Short Communication

Conventional Chiralpak ID vs. Capillary Chiralpak ID-3 Amylose Tris-(3-Chlorophenylcarbamate)-Based Chiral Stationary Phase Columns for the Enantioselective HPLC Separation of Pharmaceutical Racemates

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ABSTRACT A comparative enantioselective analysis using immobilized amylose tris-(3-chlorophenylcarbamate) as chiral stationary phase in conventional high-performance liquid chromatography (HPLC) with Chiralpak ID (4.6 mm ID × 250 mm, 5 μm silica gel) and micro-HPLC with Chiralpak ID-3 (0.30 mm ID × 150 mm, 3 μm silica gel) was conducted. Pharmaceutical racemates of 12 pharmacological classes, namely, α- and β-blockers, anti-inflammatory drugs, antifungal drugs, dopamine antagonists, norepinephrine-dopamine reuptake inhibitors, catecholamines, sedative hypnotics, diuretics, antihistaminics, anticancer drugs, and antiarrhythmic drugs were screened under normal phase conditions. The effect of an organic modifier on the analyte retentions and enantiomer recognition was investigated. Baseline separation was achieved for 1-acenaphthenol, carprofen, celiprolol, cizolirtine carbinol, miconazole, tebuconazole, 4-hydroxy-3-methoxymandelic acid, 1-indanol, 1-(2-chlorophenyl)ethanol, 1-phenyl-2-propanol, flavanone, 6-hydroxyflavanone, 4-bromogluthethimide, and pentobarbital on the 4.6 mm ID packed with a 5 μm silica column using conventional HPLC. Nonetheless, baseline separation was achieved for aminoglutethimide, naftopidil, and thalidomide on the 0.3 mm ID packed with a 3 μm silica capillary column. *Chirality 26:677–682, 2014.*

KEY WORDS: Chiralpak ID-3; chiral separations; micro-HPLC; amylose tris-(3-chlorophenylcarbamate); normal phase chromatography

INTRODUCTION

Since the thalidomide tragedy in late 1950s, the pharmaceutical community has been aware of the hazardous implications of chirality.[1] Consequently, regulatory bodies such as the US FDA recommends complete pharmacological and toxicological evaluation of each individual enantiomer even if the drug product will be marketed as a racemate.[2] Therefore, pharmaceutical companies are now shifting toward the development of single pure enantiomer drugs via preparative chiral separation techniques rather than the time-consuming chiral syntheses.[3] This in turn has created an immense need for a robust, reliable, high-throughput, environmentally benign and economically feasible separation technique of chiral pharmaceuticals to enable their availability on a commercial scale.[4,5]

Chiral separations utilizing high-performance liquid chromatography (HPLC) remain the most common technique of the enantioseparations, where the separation can be achieved by the use of chiral stationary phases (CSPs) or chiral mobile phase additives (CMPAs).[6] The liquid chromatography separation technique allows the analysis of volatile and nonvolatile samples and therefore eliminates the drawbacks of sample derivatization required for gas chromatography (GC). Conventional HPLC with chiral columns of 4.0–4.6 mm ID is the most widely used technique for analytical scale enantioseparation for industrial applications.[7] Nonetheless, conventional chiral columns are expensive, consume large volumes of hazardous solvents, and due to the large columns dimensions they are of limited throughput. Furthermore, they are less suitable for parallel and multidimensional analysis, which has become increasingly important due to increasing sample complexity.[7] One of the possible solutions to enhance the speed of the analysis is to use columns with smaller dimensions filled with a stationary phase of smaller particles and hence a smaller theoretical plates height.[8] This will allow pumping of the mobile phase at higher flow rates while maintaining column efficiency. Therefore, downscaling of 4.0–4.6 mm ID into smaller columns including the capillary format represents a promising approach for high-throughput screening and multidimensional analyses.[7]

Polysaccharide CSPs are one of the most efficient CSPs for HPLC enantioseparations, with proven broad enantioselectivities under multimodal chromatographic conditions.[7,9–12] Several amylose and cellulose derivatives with structural differences are commercially available. These structural differences lead to peculiar chiral resolving capabilities which expand the possibilities to explore enantioselective conditions for diverse classes of chiral

