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Predictability of Enantiomeric Chromatographic Behavior on Various Chiral Stationary Phases Using Typical Reversed Phase Modeling Software†

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ABSTRACT Pharmaceutical companies worldwide tend to apply chiral chromatographic separation techniques in their mass production strategy rather than asymmetric synthesis. The present work aims to investigate the predictability of chromatographic behavior of enantiomers using DryLab HPLC method development software, which is typically used to predict the effect of changing various chromatographic parameters on resolution in the reversed phase mode. Three different types of chiral stationary phases were tested for predictability: macrocyclic antibiotics-based columns (Chirobiotic V and T), polysaccharide-based chiral column (Chiralpak AD-RH), and protein-based chiral column (Ultron ES-OVM). Preliminary basic runs were implemented, then exported to DryLab after peak tracking was accomplished. Prediction of the effect of % organic mobile phase on separation was possible for separations on Chirobiotic V for several probes: racemic propranolol with 97.80% accuracy; mixture of racemates of propranolol and terbutaline sulphate, as well as, racemates of propranolol and salbutamol sulphate with average 90.46% accuracy for the effect of percent organic mobile phase and average 98.39% for the effect of pH; and racemic warfarin with 93.45% accuracy for the effect of percent organic mobile phase and average 99.64% for the effect of pH. It can be concluded that Chirobiotic V reversed phase retention mechanism follows the solvophobic theory. *Chirality 25:506–513, 2013.*

KEY WORDS: predictability of enantiomeric separations; DryLab; Chirobiotic V; Chirobiotic T; Chiralpak AD-RH; Ultron ES-OVM

INTRODUCTION

Nowadays, studying the stereoselectivity profiles of marketed chiral drugs is compulsory in order to understand the pharmacokinetics and dynamics of each enantiomer. Considering that enantioselective synthesis is expensive and time consuming, separation techniques on the preparative scale are preferred by the pharmaceutical companies if the eutomer is to be marketed. Almost 90% of chiral separations have been achieved by HPLC compared to a lower percentage by other analytical techniques such as gas chromatography or capillary electrophoresis.[1]

Variations in the chiral stationary phases (CSPs) used are the predominant tool for method development in almost all analytical, biochemical, pharmaceutical, and pharmacological applications. More than 100 CSPs are currently commercially available; among them 20 to 30 CSPs are the most frequently employed, covering nearly all enantiomeric separations.[2] These CSPs are polysaccharides, synthetic polymers, proteins, cyclodextrin, macrocyclic glycopeptide, crown ethers, donor–acceptor chiral-ion exchangers, and ligand exchangers. The retention mechanism involved in chiral recognition of the CSPs was studied in many works.[2–8] The "three-point attachment model" was one of the early attempts to rationalize the enantiospecific interactions between enantiomers with the chiral selector. It was hypothesized that the enantiomer that will display the best optimum fit, by the formation of the three interactions, will elute later than the other enantiomer that will be less fitting. This model was too simple, however, and did not reflect the overall nature of interaction, whether it is attraction or repulsion. Nevertheless, this model is still used, taking in consideration that the chiral selector is three dimensional, not planar. Accordingly, it can be concluded that chiral recognition is mediated via multiple interactions: for example, π–π and dipole–dipole interactions.[2]

On the other hand, computer-assisted HPLC method development has received much attention since late 1970s, especially in the pharmaceutical industry in order to save time and resources; furthermore, it achieves a robust analytical method. One of the most commonly used computer programs is DryLab, which has many applications in the reversed phase mode.[9–17] Its predictions are based on mathematical calculations of the solvophobic theory, which has been studied and explained in detail by Horváth et al.[18–20] In solvophobic theory, the nature of both the stationary phase and the mobile phase are important for prediction of retention.[21]

The aim of this study is to use DryLab® to investigate the predictability of chromatographic behavior of chiral compounds on different chiral stationary phases which is hypothesized to be of considerable interest for drug development industry, food

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industry, environmental and agricultural fields as it will reduce the time required for the chiral chromatographic method development. Three CSPs are investigated in the present study namely, macrocyclic glycopeptides (Chirobiotic V and T), polysaccharides (Chiralpak® AD-RH) and proteins (Ultron ES-OVM).

Few reported trials have aimed to predict the enantioselectivity of chiral drugs. One of the earliest studies predicted the enantioselectivity of a number of chiral drugs on derivatized ß-cyclodextrin stationary phase based on free energy calculations of substituents present on the stereogenic center. This study was able to predict the possibility of enantiomeric separation on a specific stationary phase rather than the elution order.[22] Previous works relating chiral separation to two types of HPLC method development software, ACD Lab and Chromsword, were reported. ACD Lab software was able to predict the optimum %B required for the separation of enantiomers of eszopiclone, using tandem mass spectrometric detection on chiral AGP, which is an α_1-acid glycoprotein stationary phase[23]; for Chromsword, chiral drugs were separated on a polysaccharide-based stationary phase using normal phase mode. DryLab was the tool for prediction in only two studies, which were performed on a quinine carbamate-based chiral anion-exchanger[24] and a *tert*-butyl carbamoylated quinine[25] stationary phase, where *N*-derivatized amino acids were successfully resolved.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol, acetonitrile (HPLC grade), trifluoroacetic acid (TFA), glacial acetic acid, monobasic sodium monophosphate (NaH_2PO_4), dibasic sodium monophosphate (Na_2HPO_4), ammonium hydroxide (NH_4OH), phosphoric acid (H_3PO_4), and triethylamine (TEA) were purchased from Sigma-Aldrich, Germany. *Rac*-Propranolol, *rac*-salbutamol sulfate, *rac*-terbutaline sulfate, *rac*-tetoprolol, and *rac*-ibuprofen were provided by SEDICO Pharmaceutical Company, Egypt. *Rac*-Warfarin was provided by GlaxoSmithKline (GSK, Cairo, Egypt).

