The Pursuit of Chiral Anion Exchange-Type Selectors: From Concept to Application

by Norbert M Maier, Chiral Technologies, Inc., 800 N Five Points Road, West Chester, PA 19380 USA.
Email: nmaier@chiraltech.com

The major progress achieved in liquid phase enantiomer separation technology and its crucial contributions to modern drug development efforts is well documented [1]. With drug discovery trends increasingly focusing on more polar "bio-like" target compounds, unmet needs concerning the resolution of polar ionizable and/or permanently charged chiral compounds are becoming apparent. Most of the established chiral stationary phases show poor chiral recognition performance for charged compounds, a situation that is often exacerbated by solubility issues, mobile phase incompatibilities and general lack of retention. In most cases, basic or acidic additives have to be used to suppress the ionic character of charged compounds to achieve efficient enantiomer separation, adding considerable levels of complexity and compromise to analytical and preparative method development.

Ion exchange-type chiral selectors capable of exploiting instead of suppressing the ionic nature of these compounds may provide an appealing solution to the problem. They can be expected to provide a number of striking benefits, such as simplified method development taking advantage of well understood retention mechanisms, and the compatibility with aqueous media, a feature that certainly facilitates the bioanalytical monitoring of charged compounds in biological fluids and the use of mass sensitive detection modes. In terms of preparative applications, ion exchange-type selectors are appealing because of their inherently strong intermolecular interactions, ensuring high loading capacities and broad mobile phase compatibility, factors that allow for flexibility in the development of highly productive separation processes with improved ecological performance characteristics.

Over the last two decades, major advances in this field of the development of broadly applicable ion exchange-type chiral selectors have been achieved by the group of Professor Wolfgang Lindner at the University of Vienna [2]. Lindner's research efforts in this area have not only contributed to a deepened understanding of molecular mechanisms governing enantioselective recognition of charged chiral molecules, but also established compelling evidence for the practical utility of chiral ion exchange-type selectors in addressing challenging real-world applications. The output of these efforts has provided scientists in pharmaceutical and industrial research settings with new chromatographic tools to tackle the challenges associated with analytical and preparative resolution of ionic chiral compounds.

The following review is an attempt to provide a brief overview of these accomplishments. Because of the limited format available for these discussions the treatment addresses only major milestones within the rich body of studies. Emphasis will be paced on demonstrating achievements by application-relevant examples rather than enumerating academic exercises. Readers interested in a more detailed coverage are referred to comprehensive reviews [2-4].

2. Cinchona Alkaloid Carbamate Anion-Type Selectors

A crucial aspect in the development of anion exchange-type selectors for chiral acids was the choice of appropriate molecular templates providing a high level of preformed chiral recognition complementarity for the target analytes. After evaluation of numerous synthetic scaffolds, Cinchona alkaloids, specifically quinine and quinidine, were selected as promising core structures. This choice was motivated by a number of considerations. Cinchona alkaloids offer basic quinuclidine functionality embedded in a stereochemically well-defined environment in close proximity to functional groups with rather diverse molecular interaction potential. In addition, Cinchona alkaloids are readily available at scale at reasonable cost. Cinchona alkaloids incorporate several functional groups readily accessible to chemical modification (C9-hydroxy and the C11 vinyl group), a feature that was considered essential for dedicated structure-based optimization of the chiral recognition potential and elaboration of suitable immobilization chemistries. And finally, quinine and quinidine provide well-documented pseudoenantiomeric behavior in many chiral recognition applications [5, 6], which allows controlling the preferences of Cinchona-mediated chiral recognition events by switching between the individual alkaloids.

Exploratory studies using chiral stationary phases incorporating native Cinchona alkaloids produced rather disappointing results. For example, when a CSP comprising native quinine attached via its C11 vinyl group to mercaptopropyl-modified silica gel was evaluated in buffered hydro-organic mobile phases very low levels of enantioselectvity ($\alpha <$ 1.2) for N-3,5-dinitrobenzoyl amino acids [7]. These observations were consistent with the results reported by others employing CSPs incorporating native cinchona alkaloids for normal phase applications [8]. It was concluded that the functional group repertoire integrated in native Cinchona alkaloids is insufficient to support effective enantioselective binding of acidic analytes. It was reasoned that the rather modest chiral recognition capabilities might be enhanced by providing supportive molecular interaction motifs by introduction of additional functional groups at the central C9-position. To test this hypothesis, a considerable number of focused libraries of Cinchona-based selectors were synthesized and evaluated in terms of their chiral separation performance under ion exchange

conditions. These classes of investigated Cinchona alkaloids covered in this effort included ester, carbamate, ether, amides, sulfonamide and hydrazide derivatives [2], and various types of Cinchona hetero- and homodimers joined by different types of bifunctional linkers [9, 10]. From this large-scale combinatorial effort, O9-carbamate derivatives of quinine and quinidine emerged as particularly efficient anion exchange-type selectors [11]. Further structure-based optimization studies within this class led to the development of tert-butylcarbamoyl quinine and quinidine as chiral stationary phases, providing an excellent compromise between broad applicability and high enantioselectivity [12]. These particular chiral stationary phases were found to very efficiently separate different classes of chiral compounds, including N-aryl, N-acyl and N-carbamoyl amino acids of the α-, β- and ψ-type, structurally related phosphoric, phosphinic and sulfonic acids, a large variety of N-protected peptides, and a broad assortment of other chiral acidic compounds of pharmaceutical and biological relevance [2]. A detailed discussion of these applications is beyond the scope of this account, but is comprehensively covered in [2]. Chiral stationary phases incorporating tert-butylcarbamoyl quinine and tert-butylcarbamoyl quinidine have been commercialized under the trade names CHIRALPAK QN AX and CHIRALPAK QD AX [13].