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compounds.[13] Moreover, immobilization of the polymeric chiral selector on the silica support is considered an efficient tool to provide solvent versatility and therefore broaden mobile phase eluents to cover harsh solvents such as dichloromethane (DCM), methyl tert-butyl ether (MtBE), tetrahydrofuran (THF), and ethylacetate (EtAc).[14]

The aim of this study was to investigate the chromatographic performance of both conventional and capillary columns packed with a similar chiral selector (CS); immobilized amylose

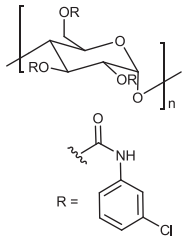

Fig. 1. Amylose tris-(3-chlorophenylcarbamate) CSP.

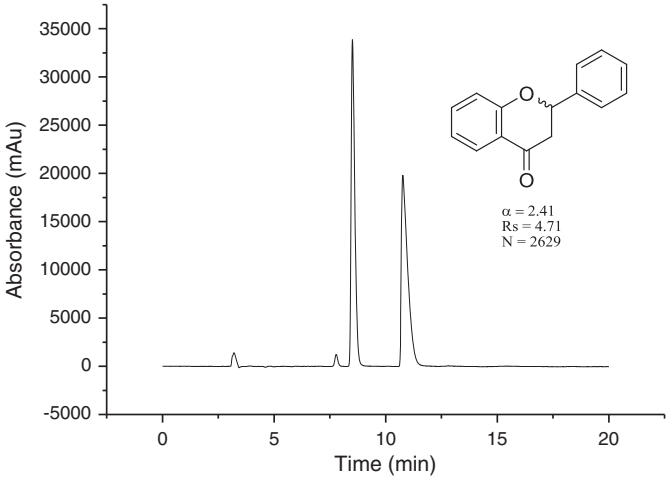

Fig. 2. Enantioselective separation of racemic flavanone on Chiralpak ID (4.6 mm ID, 250 mm length). Mobile phase: *n*-hexane/2-propanol 90:10 v/v, UV: 254 nm, flow rate: 1 mL/min.

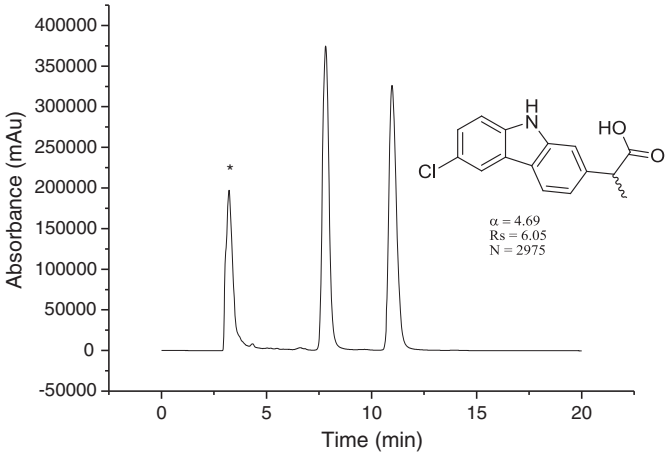

Fig. 3. Enantioselective separation of racemic carprofen on Chiralpak ID (4.6 mm ID, 250 mm length). Mobile phase: *n*-hexane/2-propanol 90:10 v/v, UV: 220 nm, flow rate: 1 mL/min. *2-propanol.

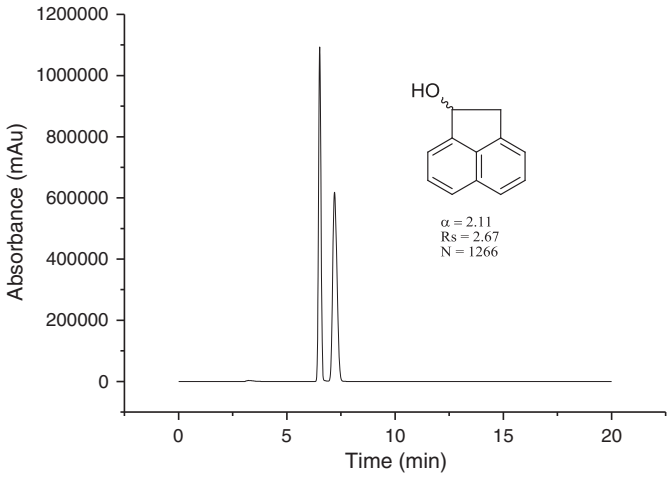

Fig. 4. Enantioselective separation of racemic 1-acenaphthenol on Chiralpak ID (4.6 mm ID, 250 mm length). Mobile phase: *n*-hexane/2-propanol 90:10 v/v, UV: 254 nm, flow rate: 1 mL/min.