Equipment and Software

HPLC (Thermo Finnigan SpectraSYSTEM, Thermo Electron Corporation, UK) consisted of: pump model P2000, detector model UV3000, autosampler model AS3000, and data acquisition for the HPLC Chromquest 4.2 data system (UK).

The columns used are Chirobiotic V bonded vancomycin-based phase, 5-µm particle size (150 × 4.6 mm) and the Chirobiotic T bonded teicoplanin-based column, 5-µm particle size (150 × 4.6 mm), both purchased from Advanced Separations Technologies, Inc. (Astec) and Supelco (Bellefonte, PA, USA); Chiralpak AD-RH amylose tris (3,5-dimethylphenylcarbamate) coated on 5-µm silica gel (150 × 4.6 mm) purchased from Daicel Chemical Industries Ltd (Tokyo, Japan); and Ultron ES-OVM: protein, ovomucoid, chemically bonded to the silica support (150 × 4.6 mm), purchased from Agilent Technologies, USA.

The computer programs used were Chromquest 4.2 data system (UK) for data acquisition, DryLab2000Plus, and PeakMatch, v. 3.60 (Molnár Institute for Applied Chromatography, Germany).

METHODS

Determination of Predictability of the CSPs

Experimental limitations. The parameters that can be optimized by DryLab software are: % organic phase, temperature, pH, and flow rate. Three different CSPs were investigated:

- Macrocyclic glycopeptides (Chirobiotic V and T) have limitations regarding operating pressure and temperature (max. 45 °C) and considerable variations of temperatures and flow rate from low to high values were not possible, limiting the ability to study these two parameters as modifiers of the chiral recognition. The % organic phase and the pH were the investigated parameters.
- Polysaccaride CSPs (Chiralpak AD-RH) are limited with respect to operating pressure; accordingly the flow rate was not a studied variable. The % organic phase and the temperature were the parameters under investigation.
- Protein CSPs (Ultron ES-OVM) are limited regarding pressure and temperature (40 °C), as well as the % organic phase, which was only studied in the limited range allowed.

Experimental preliminary runs. The preliminary-run conditions required to start predictions via computer simulations were selected according to the column restrictions previously mentioned and the corresponding DryLab operational mode. These conditions are presented in Table 1 as follows: for Chirobiotic V and T, two isocratic runs at 75% and 95%B; for Chiralpak AD-RH, four isocratic runs at 40% and 60%B each at two different temperatures (30 °C and 60 °C); and for Ultron ES-OVM, two isocratic runs at 5% and 25%B.

The preliminary runs were exported to PeakMatch software in artificial intelligence assemblage (AIA) format for peak tracking where the peak areas were taken as identifiers for the peaks. These were subsequently exported to DryLab to build the resolution map of the method.

The optimum chromatographic separation conditions were selected via the resolution map to achieve the best resolution for the enantiomers in the least possible run time. Then the computed model was experimentally checked for accuracy of the retention time via comparison between predicted and experimental chromatograms.

Determination of the Effect of pH

Three preliminary runs were performed each at a different pH value (differing by 0.5–0.6 pH units).[17] After peak tracking and obtaining the resolution map, retention time was tested for accuracy between virtual and experimental chromatograms.

RESULTS AND DISCUSSION

Chirobiotic V

Results of retention time at various conditions are shown in Table 2. The resolution map (plot of minimum resolution for the poorest-resolved band pair in the sample versus % organic), presented in Figure 1, showed that the best resolution for propranolol enantiomers was obtained at 100%B, proven by comparison of the results of the experimental run and the predicted. A difference in retention times between predicted and experimental of only 1.50 min and 0.51 min, for the first and the second propranolol enantiomers, respectively, was found, resulting in 97.80% average accuracy between experimental and predicted as demonstrated in Table 3.

The accuracy of the predictions was tested for warfarin as well—both the effect of the % organic phase and the pH were studied. For the effect of % organic phase, the retention time of the two enantiomers for each of the two preliminary runs, 5% and 25%B, are shown in Table 2, and indicate that when %B (organic phase) increased, both the retention time and the Rs decreased. Optimum separation conditions, according to the computed resolution map, were obtained when the %B was at 10%, as shown in Figure 2. A comparison of the in-silico model chromatogram results with the experimental results is presented in Table 3, indicating a difference of 1.188 min and 0.585 min for the first and second eluted warfarin enantiomers, which proved an average accuracy on prediction of the retention time by 93.45%. Some peak tailing was observed in the later enantiomer of warfarin.