2.1. Chiral Recognition Mechanism

Cinchona carbamate-type selectors were found to produce exceptionally high levels of chiral recognition for N-acylated amino acids bearing pi-acidic substituents, e.g. a CSP based on tert-butylcarbamoyl quinine gave for N-3,5-dinitrobenzoyl amino acids in buffered hydro-organic mobile phase enantioselectivities up to α = 15. This exceptionally favorable selector-analyte combination was subsequently used as a suitable model system for the elucidation of molecular recognition mechanisms governing enantioselective binding of acidic analytes to cinchona carbamate-type selectors. This task involved a multidisciplinary approach, utilizing modern solution-phase and solid phase spectroscopic tools (IR [14,15], H-NMR [16-18], UV and CD [19], VCD [20]), molecular modeling approaches (molecular dynamics simulations [16-18] and density functional theory calculations [15]), thermodynamic studies (variable temperature chromatography [21], isothermal titration calorimetry [22]) and solid phase structure elucidation techniques, such as X-ray crystal structure analysis [12, 16-18]. The combined experimental evidence emerging from these studies provided a very detailed picture of the chiral recognition mechanism. The crucial structural requirements and intermolecular interaction forces governing

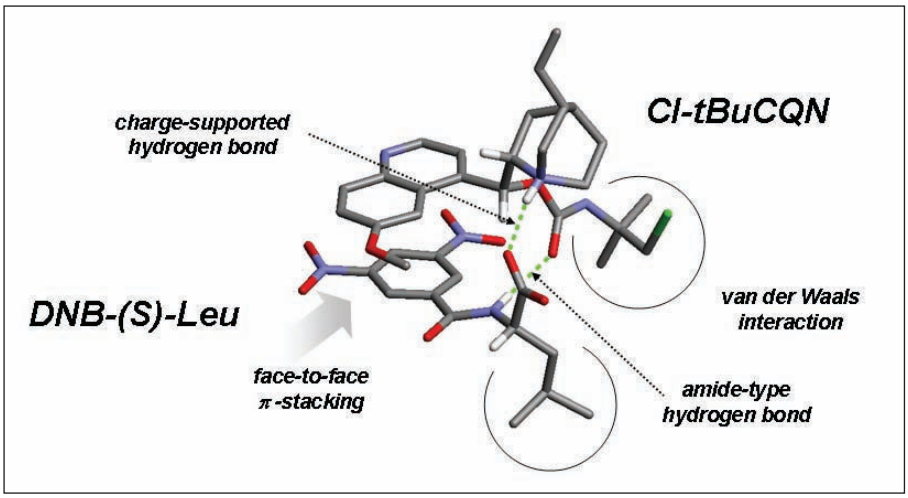

Figure 1. X-ray crystal structure of the more stable selector–selectand ion-pair complex of O-9-(-chloro-tert-butylcarbamoyl)quinine with N-(3,5-dinitrobenzoyl)-(S)-leucine. The intermolecular interactions contributing simultaneous and in co-operative fashion to the enantioselective binding process are indicated.

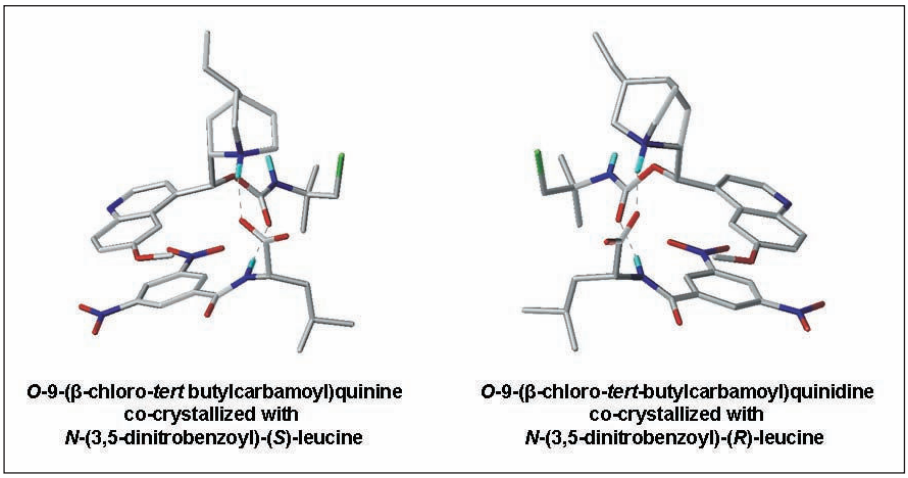

Figure 2. X-ray crystal structures of selector–selectand ion-pair complexes of (left) O-9-(β-chloro-tert butylcarbamoyl)quinine with N-(3,5-dinitrobenzoyl)-(S)-leucine, and (right) the pseudoenantiomeric complex of O-9-(β-chloro-tert-butylcarbamoyl)quinidine with N-(3,5-dinitrobenzoyl)-(R)-leucine. Note the mirror imiage-like relationship between the solid-phase structures of ion-pair. Adapted from [2] with permission.

these highly efficient enantioselective binding events are clearly evident from solid phase structures of the more stable diastereomeric ion-pair complex of appropriate model systems. Figure 1 exemplifies these important structural and functional features based upon the X-ray crystal structure of the O9-tert-butylcarbamoyl quinine with (S)-N-3-5-dinitrobenzoyl leucine.