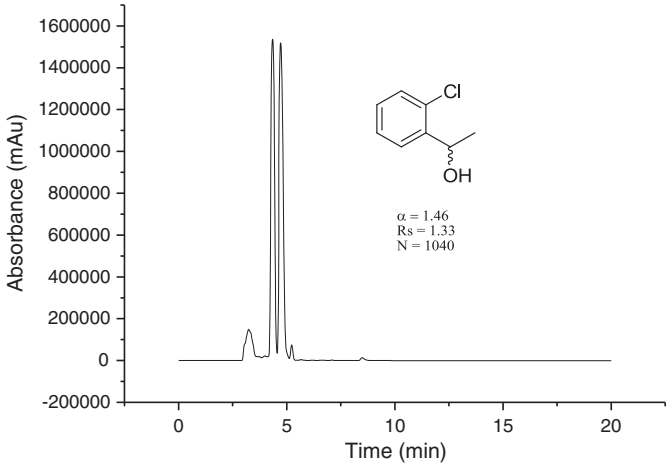

Fig. 5. Enantioselective separation of racemic 1-(2-chlorophenyl)ethanol on Chiralpak ID (4.6 mm ID, 250 mm length). Mobile phase: n-hexane/2-propanol 90:10 v/v, UV: 254 nm, flow rate: 1 mL/min.

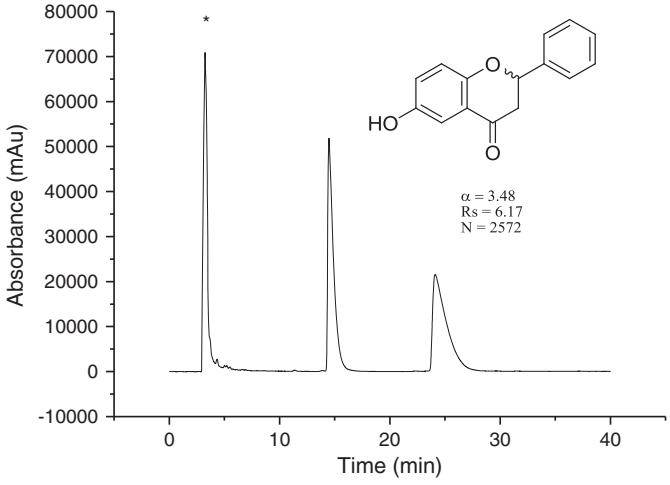

Fig. 6. Enantioselective separation of racemic 6-hydroxyflavanone on Chiralpak ID (4.6 mm ID, 250 mm length). Mobile phase: n-hexane/2-propanol 90:10 v/v, UV: 254 nm, flow rate: 1 mL/min.

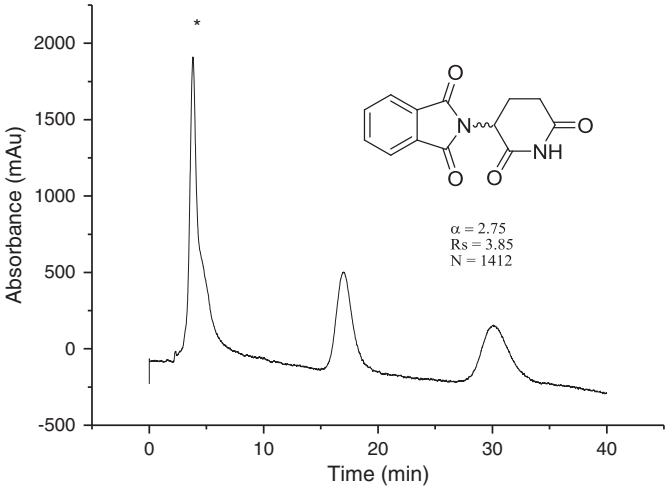

Fig. 7. Enantioselective separation of racemic thalidomide on Chiralpak ID-3 (0.3 mm ID, 150 mm length). Mobile phase: n-hexane/2-propanol 30:70 v/v, UV: 219 nm, flow rate: 10 μL/min. *2-propanol.

tris-(3-chlorophenylcarbamate) is an example of a widely used polysaccharide CSP. The CS was immobilized on 5 μm or 3 μm silica particles in conventional or capillary columns, respectively, and the enantioselective analyses were carried out using conventional or capillary liquid chromatography (CLC). So far, there are not enough data about correlating CSPs performance in different columns platforms.