The effect of pH on separation of warfarin enantiomers on Chirobiotic V was studied. The three isocratic runs at 10%B at three different pH values (3.30, 3.90, and 4.50) showed that

TABLE 1. Preliminary-run conditions on each of the four columns to build the resolution map

Columns	Adsorbents	Analyte	Mobile phase	Preliminary runs	Flow (ml/min)	Temp (°C)
Chirobiotic V*	Vancomycin	1mg/ml Propranolol	A = ultrapure water B = methanol with 0.01% trifluoroacetic acid and 0.01% ammonium hydroxide	2 isocratic runs: 75%B, 95%B	1.0	30 °C
		1mg/ml Warfarin	***Effect of % organic phase***: A = 1%Triethylammonium acetate (TEAA), pH 4.10 B = Acetonitrile.	2 isocratic runs: 5%B, 25%B		
			Effect of pH: A = 1% triethylammonium acetate (TEAA) B = acetonitrile	1 isocratic run: 10%B at 3 different pH (3.30, 3.90, 4.50)		
		Racemic mixtures**: ***1st mixture:*** Propranolol (1 mg/ml) and Terbutaline sulphate (1 mg/ml).	***Effect of % organic phase***: A = ultrapure water B = methanol with 0.01% trifluoroacetic acid and 0.01% ammonium hydroxide	2 isocratic runs: 75%B, 95%B		
		2nd mixture: Propranolol (1 mg/ml) and salbutamol sulphate (1 mg/ml)	***Effect of pH***: A = ultrapure water (adjusted to desired pH using 0.1 M phosphoric acid) B = methanol with 0.01% trifluoroacetic acid and 0.01% ammonium hydroxide	1 isocratic run: 95%B at 3 different pH (3.00, 3.60, 4.20)		
Chirobiotic T*	Teicoplanin	1mg/ml propranolol	A = ultrapure water B = acetonitrile:methanol (55%:45%) with 0.3% acetic acid and 0.2% Triethylamine (TEA)	2 isocratic runs: 75%B, 95%B	1.0	30 °C
		1mg/ml metoprolol	A = ultra pure water B = methanol with 0.1% trifluoroacetic acid and 0.1% ammonium hydroxides	2 isocratic runs: 75%B, 95%B		
CHIRALPAK® AD-RH	Amylose carbamate	0.1 mg/ml propranolol	A = 20 mM phosphate buffer pH 8.00 B = acetonitrile	2 isocratic runs: 40%B & 60%B each at 2 different temperatures	0.6	30 °C and 60 °C
Ultron ES-OVM*	Ovomucoid protein	0.025 mg/ml ibuprofen	A = 25 mM phosphate buffer pH 4.50 B = methanol	2 isocratic runs 5% and 25%B	0.2	30 °C

*These columns have a restriction as the maximum operating temperature is 45 °C.
**The effect of % organic phase and pH were studied on both mixtures.

when the pH increased, the retention time increased, as presented in Table 2. As predicted by the resolution map in Figure 3, the pH increase is associated with improvement in resolution, up to pH 3.50, followed by a decrease in resolution after that value. Retention times were 8.336 min and 12.572 min for the first and the second eluted enantiomers, respectively, in the predicted run as demonstrated in Figure 3; the retention times in the experimental run were 8.383 min and 12.591 min for the first and the second eluted warfarin enantiomers, respectively, leading to an average accuracy for of 99.64%.

The method was also applied to two mixtures composed of propranolol/terbutaline sulfate (first mixture) and propranolol/salbutamol sulfate (second mixture) to determine whether the behavior of chiral compounds on Chirobiotic V is predictable by DryLab. Two isocratic preliminary runs, 75% and 95% B, for each mixture were performed and the retention times calculated, as shown in Table 2. It was observed that when the %B decreased, the retention time increased while the resolution decreased. In Figures 4 and 5, the two resolution maps showed that the best resolution is 100%B for both mixtures. The percent averages for prediction accuracy were 92.27% and 88.67% for propranolol/terbutaline sulfate and propranolol/salbutamol sulfate, respectively, as presented in Table 3.

Further, it was found that the change in pH has a minor effect on both the retention time and resolution as shown in Table 2. Figures 6 and 7 show that the optimum pH is 3.50 for the first mixture and 4.10 for the second mixture. Both predicted and experimental runs were compared and matched with 99.12% and 97.16% accuracy for the propranolol/terbutaline sulfate and propranolol/salbutamol sulfate, respectively, as demonstrated in Table 3. It is worth mentioning that the average accuracy between experimental and virtual runs on Chirobiotic V was 94.85%, which represents a significantly good match.

According to the previous results, vancomycin (the Chirobiotic V adsorbent) chromatographic chiral recognition behavior can be predicted by DryLab. Chirobiotic V, as with other macrocyclic glycopeptide CSPs, was introduced as a chiral selector by the pioneering work of Armstrong.[26] It acts in a variety of ways in different chromatographic modes: normal phase, reversed phase, and polar ionic. The chiral recognition is due to hydrophobic

TABLE 2. The retention times of the preliminary-run conditions on each of the four columns