It becomes evident that the Cinchona carbamate selector forms a tight ion pair-type complex with the acidic analyte, with the latter being situated within a stereochemically well-defined "binding pocket" and stabilized by multiple noncovalent intermolecular interactions. Specifically, complex stabilization is achieved by a charge-supported hydrogen bond existing between the protonated quinuclidine nitrogen at the selector and the deprotonated carboxylic group of the acidic analyte; an amide-type hydrogen bond established between the carbamate carbonyl group of the selector and the amide group of the analyte; face-to-face pi-pi stacking interactions occurring between the aromatic portions of the selector and selectand; and finally, subtle steric interactions between the bulky side chains of the selector and selectand. The solid phase structure also provides compelling evidence for the crucial contributions of the O9-carbamate group of the selector to chiral recognition event, fulfilling a dual function in providing an important stabilizing intermolecular interaction and as an essential structural element to the formation of a spatially well-defined binding site. The high level of enantioselectivity seen with this selector analyte system is the consequence of a steric exclusion process, with the mismatched enantiomer being evidently incapable of being accommodated within the selector binding site and experiencing stabilizing secondary interactions.

An X-ray crystal structure obtained for quinidine tert-butylcarbamate/(R)-N-3,5-dinitrobenzoyl leucine model system was

essentially a mirror-image of the structure discussed above, providing compelling evidence that pseudoenantiomeric molecular recognition characteristics of Cinchona alkaloids is also fully preserved at the molecular level [23] (Figure 2).

2.2. Retention Mechanisms

The chromatographic retention behavior of cinchona carbamate-type selectors for acidic analytes shows, independent from the mobile phase media, classical ion-exchange characteristics. Retention can be conveniently modeled on the basis of the well-established stoichiometric displacement model [24], predicting an inverse linear relationship between binding affinity and the concentration of a given counterion present as displacing species in the mobile phase. Provided that constant pH is maintained, the level of chiral discrimination of a given analyte generally remains uncompromised by changes of the counterion concentration in the mobile phase, allowing a convenient manipulation of analysis time without sacrificing enantioselectivty.

The nature of the employed counterion, however, was found to affect both retention and enantioselectivity [2]. Generally, the elution strength of the counterions in the RP mode decreases roughly in the order citrate > phosphate > formate > acetate. The impact of the nature of the counterion on enantioselectivity is difficult to generalize and appears to involve specific competitive interactions at the binding site of the selectors.

The impact of the mobile phase pH on the chromatographic performance characteristics of cinchona carbamate-type selectors can be readily rationalized considering that the quinuclidine unit serving as the active anion exchange functionality is a relatively weak base, rendering these selectors as weak anion exchanger systems [11]. Consequently, variations of the mobile phase pH value will affect the degree of protonation for both the basic quinuclidine integrated within the selectors and the acidic function in the analyte. Highest levels of chiral recognition and affinity are generally obtained at pH values ensuring the co-existence of maximum concentrations of protonated selector and deprotonated analyte species. With hydro-organic mobile phases and simple carboxylic acids this ideal pH value is in the range of pH 4 to 6. For acids with lower pKa values, such as phosphoric acids or sulfonic acids, the optimal pH values may be shifted to lower values.

An attractive feature of Cinchona carbamate anion exchange-type selectors is the fact that chiral recognition is not restricted to certain mobile phase environments [2]; successful enantiomer separation can be achieved in essentially all major mobile phase modes, including reversed phase (RP), polar organic (PO), nonpolar (NP) and even in supercritical fluid mobile phases.

In hydro-organic mobile phase environments cinchona alkaloid-based CSPs exhibit mixed-mode RP/weak anion exchange retention characteristics [11]. The relatively hydrophobic nature of cinchona carbamates along with the linker functionalities employed for immobilization renders the surface of these phases lipophilic, and expresses in water-rich mobile phase systems RP retention increments that contribute significantly to the global retention characteristics. At constant ionic strength and pH the global retention behavior follows trends consistent with the linear solvent strength theory, predicting an inverse relationship between log k and the volume fraction of organic modifier in the mobile phase. This hydrophobic retention increment can be effectively attenuated via an increase of the content of the organic modifier in the mobile phase. For most applications strong hydrophobic interactions are unfavorable as they add retention and generally compromise enantioselectivity. Enforcing hydrophobic interactions by using water-rich hydro-organic mobile phase systems, however, might be favorable for applications requiring enhanced levels of chemoselectivity, e.g., for the separation of complex (diastereomeric) mixtures [25].

For most applications, PO mobile phases, consisting of methanol and/or acetonitrile and a low concentration of acetic acid and ammonium acetate as displacing ionic species, are preferable over other RP mobile phase modes [2]. Specifically for highly lipophilic acidic analytes, PO mobile phases provide superior levels of enantioselectivity and fast elution through efficient suppression of non-specific interactions. Also, the lower viscosity of PO solvents as compared to water-rich RP mobile phases favors mass transfer and reduces column pressure drop, resulting in higher efficiencies and improved longevity of the column packing. Polar organic mobile phases composed of acetonitrile and/or methanol and volatile carboxylic acids, devoid of difficult to remove salt additives, are good options for preparative applications, facilitating product recovery.

Certain classes of cinchona-type selectors were found to operate successfully even with NP mobile phases systems. For example, a hybrid urea-linked epiquinine-calixarene-type CSP operated with a mobile phase composed of chloroform and acidic acid as an acidic displacer resolved the enantiomers of N-tert-butoxycarbonylproline with high levels of selectivity [26]. No elution was observed in the absence of acetic acid. Upon increasing the acetic acid concentration, linear ln *k* vs. ln[CH3COOH] dependencies were observed with this specific separation system, suggesting that anion-exchange processes may still operative even in relatively NP mobile phase environments.

Retention mechanisms related to those seen in NP mobile phase systems also may underlie the successful chiral separation of acidic analytes with cinchona carbamate-type CSPs in mobile phases composed of supercritical carbon dioxide and polar alcoholic modifiers. The separation of a mixture of the four diastereomers of a hydrophobic acidic drug intermediate could be achieved using a commercial quinine tert.-butylcarbamate CSPs in combination with methanol modified carbon dioxide [26] (Figure 3). It is interesting to note the elution of the acidic analyte could be effected without additives. This may be seen as evidence that carbon dioxide is sufficiently acidic to function as an efficient displacer for anion exchange applications.