EXPERIMENTAL

Instrumentation

Conventional HPLC analysis was performed using a Shimadzu XFLC model with LC-20AD pump, autosampler SIL-20A, and SPD-20A UV/VIS detector. CLC analysis was carried out using a Prominence Shimadzu System that consists of an LC-10 AD VP pump (Kyoto, Japan), SIL-20AHT auto sampler, a GL Science UV-vis detector model MU 701 UV-VIS (Tokyo, Japan), and a Shimadzu CDM-20A communications bus module (Kyoto, Japan). All analyses were performed at room temperature. Chiralpak ID (4.6 mm ID × 250 mm, 5 μm silica gel) was supplied by Daicel (Tokyo, Japan) and Chiralpak ID-3 (0.30 mm ID × 150 mm, 3 μm silica gel) was a gift from Eskigent (Redwood City, CA).

Sample Preparations

Stock solutions of the racemic analytes at concentrations of 1 mg/mL in filtered HPLC-grade 2-propanol were prepared. For conventional HPLC analyses, stock solutions were used after filtration through Sartorius Minisart RC 15 0.2-μm pore size filters (Goettingen, Germany) without further dilution; the injection volume was 10 μL. For CLC analyses, the stock solutions were further diluted 10x and filtered; the injection volume was 1 μL.

HPLC Conditions

The enantioselective analyses were conducted using mobile phase comprised of *n*-hexane and 2-propanol mixtures. Preliminary UV analyses were performed at several different wavelengths (219–270 nm) for each compound, in order to select the optimum wavelength for all the analytes and best utilize a single wavelength UV detector.

RESULTS AND DISCUSSION

Albeit there is increased demand for green and less expensive analytical methods for chiral separations, the development of commercial capillary columns is still in its infancy and cannot compete with their full-sized counterparts. In this study, the immobilized polysaccharide-type CS amylose tris-(3-chlorophenylcarbamate) (Fig. 1) was chosen to compare the column performance in conventional size (4.6 mm ID × 25 cm) and capillary format (0.3 mm ID × 15 cm); both columns were provided by their commercial suppliers. Amylose tris-(3-chlorophenylcarbamate) was immobilized on 5 μm or 3 μm silica particles for the conventional column or the capillary antipode, respectively. Immobilized CSPs provide robust adsorbents with high compatibility to drastic solvents.[9]

Polysaccharide CSPs displayed their highest enantioseparation under normal phase and polar organic elution conditions where the formation of hydrogen bonding between the CSPs and the analytes is highly predominant.[15] However, the enantioselective separation of polysaccharide CSPs was also achievable under reversed phase conditions.[16,17]

TABLE 1. Chromatographic conditions, separation, and resolution factors for the baseline resolved compounds on Chiralpak ID (flow rate 1 mL/min) and ID-3 (flow rate 10 μL/min for aminoglutithimide and thalidomide and 50 μL/min for naftopidil)