Columns	Analyte	Preliminary runs	t_R of the enantiomers (min)
Chirobiotic V*	1mg/ml propranolol	75%B	t_{R1} = 112.731 t_{R2} = 117.99
		95%B	t_{R1} = 53.657 t_{R2} = 58.221
	1mg/ml warfarin	5%B	t_{R1} = 19.965 t_{R2} = 31.736
		25%B	t_{R1} = 3.672 t_{R2} = 4.219
		pH 3.30	t_{R1} = 7.294 t_{R2} = 10.814
		pH 3.90	t_{R1} = 10.082 t_{R2} = 14.919
		pH 4.50	t_{R1} = 11.828 t_{R2} = 15.497
		75%B	t_R = 61.131 (propranolol enantiomers coeluted)
			t_R = 64.237 (terbutaline sulfate enantiomers coeluted)
	Racemic Mixtures*:** ***1st Mixture:	95%B	*t_{R1} = 34.550 t_{R2} = 37.398
	Propranolol* (1 mg/ml) and terbutaline sulfate** (1 mg/ml)		**t_{R1} = 41.217 t_{R2} = 42.772
		pH 3.00	*t_{R1} = 32.924 t_{R2} = 35.501
			**t_{R1} = 41.190 t_{R2} = 42.820
		pH 3.60	*t_{R1} = 32.780 t_{R2} = 35.368
			**t_{R1} = 41.556 t_{R2} = 43.154
		pH 4.20	*t_{R1} = 29.656 t_{R2} = 31.990
			**t_{R1} = 38.084 t_{R2} = 39.463
		75%B	*t_{R1} = 61.903 t_{R2} = 64.665
			***t_{R1} = 76.651 t_{R2} = 80.481
		95%B	*t_{R1} = 31.443 t_{R2} = 33.924
			***t_{R1} = 48.097 t_{R2} = 52.737
	2nd Mixture:		
	Propranolol* (1 mg/ml) and salbutamol sulphate*** (1 mg/ml)	pH 3.00	*t_{R1} = 32.819 t_{R2} = 35.485
			***t_{R1}= 47.547 t_{R2} = 51.896
		pH 3.60	*t_{R1} = 33.029 t_{R2} = 35.618
			***t_{R1} = 49.614 t_{R2} = 54.193
		pH 4.20	*t_{R1} = 33.481 t_{R2} = 36.140
			***t_{R1} = 50.750 t_{R2} = 55.413
Chirobiotic T*	1mg/ml propranolol	75%B	t_{R1} = 2.884 (propranolol enantiomers coeluted)
		95%B	t_{R1} = 3.369 t_{R2} = 3.513
	1 mg/ml metoprolol	75%B	t_{R1} = 17.892 (metoprolol enantiomers coeluted)
		95%B	t_{R1} = 31.499 t_{R2} = 34.012
CHIRALPAK AD-RH		40%B, T = 30 °C	t_{R1} = 10.190 t_{R2} = 11.123
		40%B, T = 60 °C	t_{R1} = 9.240 t_{R2} = 10.317
	0.1 mg/ml propranolol	60%B, T = 30 °C	t_{R1} = 5.015 t_{R2} = 5.885
		60%B, T = 60 °C	t_{R1} = 4.698 t_{R2} = 5.571
Ultron ES-OVM*	0.025 mg/ml ibuprofen	5%B	t_{R1} = 11.046 t_{R2} = 13.271
		25%B	t_{R1}= 10.857 (ibuprofen enantiomers coeluted)

*These columns have a restriction as the maximum operating temperature is 45 °C.

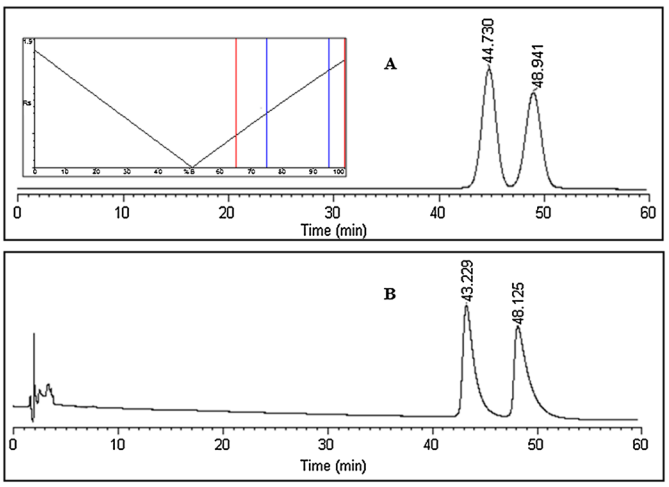

Fig. 1. The chromatograms of 1 mg/ml of propranolol on Chirobiotic V at optimum conditions, namely: 100% methanol with 0.01% TFA and 0.01% ammonium hydroxide, at flow rate 1.0 ml/min, and temperature 30 °C. **(A)** Predicted run and the corresponding resolution map; **(B)** experimental chromatogram at optimized conditions.

interactions (hydrophobic inclusion complexes or merely an association with a hydrophobic cleft or pocket), hydrogen-bonding, dipole stacking, steric interactions, and π–π complexation.[27]

In our study, Chirobiotic V showed a mixed retention mechanism: reversed phase and polar ionic modes. As the LC module of DryLab is based on a reversed phase retention mechanism approximating the linear solvent strength theory (increasing % organic modifier decreases retention factors), it was successful in predicting retentions of enantiomers on Chirobiotic V under different conditions of %B and pH. Hence we can conclude that retention on Chirobiotic V is reversed, to a considerable extent, at the different RP mobile phases used and with the variety of drugs studied, which were acidic (warfarin, pK_a = 5.0) and basic (propranolol, pK_a = 9.5; salbutamol sulfate, pK_a = 9.3; and terbutaline sulfate, pK_a = 8.7).