2.3. Analytical Applications

2.3.1. HPLC Applications

A chiral stationary phase based quinine 09-tert-butylcarbamate has been successfully applied for the enantiomer separation of thyroid hormone (T4) thyroxin and its mono-deiodine analog triiodothyronine (T3) [25]. Sensitive and robust assays for the enantiomer

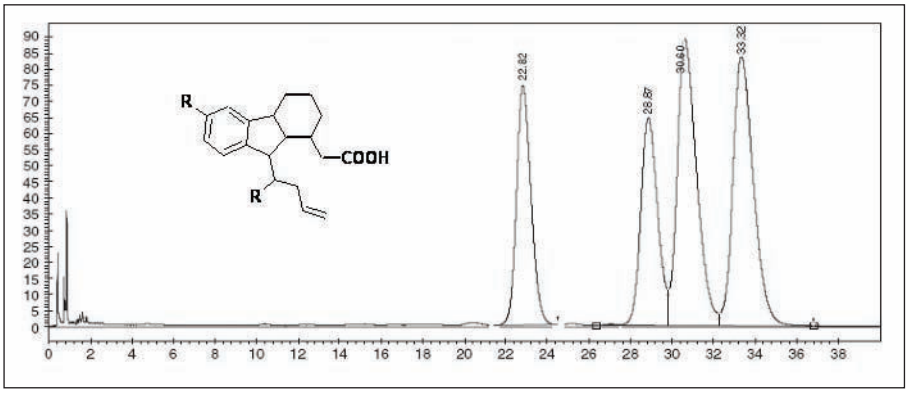

Figure 3. Separation of stereoisomers of an acidic drug intermediate on a CHIRALPAK QN-AX column under supercritical fluid conditions employing carbon dioxide methanol as mobile phase. Reproduced from [27] with permission.

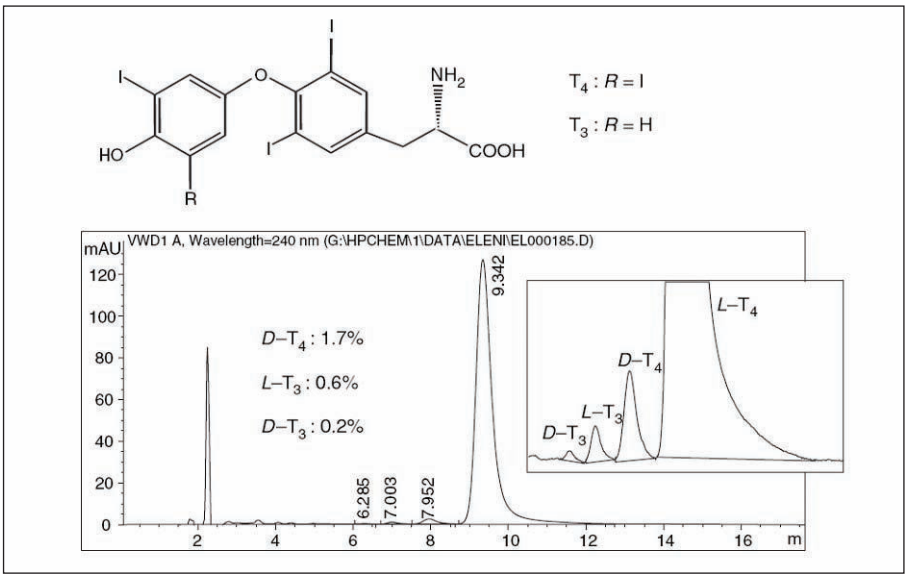

Figure 4. Enantioselective HPLC monitoring of impurities in a levothyroxin (L-T4) formulation. Experimental conditions: Column, Chiralpak QN-AX (150 mm × 4 mm ID); mobile phase, acetonitrile-50 mM ammonium acetate (60:40, v/v) (pHa 4.5); flow rate, 0.7 mLmin−1; UV detection, 240 nm; temperature, 25 C. Reproduced from [25] with permission.

