases, the retention factors decreased with increased temperature. For all selected analytes, linear plots ($\ln\alpha$ versus $1/T$) were obtained with a correlation coefficient $r^2$ higher than 0.9972 as depicted in Fig. 8. The thermodynamic parameters calculated by means of the equations above are listed in Table 4. The $\Delta\Delta S$ values were negative in all cases, indicating that the second eluted enantiomers have less degrees of freedom on binding to the SO and CSP compared to the first eluted enantiomers. The negative $\Delta H$ and $\Delta\Delta H$ values reflect that the transfer of the enantiomers from the mobile phase to the CSP is enthalpically favored for all selected analytes.

Table 4
Thermodynamic parameters $\Delta\Delta H$, $\Delta\Delta S$, $\Delta\Delta G$ and correlation coefficients ($r^2$) of the enantiomer separations

| Analyte | $\Delta\Delta H$ (kJ/mol) | $\Delta\Delta S$ (J/mol K) | $\Delta\Delta G^{298\,K}$ (kJ/mol) | $r^2$ |
|---|---|---|---|---|
| **7**[a] | −3.0 | −8.0 | −0.6 | 0.9961 |
| **9**[b] | −2.2 | −5.9 | −0.4 | 0.9972 |
| **10**[b] | −1.7 | −4.6 | −0.3 | 0.9970 |

Chromatographic conditions: stationary phase: CSP 1;. other conditions given in Section 2.
[a] Mobile phase: ammonium acetate (0.1 M; $pH_a$ 6.0):MeOH 60:40 (v/v).
[b] Mobile phase: ammonium acetate (0.1 M; $pH_a$ 6.0):ACN 80:20 (v/v).

## 4. Conclusions

Practical and mechanistic aspects of the retention and chiral discriminating ability of quinine carbamate type CSPs for a set of neutral polar imidazo-quinazoline-diones as potential NMDA and/or AMPA receptor antagonists were investigated. The influence of ionic strength of the mobile phase, type and concentration of organic additive, structural variation of analytes and column temperature on the overall enantiomer separations were analysed. The *tert*-butyl carbamoyl quinine SO proved to be suitable for the direct enantiomer separation of neutral analytes under hydro-organic conditions. Varying the structure of the SO, the chiral recognition was influenced significantly, indicating the importance of steric requirements for the binding sites. It was shown that the buffer concentration does not play a major role in the retention, however it has reasonable influence on overall enantioselectivity and efficiency. Acetonitrile has a stronger effect on elution than methanol; this supports an enhanced chiral discrimination process for most of the investigated analytes. Apart from van der Waals interactions and hydrogen bond formations, π–π interactions seem to play a crucial role in the enantiomer separation. Regarding the strong effect of the type and position of substituents on the phenyl ring, steric hindrance phenomena and conformational aspects seem to be responsible for the lack of enantiomer separation obtained for analyte **6**. These results were also demonstrated by molecular modeling. Thermodynamic analysis showed that enantiomer separation was predominantly enthalpically driven for the selected analytes at the temperature employed.

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