It is worth mentioning that although DryLab was previously used to predict behavior on two chiral stationary phases not commonly used—quinine and quinidine carbamate derivatives—the analytes still needed to be derivatized prior to separation. The derivatizing agents 2,4-dinitrofluorobenzene [24] and 3,5-dinitrobenzyloxycarbonyl[25] for the amino acid analytes

TABLE 3. Experimental versus DryLab predicted retention of Chirobiotic V under isocratic conditions for optimum %B and pH

Analyte		Optimum Condition	Predicted t_R	Experimental t_R,	Difference (min)	% Accuracy
	Enantiomer 1	100%B	44.730	43.229	1.50	96.65
Propranolol	Enantiomer 2		48.941	48.125	0.51	98.95
	Enantiomer 1	10%B	12.285	11.097	1.188	90.32
	Enantiomer 2		17.110	16,525	0.585	96.58
	Enantiomer 1	pH = 3.50	8.336	8.383	0.047	99.44
	Enantiomer 2		12.572	12.591	0.019	99.84
Warfarin	Enantiomer* 1	100%B	29.657	26.699	2.958	90.03
	Enantiomer* 2		32.724	29.222	3.502	89.30
	Enantiomer** 1		36.447	33.329	3.118	91.45
	Enantiomer** 2		38.160	35.212	2.948	92.28
Propranolol* and terbutaline sulfate**	Enantiomer* 1	pH = 3.50	33.010	32.581	0.429	98.70
	Enantiomer* 2		35.617	35.196	0.421	98.82
	Enantiomer** 1		41.766	41.546	0.220	99.47
	Enantiomer** 2		43.374	43.154	0.220	99.49
	Enantiomer* 1	100%B	26.624	23.785	2.839	89.33
	Enantiomer* 2		28.942	26.004	2.938	89.85
	Enantiomer*** 1		42.853	37.460	5.393	87.42
	Enantiomer*** 2		47.487	41.828	5.659	88.08
Propranolol* and salbutamol sulfate***	Enantiomer* 1	pH = 4.10	32.774	32.053	0.721	97.80
	Enantiomer* 2		35.386	34.618	0.768	97.83
	Enantiomer*** 1		50.336	49.107	1.227	97.56
	Enantiomer*** 2		55.084	53.681	1.403	97.45

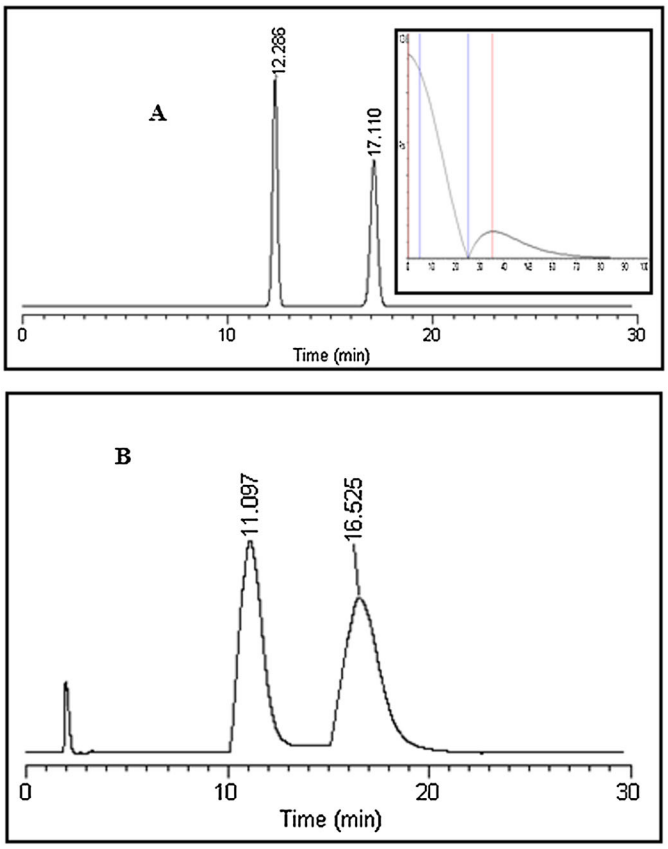

Fig. 2. The chromatograms of 1 mg/ml warfarin on Chirobiotic V at optimum conditions, namely: 10%B (where **A**: 1% TEAA, pH = 4.10, **B**: acetonitrile), at flow rate 1.0 ml/min, and temperature 30 °C. **(A)** Predicted run and the corresponding resolution map; **(B)** experimental chromatogram at optimized conditions.