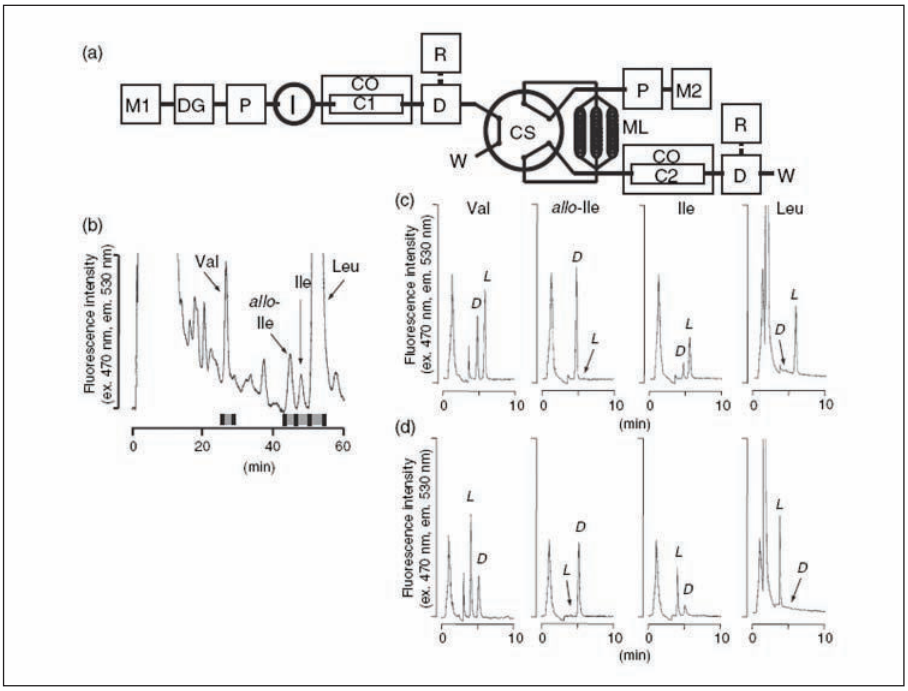

Figure 5. Enantioselective monitoring of hydrophobic D-amino acids as NBD-derivatives (obtained by precolumn derivatization with 4-fluoro-7-nitro-2,1,3-benzoxadiazole NBD-F) in rat tissue using an online 2-dimensional HPLC system combining RP18 and chiral anion-exchanger columns with fluorescence detection. Experimental conditions: (b) first dimension: M1: THF-TFA-H2O (25:0.05:75; v/v); flow rate, 75 µL min−1; C1: Capcellpak C18 MG II (150 mm × 1.0 mm ID, 40 C); (c) second dimension: M2: acetonitrile–methanol (50:50; v/v) containing 10 mM citric acid; flow rate, 1.5 mLmin−1; C2: Chiralpak QN-AX (150 mm × 4.0 mm ID, 40 ○ C); (d) same as (c) but C2: Chiralpak QD-AX (150 mm × 4.0 mm ID, 40 ○ C). Fluorescence detection, λex 470 nm, λem 530 nm. Legend: M1 and M2, mobile phase 1 and 2; C1 and C2, column 1 and 2; DG, degasser; P, pump; I, injector; CO, column oven; D, detector; R, integrator; W, waste; ML, multiloop trapping device; CS, column selection valve. Reproduced from [28] with permission.

purity of these compounds are mandatory as the individual thyroid hormones display very different biological activity spectra. While the L-enantiomer stimulates the metabolic rate and regulates growth and the development in infants and is used for the treatment of thyroid disorders, the D-enantiomer has seen applications as antihyperlipidemic agent.

Simultaneous separation of the enantiomers of T4 and T3 could be achieved using a hydro-organic mobile phase conditions. This robust method was subsequently employed to monitor the quality of levothyroxin in tablets. A typical chromatogram is depicted in Figure 4. It could be shown that the investigated formulation contained not only an significant amount of the undesired D-T4 (1.7%) but also significant amounts of both enantiomers of T3 amounts (0.6% and 0.2% of L- and D-T3).

Recently, Cinchona carbamate-type CSPs have been implemented as stereoselective separation tools for the monitoring of fluorescence-labeled D-amino acids in various mammalian tissues, employing a sophisticated 2D column switching method [25]. The operational scheme is given in Figure 5.

The sensitive detection and quantification of D-amino acids in biological samples are currently of considerable interest because of their relevance as potential disease biomarkers. After extraction from tissue samples and fluorescence labeling the amino acid derivatives were first chromatographed in an "achiral dimension" using a RP18 narrow bore column to achieve the chemoselective resolution of the four hydrophobic racemic amino acid derivatives of interest (Val, allo-Ile, Ile, Leu). Fractions of each peak were collected in a multiloop trapping device and then transferred sequentially into the second "chiral" chromatographic dimension, presented by either a Chiralpak QN-AX column or a Chiralpak QD-AX column, to achieve the separation of the enantiomers. The incorporation of both pseudoenantiomeric cinchona carbamate-type CSPs allowed reversal of enantiomer elution orders on demand, facilitating the peak integration of the minor enantiomers and thus the quality of the quantification. The 2D-method was fully validated for the concentration range between 0.005 and 0.5 pmol for D-amino acids and 0.05–5 pmol for L-amino acids, providing excellent performance characteristics. LODs and LOQs were reported to be as low as 3 fmol and 5 fmol, making this method to one of the most sensitive analysis method for amino acid enantiomers currently available for mammalian tissue samples.

2.3.2. Capillary Electrophoresis

Cinchona carbamate-type selectors have been shown to be highly efficient chiral background electrolyte (BGE) additives for the enantiomer separation of chiral acids via capillary electrophoresis (CE) [29]. The enantiomer separation by CE involves stereoselective ion-pair formation of oppositely charged cationic selector and anionic solutes, which leads to a difference of net migration velocities of both enantiomers in the electric field. Under acidic conditions a favorable countercurrent-like migration scenario of free (anodic direction) and complexed solute species (cathodic migration of ion-pairs with EOF) can be obtained, facilitating enantiomer separation. Ion-pair CE with cinchona carbamate-type chiral