Column	Separated compound	Chromatographic conditions	Separation factor (α)	Resolution (Rs)
Chiralpack ID	1-Acenaphthenol	MP: *n*-hexane/2-propanol 90:10 v/v, UV: 254 nm	2.11	2.67
	Carprofen	MP: *n*-hexane/2-propanol 90:10 v/v, UV: 220 nm	4.69	6.05
	Celiprolol	MP: *n*-hexane/2-propanol 88:12 v/v, UV: 254 nm	1.94	1.61
	cizolirtine carbinol	MP: *n*-hexane/2-propanol 90:10 v/v, UV: 254 nm	1.59	3
	Miconazole	MP: *n*-hexane/2-propanol 85:15 v/v, UV: 254 nm	2.83	2.2
	Tebuconazole	MP: *n*-hexane/2-propanol 90:10 v/v, UV: 254 nm	2.73	4.22
	4-hydroxy-3-methoxymandelic acid	MP: *n*-hexane/2-propanol 90:10 v/v, UV: 254 nm	3.52	5.34
	1-indanol	MP: *n*-hexane/2-propanol 90:10 v/v, UV: 254 nm	1.92	1.53
	1-(2-chlorophenyl)ethanol	MP: *n*-hexane/2-propanol 90:10 v/v, UV: 254 nm	1.46	1.33
	1-phenyl-2-propanol	MP: *n*-hexane/2-propanol 90:10 v/v, UV: 254 nm	1.74	1.88
	Flavanone	MP: *n*-hexane/2-propanol 90:10 v/v, UV: 254 nm	2.41	4.71
	6-hydroxyflavanone	MP: *n*-hexane/2-propanol 90:10 v/v, UV: 254 nm	3.48	6.17
	4-bromogluthethimide	MP: *n*-hexane/2-propanol 85:15 v/v, UV: 219 nm	3.23	7.71
	Pentobarbital	MP: *n*-hexane/2-propanol 90:10 v/v, UV: 254 nm	1.24, 1.43	1.65, 1.18
Chiralpak ID-3	Aminoglutethimde	MP: *n*-hexane/2-propanol 40:60 v/v, UV: 219 nm	3.34	2.25
	Naftopidil	MP: *n*-hexane/2-propanol 90:10 v/v, UV: 219 nm	2.7	2.78
	Thalidomide	MP: *n*-hexane/2-propanol 30:70 v/v, UV: 219 nm	2.75	3.85

MP: mobile phase.

For pharmaceutical applications, the enantioselective separation has mostly been reported under normal phase elution.[15] Consequently, both columns were investigated for the enantioselective separation of 13 classes of racemic pharmaceuticals using conventional HPLC and CLC under normal phase chromatographic conditions using a mobile phase comprised of *n*-hexane-2-propanol mixtures, which has been widely explored in the literature.[10,12,15]

Miniaturized polysaccharide-based CSPs have been explored for nano-LC and CEC applications in both particle-packed and monolithic forms. Fast, subminute baseline separation of enantiomers using few nanoliters of the mobile phase was accomplished in state of the art capillary columns.[7]

Enantioselective Separation of the Pharmaceutical Racemates

Chiralpak ID and ID-3 were investigated for the enantioselective liquid chromatographic separation of a set of different classes of racemic pharmaceuticals: β-blockers, α-blockers, anti-inflammatory drugs, antifungal drugs, dopamine antagonists, norepinephrine-dopamine reuptake inhibitors, catecholamines, sedative hypnotics, diuretics, antihistaminics, anticancer drugs, flavonoids, and antiarrhythmic drugs. The choice of compounds was arbitrary and guided by preliminary investigations. Baseline separation ($Rs \geq 1.5$) was achieved for 1-acenaphthenol, 4-bromogluthethimide, carprofen, celiprolol, 1-(2-chlorophenyl)ethanol, cizolirtine carbinol, flavanone, 6-hydroxyflavanone, 4-hydroxy-3-methoxymandelic acid, 1-indanol, miconazole, pentobarbital, 1-phenyl-2-propanol, and tebuconazole on the conventional column Chiralpak ID. On the other hand, baseline separation was achieved for aminoglutethimide, naftopidil, and thalidomide on the capillary counterpart Chiralpak ID-3 (Figs. 2–7). Separation (α) and resolution (Rs) factors for the baseline resolved compounds are listed in Table 1.

It was expected that a larger number of racemates would be resolved on the capillary column, as it was packed with smaller particles, in agreement with a previous study which emphasized the importance of free silanol groups in enhancing the resolution (more silanol groups in the smaller particle size bed).[18] However, none of the compounds resolved on the conventional column showed any similar/comparable enantioselectivity on the capillary column (Fig. 8). Naftopidil showed partial separation on the conventional column ($Rs = 1.17$) and baseline separation on the capillary antipode ($Rs = 2.78$) (Fig. 9). Moreover, the conventional column could recognize pentobarbital diastereomers, which was not achievable with the capillary column. All these observations suggest the importance of the CS concentration in achieving/enhancing enantiomer recognition, in agreement with previous studies.[19,20] It was also expected that the peak efficiency would increase with decreasing the particle size due to decreased longitudinal inhomogeneity of mobile