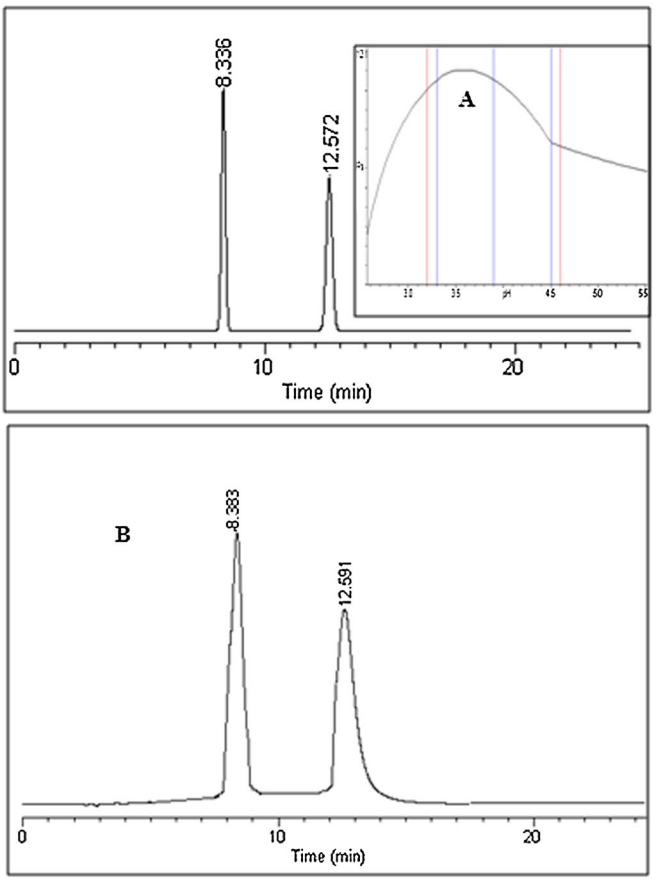

Fig. 3. The chromatograms of 1 mg/ml warfarin on Chirobiotic V at optimum pH = 3.50, at 10%B (where **A**: 1% TEAA, pH = 3.50, **B**: acetonitrile), at flow rate 1.0 ml/min, and temperature 30 °C. **(A)** Predicted run and the corresponding resolution map; **(B)** experimental chromatogram at optimized conditions.

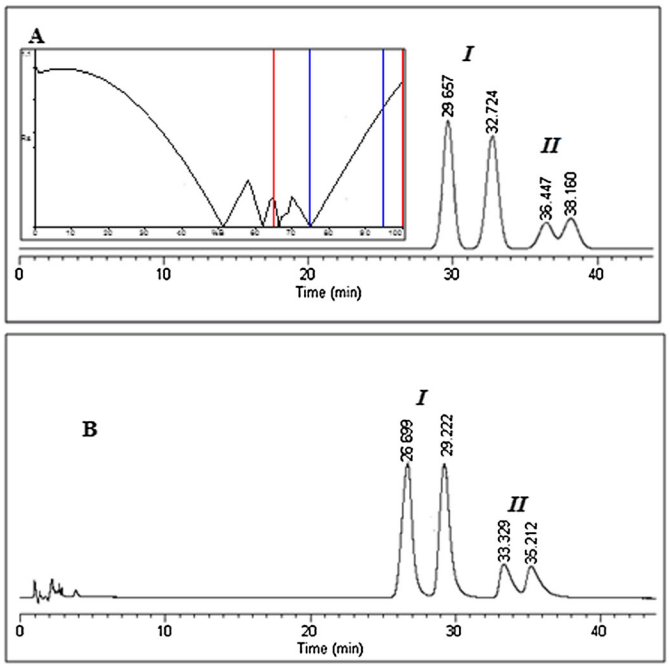

Fig. 4. Chromatograms at optimum conditions for 1 mg/ml propranolol (I) and 1 mg/ml terbutaline sulfate (II) mixture on Chirobiotic V, namely: 100% methanol with 0.01% TFA and 0.01% ammonium hydroxide, at flow rate 1.0 ml/min, and temperature 30 °C. **(A)** Predicted chromatogram at optimized conditions and the corresponding resolution map; **(B)** experimental chromatogram at optimized conditions.

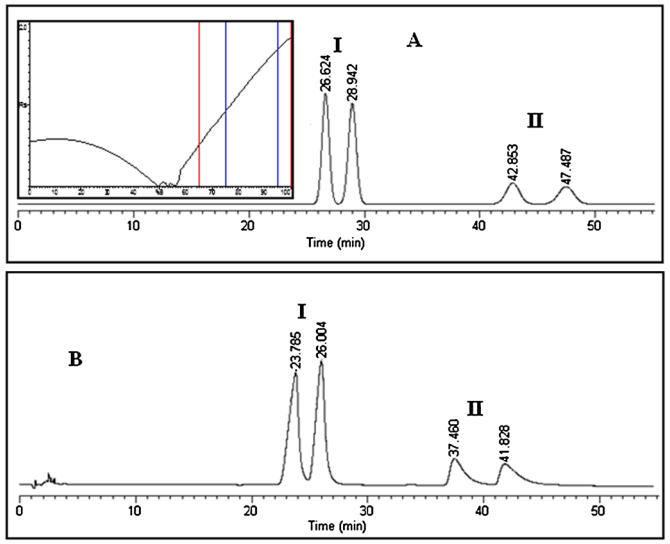

Fig. 5. Chromatograms at optimum conditions for 1 mg/ml propranolol (**I**) and 1 mg/ml salbutamol sulfate (**II**) mixture on Chirobiotic V, namely: 100% methanol with 0.01% TFA and 0.01%ammonium hydroxide, at flow rate 1.0 ml/min, and temperature 30 °C: **(A)** predicted chromatogram at optimized conditions and the corresponding resolution map; **(B)** experimental chromatogram at optimized conditions.

imparted more hydrophobic character to fit the applications scope of DryLab® and, in fact changed dramatically the original nature of the chiral analytes which highlights the importance of our study that applies to the broadly used type Chirobiotic V with no need for an extra derivatization step that modifies the chemical characteristics thus providing more information on the chiral recognition mechanisms on this stationary phase under the studied conditions.