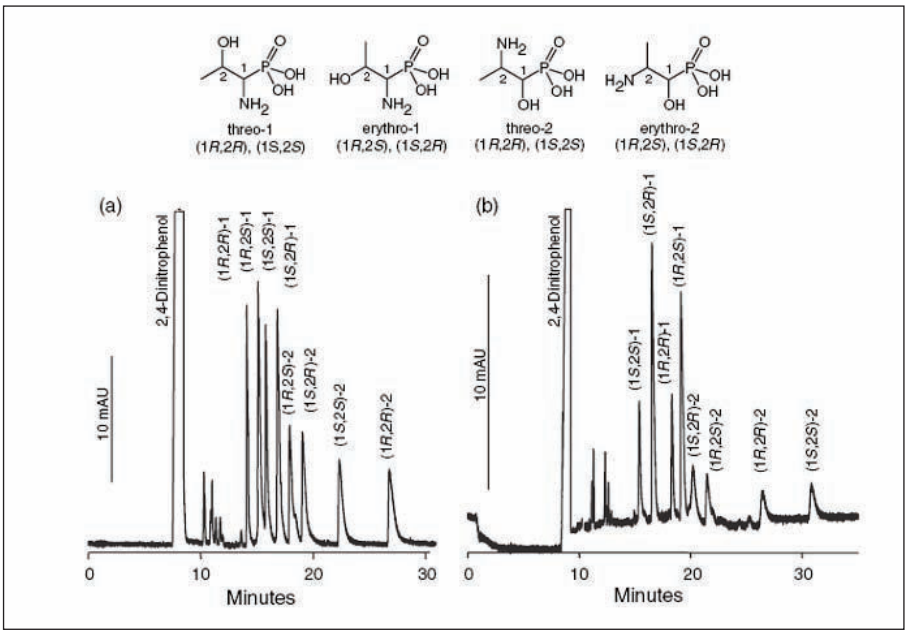

Figure 6. Capillary electrophoretic separation of the stereoisomers of 1-amino-2-hydroxypropane phosphonic acid and 2-amino-1-hydroxypropane phosphonic acid after derivatization with Sanger's reagent as N-2,4-dinitrophenyl derivatives by nonaqueous CE with O-9-(tertbutylcarbamoyl)quinine (a) and O-9-(tert-butylcarbamoyl) quinidine (b) as counterions. Note the reversal of elution order induced upon switching the pseudoenantiomeric counterions. Experimental conditions: Fused-silica capillary, 50 µm i.d., 45.5 cm total length, 37 cm to detection window; background electrolyte, 100 mM acetic acid and 12.5 mM triethylamine in ethanol-methanol (60:40, v/v); selector solution, 10 mM counterion in background electrolyte; partial-filling technique, filling of the selector solution with 50 mbar for 5 min (corresponds to ca. 30 cm selector plug length); injection, 50 mbar for 5 s; applied voltage, −25 kV (plain background electrolyte at both inlet and outlet electrode vessels); temperature, 15°C. Reproduced from [32] with permission.

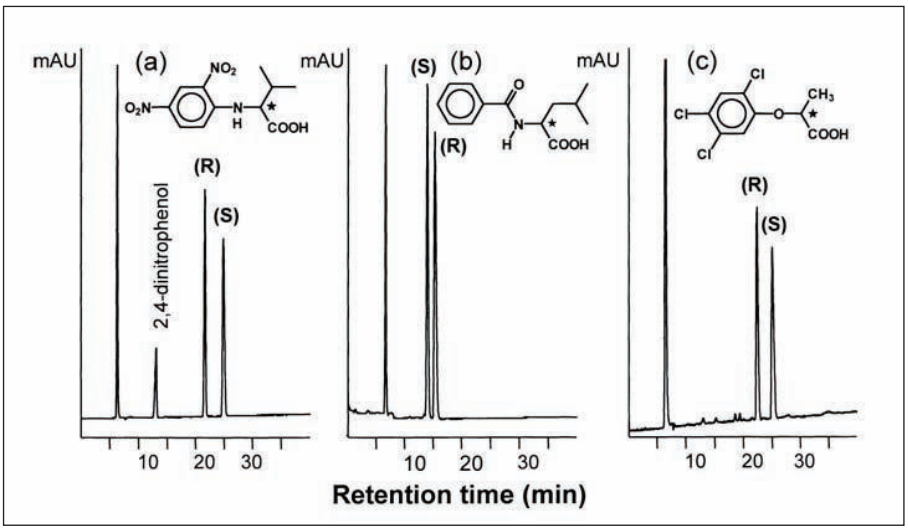

Figure 7. Capillary electrochromatographic separations of DNP-Val (a), Bz-Leu (b), and Fenoprop (c) enantiomers on a 150-mm-long quinidine-functionalized chiral monolith. Conditions: polymerization mixture, chiral monomer 8 wt %, 2-hydroxyethyl methacrylate 28 wt %, ethylene dimethacrylate 4 wt %, 1-dodecanol 30 wt %, and cyclohexanol 30 wt %; UV-initiated polymerization for 16 h at room temperature; pore diameter, 1097 nm; capillary column, 335 mm (250-mm active length) _ 0.1 mm i.d.; EOF marker, acetone; mobile phase, 0.6 mol/L acetic acid and 6 mmol/L triethylamine in 80:20 mixture of acetonitrile and methanol; separation temperature, 50 °C; voltage, -25 kV. Reproduced from [36] with permission.

counterions has preferentially been carried out in nonaqueous mode (NACE) to address solubility issues and exploit the tendency for enhanced ion pair formation in these media. Generally, countercurrent [30, 31] and partial-filling techniques (PFT) [32] have been employed to avoid detection issues associated with the inherently strong UV absorbance of cinchona carbamates. The exceptional resolution power of this technique has been exploited for the separation of a complex mixture of phosphonic acid stereoisomers obtained by aminolysis of fosfomycin [32].

To facilitate sensitive detection, the UV-transparent analytes were converted into the respective N-2,4-dinitrophenyl derivatives prior to separation. Employing O-9-(tert-butylcarbamoyl)quinine as chiral counterion allowed development of a NACE PTF protocol capable of simultaneously separating all eight phosphonic acid stereoisomers of interest. Again, reversal of enantiomer elution order could be conveniently effected by the use of the pseudoenantiomeric O-9-(tert-butylcarbamoyl) quinidine selector to facilitate sensitive peak detection and quantification. The achieved separations are depicted in Figure 6.

2.3.3. Capillary Electrochromatography

Cinchona carbamate-type selectors have been successfully implemented as chiral recognition elements for capillary electrochromatographic (CEC) separation techniques [33, 34]. CEC represents a hybrid separation technique combining the favorable methodological aspects of HPLC and CE. In contrast to HPLC, CEC utilizes electro-osmotic flow (EOF) phenomena rather than pressure-driven transport to effect analyte migration through a chromatographic bed, leading to a plug-like instead of a parabolic flow profile, with the benefit of tremendously enhanced separation efficiencies. Additional enhancements in performance may result from EOF-induced pore flow phenomena, leading to a total improvements in plate numbers by a factor up to 10 as compared to HPLC.