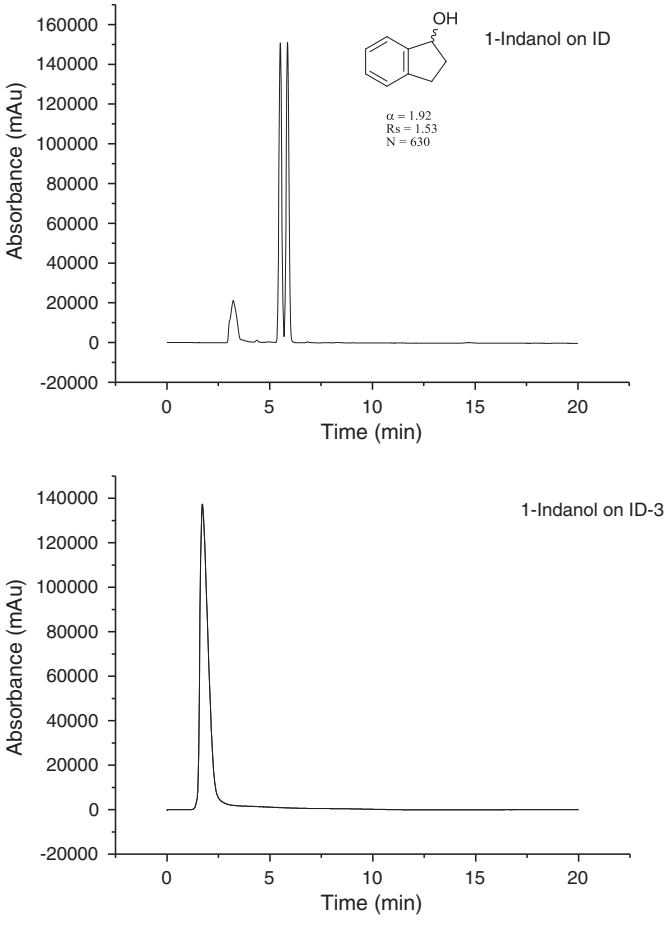

Fig. 8. Enantioselective separation of racemic 1-indanol on the conventional Chiralpak ID (top) vs. the capillary Chiralpak ID-3 (bottom). Mobile phase: *n*-hexane/2-propanol 90:10 v/v, UV: 254 nm, flow rate: 1 mL/min and 10 μL/min, respectively.