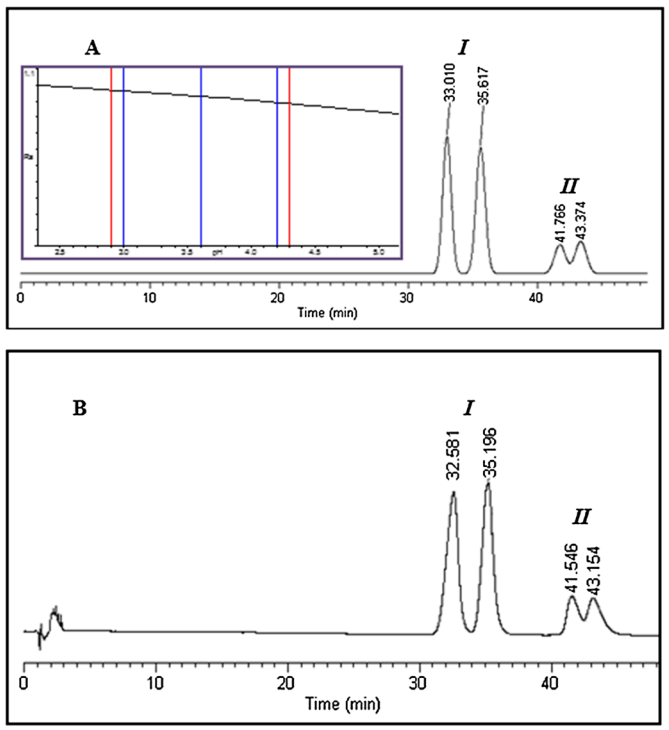

Fig. 6. Chromatograms at optimum pH 3.50 for 1 mg/ml propranolol (**I**) and 1 mg/ml terbutaline sulfate (**II**) mixture on Chirobiotic V, at 95%B (where **A**: ultrapure water adjusted at pH 3.50; **B**: methanol with 0.01% TFA and 0.01% ammonium hydroxide), at flow rate 1.0 ml/min, and temperature 30 °C. (**A**) Predicted chromatogram at optimized conditions and the corresponding resolution map; (**B**) experimental chromatogram at optimized conditions.

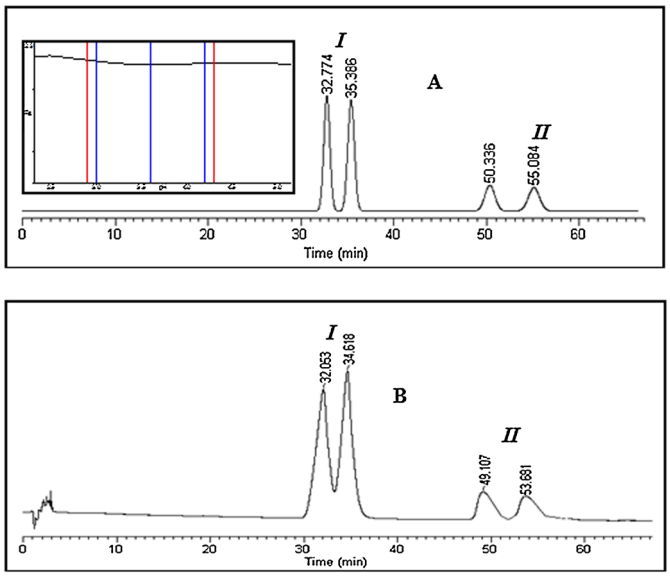

Fig. 7. Chromatograms at optimum pH 4.10 for 1 mg/ml propranolol (**I**) and 1 mg/ml salbutamol sulfate (**II**) mixture on Chirobiotic V, at 95%B (where **A**: ultrapure water adjusted at pH 4.10; **B**: methanol with 0.01% TFA and 0.01% ammonium hydroxide), at flow rate 1.0 ml/min, and temperature 30 °C. (**A**) Predicted chromatogram at optimized conditions and the corresponding resolution map; (**B**) experimental chromatogram at optimized conditions.

Chirobiotic T

Considering that Chirobiotic T has the same restriction as Chirobiotic V regarding the maximum operating temperature, 45 °C, the same approach followed with Chirobiotic V was applied to Chirobiotic T. Separation of enantiomers of propranolol

and metoprolol with different mobile phases were conducted and the results are presented in Table 1. The results showed prolongation of retention upon decrease in percentage of aqueous component of the mobile phase, as shown in Table 2.