Particularly appealing results could be achieved with CEC phases obtained by in-situ integration of polymerizable versions of quinine carbamate-type selectors in polymethacrylate-type monoliths [35, 36]. These phases were generated by copolymerization O-9-[2-(methacryloyloxy)ethylcarbamoyl]-10,11-dihydroquinine with a hydrophilic co-monomer and a crosslinking agents with optimized binary porogenic solvent mixture within the confines of fused-silica capillaries. Comprehensive optimization of all experimental parameters gave rise to robust synthesis protocols, allowing the fabrication of CEC phases with excellently reproducible performance characteristics in terms of retention time, enantioselectivity and column efficiency. As is evident from the chromatograms depicted in Figure 7, the resulting enantioselective capillary columns produced enantiomer separations with extremely good performance in the CEC mode for chiral acidic analytes. Separation efficiencies in excess of 100,000 plates/meter could be routinely achieved for a variety of amino acid derivatives (with chromophoric and fluorophoric labels) as well as other chiral acids such as 2-aryloxycarboxylic acids.

2.4. Preparative Applications

2.4.1. HPLC

One of the most appealing features of Cinchona carbamate-type stationary phases, in addition to their broad chiral recognition capabilities for chiral acids, is their inherently high loading capacity.

Recently, the preparative potential of a commercially available quinidine tert-butylcarbamate CSP was evaluated using Fmoc-allylglycine enantiomers as model analyte and methanol–glacial acetic acid–ammonium acetate as eluent [37]. In this particular study, the properties of the mobile phase were incorporated into the adsorption model and the inverse method was used to measure the competitive adsorption isotherms of both the solute enantiomers as well as of the invisible adsorbing acetic acid additive. The adsorption of the Fmoc-allylglycine enantiomers could be sufficiently well described by a non-heterogeneous adsorption model, indicating a close-to-homogenous interaction in this chiral preparative phase system. The saturation capacities under these conditions were exceptionally high, amounting to 185 mg/mL for the more strongly retained (R)-enantiomer and 110 mg/mL of the less strongly (S)-enantiomer. The effective loading capacity, corresponding to a touching band chromatogram, was estimated to be in the range of 20.0 mg/g CSP for a methanol–acetic acid–ammonium acetate 99:1:0.25 (v/v/w) mobile phase. This figure ranks amongst the highest values reported so far for chiral stationary phases. Considering that the studied analytes show a relatively modest enantioselectivity value (α = 2.0) higher loading capacities may be well in reach with cinchona carbamate-type CSPs.

2.4.2. Liquid-liquid Extraction Based Enantiomer Separation

The exceptional high enantioselectivity values and binding affinities achievable with cinchona carbamate-type selectors makes these molecular recognition elements attractive candidates for the development of preparative liquid-liquid extraction based enantiomer separation technologies.

Specifically for these applications a special class of chiral extractants has been developed by enhancing the lipophilicity of the parent Cinchona carbamate-type selectors by attachment of highly lipophilic alkyl chains. This type of modification ensures high solubility of the extractants in apolar organic solvents and effective retention in organic solvents even upon protonatation. Several of these chiral extractants have been employed to study the feasibility of various liquid-liquid extraction enantiomer separation formats, including supported liquid membrane [38], centrifugal partition chromatography [39, 40] and countercurrent liquid-liquid extraction techniques [41].

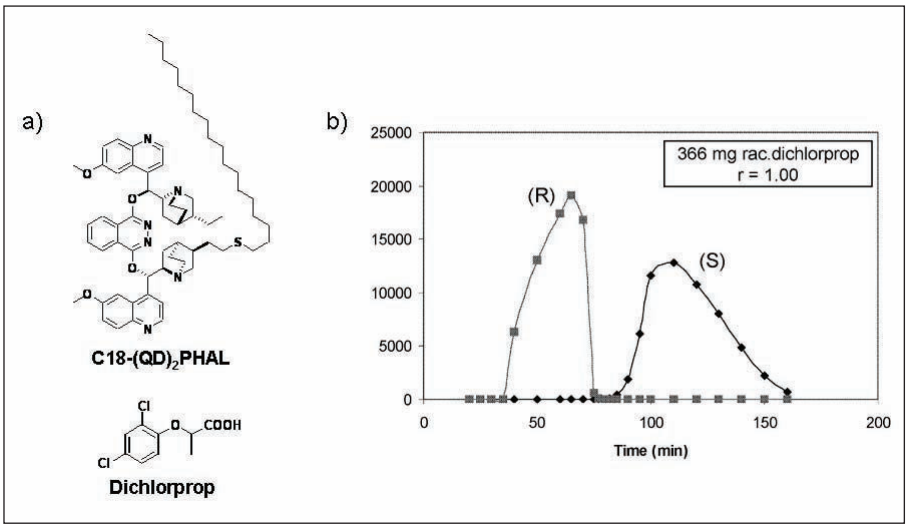

Figure 8. Enantiomer separation of Dichlorprop by centrifugal partition chromatography (CPC) using Cinchona-type extractant as a chiral stationary phase additives. a) Chemical structures of Dichlorprop and the employed quinine-based chiral extractant. b) Preparative CPC enantiomer separation of 366 mg dichlorprop achieved under optimized operation conditions. Conditions: flow rate of 3 mL/min, mobile phase: aqueous sodium phosphate buffer (100 mM, pH 8.0) as mobile phase; stationary phase: 10 mM (DHQD)2PHAL-type CSPA in MTBE; rotor speed: 1100 rpm; T: 25 °C. r refers to the molar ratio of the loaded amount of racemic dichlorprop and the total amount of extractant employed. Reproduced from [39] with permission.