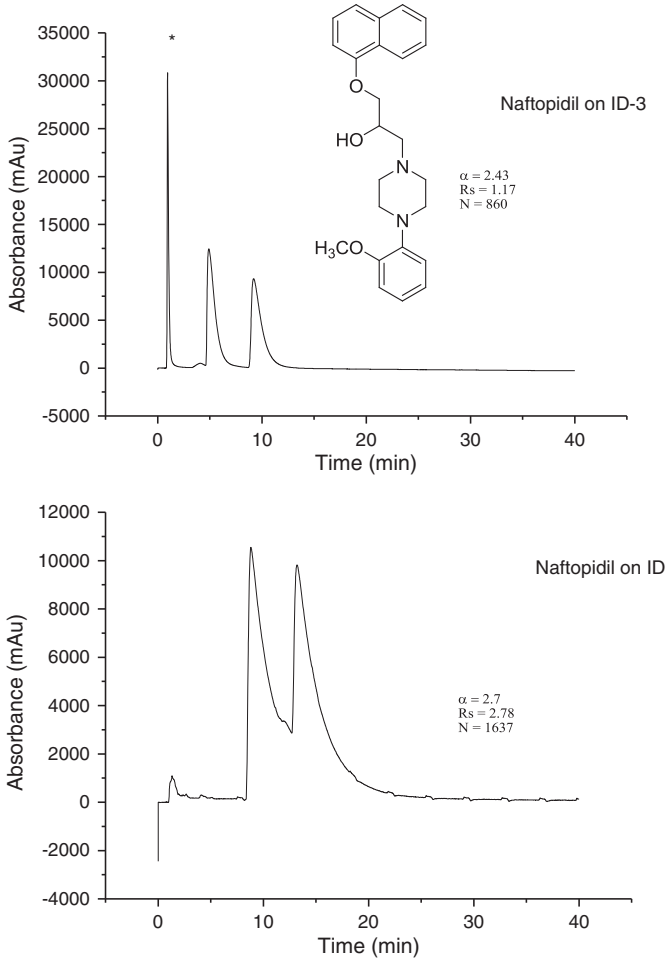

Fig. 9. Enantioselective separation of racemic naftopidil on the capillary (top) vs. the conventional Chiralpak ID (bottom). Mobile phase: *n*-hexane/2-propanol 90:10 v/v, UV: 219 nm, flow rate: 50 μL/min and 1 mL/min, respectively. *2-propanol.

phase flow with smaller particles.[21] Nonetheless, due to insufficient baseline separations on the capillary column, conclusions about peak efficiency cannot be drawn.

It is evident that direct method transfer between the conventional column Chiralpak ID and its antipode Chiralpak ID-3 was not applicable in this study. However, this sheds light on the potential use of miniaturized techniques to study the interactions between the CS and the analytes from a thermodynamics point of view and trying to maintain the interactions on the larger scale. The use of miniaturized techniques might help in better understanding the CS-analyte interaction on the molecular level when the chiral selector is firmly immobilized on a solid support.

Miniaturization of Chiral Separations: A Future Perspective

Downscaling of the chromatographic techniques from conventional to micro to nano- HPLC is considered a green analysis approach. Miniaturization of instrumentation results in small solvent consumption and reduced waste production compared to the full-size laboratory instruments.[22] An early study attempted the simulatenous enantioseparation of thalidomide and its hydroxylated metabolites using three different polysaccharide-type CSPs using conventional HPLC, CLC, and capillary electrophoresis (CE).[23] Baseline separation of the six peaks in one run was achieved using conventional HPLC; however, for CLC and CE, this was not achievable using the same column length. Hence, the length of the packed bed of the capillary was increased to provide the same baseline separation previusoly displayed by the coventional column. This implies a role for the CS content in providing a baseline resolution for the racemates, which suggests certain requirements for the chiral recognition ability of a CS. Another insight involves the feasibilty of miniaturization from conventional HPLC to nano-LC and CEC for the chiral separations upon taking the CS content into consideration. Another study demonstrated high peak efficiencies in CEC upon reducing the particle diameter of the packing material; this observation was hardly detectable in CLC mode.[24] A recent study investigated the separation performance of polysaccharide-based CSPs when coated on superficially porous silica supports of different pore sizes.[19] The authors investigated the influence of varying the amount of CS (1%–5%) on the enantioselective separation of six racemates using nano-LC and CEC. Similar results have shown that the resolution was enhanced with increasing the amount of the CS; nonetheless, pore size affected the generation of EOF required for CEC but it did not display any improvement in the separation performance of nano-LC mode.

Based on these results, one can assume that the performance of the conventional column tested in this study was not comparable to the capillary counterpart mainly because of the different CS content. In order to expand the results of the previous investigations into the scope of this study, it can be inferred that the amount of CS has an impact on the resolution of the racemates, which depends on the type and nature of CS.

CONCLUSIONS

This study demonstrates that the prediction of column performance when miniaturized cannot be based on the performance of its conventional antipode. Amylose tris-(3-chlorophenylcarbamate) immobilized on silica gel was investigated for the enantioselective separation of a set of 13 different classes of racemic pharmaceuticals using conventional HPLC and capillary-LC under normal phase chromatographic conditions. Baseline separation was achieved for 1-acenaphthenol, 4-bromogluthethimide, carprofen, celiprolol, 1-(2-chlorophenyl)ethanol, cizolirtine carbinol, flavanone, 6-hydroxyflavanone, 4-hydroxy-3-methoxymandelic acid, 1-indanol, miconazole, pentobarbital, 1-phenyl-2-propanol, and tebuconazole on the conventional column and for aminoglutethimide, naftopidil, and thalidomide on the capillary counterpart. Parameters such as the content and conformation of the CS in both column formats need to be considered. Further studies on different CSs/solid support combinations is recommended so that conclusions can be drawn regarding columns performance. The complexity of the chiral recognition mechanisms could be simplified using miniaturized models to study the mechanism on the molecular level and explore the possibilities of scaling up the results.

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