Even though Chirobiotic T is reported to be multimodal and can work as a reversed stationary phase, the behavior observed was untypical of that of the reversed mode with the conditions of mobile phase and analytes studied. In fact, it was reported that if water soluble analytes are eluted in a mobile phase rich in water, analytes will elute faster.[28] This retention mode is based on hydrophilic interaction chromatography (HILIC) rather than reversed phase liquid chromatography (RPLC). Consequently, in this chromatographic mode, increasing aqueous mobile phase is accompanied by faster elution of polar analytes. HILIC may be confused sometimes with RPLC because similar mobile phase compositions are used in both, yet it should be noted that the turning points between both modes do not only depend on the nature of the mobile phase, but also on the nature of both the analyte and the stationary phase.[29] This was clear when the same analytes and mobile phase conditions were used for both Chirobiotic V and T in one of the experimental trials. The difference in the nature of the two stationary phases has led to different modes of interactions: RPLC on Chirobiotic V and HILIC on Chirobiotic T, as explained previously. DryLab mainly predicts RPLC behavior and does not apply to the HILIC mode. As a consequence, the chiral recognition of Chirobiotic T could not be predicted by this software.

Chiralpak AD-RH

Since high temperature is not a critical parameter for the stability of this column, a two- dimensional study included the effect of simultaneous variations of temperature and % organic phase (B) on the resolution where four preliminary runs are required (40%B at *T* 30 °C and 60 °C; 60%B at *T* 30 °C and 60 °C). The hydrophobic retention mechanism was typically obeyed as presented in Table 2, where an increase in % organic phase is associated with a decrease in retention. Also when the temperature decreases, retention is increased. The resolution map at Figure 8 demonstrated that the optimum conditions for best resolution were at 2%B at 30 °C. The virtual and the experimental run, manifested in Figure 8, were compared. The virtual run showed that the retention time of the first and second eluted propranolol enantiomers are 39.545 min and 54.160 min, respectively, while the experimental run showed that the two enantiomers coeluted at 41.462 min.

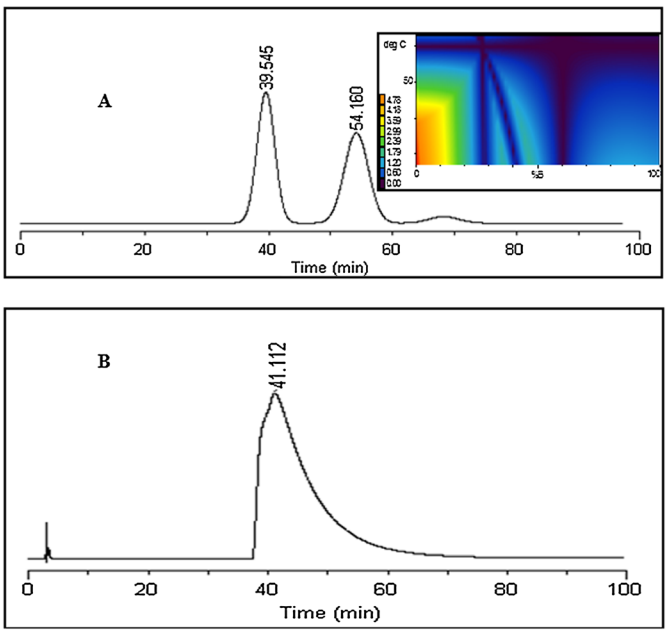

Fig. 8. Chromatograms of 0.1 mg/ml propranolol on Chiralpak AD-RH at optimized conditions, namely: 2%B (where **A**: 20mM phosphate buffer pH 8.00; **B**: acetonitrile) at 30 °C, at flow rate 0.6 ml/min. **(A)** Predicted chromatogram and the corresponding resolution map; **(B)** Experimental chromatogram at optimized conditions.

The amylose phenyl carbamate interaction is mediated via hydrogen bond (to the CO or NH of the carbamate moieties) and π–π interaction (between the phenyl moieties). It has been reported that hydrogen bonding was found to be more significant to the interaction than the π–π interaction.[3] Considering that DryLab can predict the chromatographic behavior of a nonpolar stationary phase such as C18, in which the main interaction is with van der Waal forces, this may explain its incapability to predict the chiral behavior on Chiralpak AD-RH CSPs.

Ultron ES-OVM

This ovomucoid protein-based stationary phase is highly unstable under the effect of temperatures higher than 40 °C and an organic modifier higher than 40%B. Two isocratic runs at 5% and 25% B were performed, as demonstrated in Table 2. No significant change occurred in the separation of ibuprofen enantiomers at different % organic phase conditions, which hindered the process of predictions.

CONCLUSION

DryLab software was able to predict the chiral chromatographic behavior of several compounds varying in their acidic/basic properties on Chirobiotic V, which can dramatically ease the method development procedure on this specific stationary phase. Also, this result leads to better understanding of the chiral recognition of the CSPs regarding the studied analytes and mobile phase/column parameters. It can be concluded that, even though Chirobiotic V and T belong to the same macrocyclic glycopeptides, different behavior was observed on both columns when the same mobile phase and analyte were applied to both columns. In fact, Chirobiotic V showed typical RPLC behavior while the retention mechanism of Chirobiotic T at the same conditions followed the HILIC mode, which accounts for the inability of software such as DryLab to predict chiral behavior on Chirobiotic T. As for the Chiralpak AD-RH, the significance of hydrogen bond- based interaction in the chiral recognition at the studied conditions hinders the ability of DryLab to predict its chiral behavior. The multiple limitations of conditions used safely on Ultron ES-OVM have made the preliminary runs required for DryLab unattainable.

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