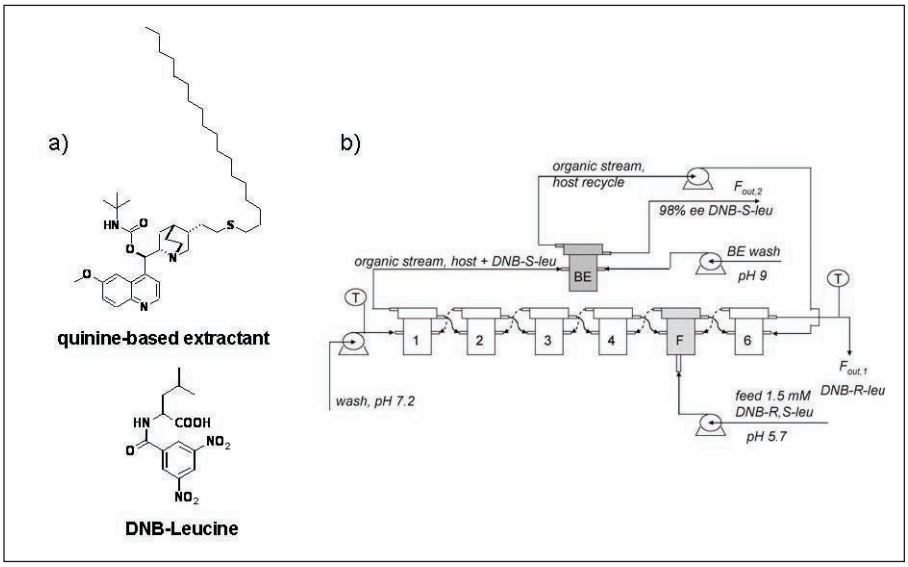

Figure 9. Enantiomer separation of 3,5-dinitrobenzoyl chloride (DND-Leu) by multistage countercurrent liquid-liquid extraction using coupled centrifugal contactors and a quinine carbamate-type extractant. a) Chemical structures of Dichlorprop and the employed quinine-based chiral extractant. b) Schematic representation of the pilot-scale process configuration employed. Reproduced from [41] with permission.

Appealing results were achieved when these enantioselective extractants were utilized as chiral selectors for preparative separation of chiral acids in centrifugal partition chromatography (CPC) applications. CPC is a carrier-free chromatographic technique employing immiscible liquids as stationary as well as mobile phase. Separation is carried out with a rotor-type column comprising a system of several hundred interconnected extraction compartments. In operational state, one of the liquid phases is kept immobilized within the column compartments by a centrifugal field generated by rotation, while the other functioning as mobile phase is forced through the stationary phase by pumping. During migration of the mobile through the rotating column separation is achieved via multiple sequential liquid-liquid extractions steps.

In studies performed with a lab-scale CPC instrument, O-9-(1-adamantylcarbamoyl)-10,11-dihydro-11-octadecylsulfinylquinine employed as chiral extractant in acetone/isobutylmethylketone (1:2, v/v) as stationary phase and 100 mM ammonium phosphate buffer pH 8.0 as mobile phase system could separate up to 300 mg of racemic 3,5-dinitrobenzoyl leucine [40]. Even

higher productivity figures were observed for the enantiomer separation of racemic dichlorprop with a phase system consisting of mono-11-octadecylthio-bis-10,11-dihydroquinidinyl)]-1,4-phthalazine in tert-butylmethylether as stationary phase and 100 mM phosphate buffer pH 8.0 as mobile phase, with baseline separation achieved for sample loads up to 370 mg [39]. A representative separation avhieved with this phase system is depicted in Figure 8. In addition to the high loading capacity, excellent selector economy could be achieved, generally with the molar amount of racemic analyte separated exceeding the molar amount chiral extractant employed.

Despite of the highly promising preparative capacity of CPC, the technique suffers from limitations concerning scalability. This problem has been addressed most recently in a study employing the readily scalable centrifugal contactor separator (CCS) devices. A CCS device is essentially a centrifuge designed for the continuous high throughput extraction of two phase systems, effecting highly efficient mixing and separation of immiscible solvent systems. Adopting O-(1-tert-Butylcarbamoyl)-11-octadecylsulfinyl-10,11-dihydroquinine as chiral extractant and racemic 3,5-dinitrobenzoyl leucine as model system, a fully optimized pilot-scale countercurrent extraction enantiomer separation process was developed [41]. The chiral extractant was employed in 1,2-dichloroethane and the racemic analyte in phosphate buffer at pH 5.7. A single back extraction stage performed with phosphate buffer at pH 9.0 was employed to recover the product with an enantiomeric enrichment of 34%ee and in 61% yield from the organic phase. To achieve complete enantiomer separation multiple extraction stages were performed by coupling 6 CCS devices in a countercurrent fashion. A schematic representation of the countercurrent extraction process is depicted in Figure 9.

This process configuration was capable of producing the (S)-enantiomer in 98% ee and 55% yield. With the pilot scale unit used the possible productivity was estimated to be 17.7 kg/week. The amount of chiral extractant required to achieve this productivity figure was favorably low (60 g), corresponding to turnover number of 400 to 700 per week, a figure that was judged to be highly economic from a process viewpoint. The authors of this study estimated that with the largest CCS units commercially available a productivity of 5 - 10 tons per week would be feasible. It was concluded that the developed extraction process has similar potential as simulated moving bed chromatography for the ton scale separation of enantiomers.

3. Conclusions

Cinchona alkaloid-derived selectors operating on anion-exchange principles present highly efficient tools for the analytical and preparative enantiomer separation of chiral acidic compounds. The combined benefits of broad mobile phase compatibility; ease of method development; adjustment of retention without compromising selectivity; convenient control of enantiomer elution orders on demand by the use of pseudoenantiomeric selectors; and the excellent compatibility with mass sensitive detection modes render cinchona-based anion-exchangers attractive options for a wide range of (bio)analytical applications. High loading capacity, often in combination with exceptional levels of enantioselectivity make these chiral selectors efficient tools for preparative chromatography, and also promising candidates for the advancement of innovative large-scale enantiomer separation techniques based on membrane and liquid-liquid extraction principles.

4. References

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