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Application and comparison of immobilized and coated amylose tris-(3,5-dimethylphenylcarbamate) chiral stationary phases for the enantioselective separation of β-blockers enantiomers by liquid chromatography

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Abstract

A direct liquid chromatographic enantioselective separation of a set of β-blocker enantiomers on the new immobilized and conventional coated amylose tris-(3,5-dimethylphenylcarbamate) chiral stationary phases (Chiralpak IA and Chiralpak AD, respectively) was studied using methanol as mobile phase and ethanolamine as an organic modifier (100:0.1, v/v). The separation, retention and elution order of the enantiomers on both columns under the same conditions were compared. The effect of the immobilization of the amylose tris-(3,5-dimethylphenylcarbamate) chiral stationary phase on silica (Chiralpak IA) on the chiral recognition ability was noted when compared to the coated phase (Chiralpak AD) which possesses a higher resolving power than the immobilized one (Chiralpak IA). A few racemates, which were not or poorly resolved on the immobilized Chiralpak IA were most efficiently resolved on the coated Chiralpak AD. However, the immobilized phase withstand solvents like dichloromethane when used as an eluent or as a dissolving agent for the analyte. The versatility of the immobilized Chiralpak IA in monitoring reactions performed in dichloromethane using direct analysis techniques without further purification, workup or removal of dichloromethane was studied on a representative example consisting of the lipase-catalyzed irreversible transesterification of a β-blocker using either vinylacetate or isopropenyl acetate as acyl donor in dichloromethane as organic solvent.

Keywords: Amylose tris-(3,5-dimethylphenylcarbamate); Chiralpak IA; Enantioseparation; LC; Kinetic resolution

1. Introduction

In pharmaceutical industry, often only one enantiomer of a racemic chiral drug is the effective agent and has a therapeutically useful action, while the second enantiomer does not or may be less effective, totally ineffective, or in the worst case even toxic [1]. The world became terribly aware of chirality in drugs when a number of children were born with severe birth defects in the late 1950s after pregnant mothers had been prescribed thalidomide for the treatment of morning sickness. Thalidomide was administered as a racemic drug that was consisting of equal amounts of both enantiomers. One of the enantiomers was the pharmaceutical agent but the other caused developmental malformations. Later, research has indicated that the enantiomers actually are interconverted in the body [2]. Legislation now requires both enantiomers of a racemate drug to be studied in detail or its pharmacological effects and consequently, the market for enantiopure drugs is rapidly expanding. For example, the worldwide revenues from chiral products reached US$ 7 billion in 2002 and about US$ 9.5 billion in 2005. The global sales of single-enantiomer drugs are expected to reach US$ 14.94 billion by the end of 2009 with an average annual growth rate of 11.4% [3]. Accordingly, a great deal of effort has been developed over the years to make the asymmetric access to enantiomerically pure drugs more appealing to the large demand of the market. Both chemical or chemoenzymatic methods can yield

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enantiomerically pure and single enantiomers [4]. The ways in which efficiency and practicality of these methods are defined are depending on a large number of factors. Among these factors are suitable equipments and reliable methods used in the determination of the enantiomeric excess (ee) of the single enantiomer [5].

The development of accurate non-chiroptic methods for the determination of enantiomeric purity has been critical for the development of enantioselective catalysis. Thus, a prerequisite in the asymmetric synthesis of drugs is a precise and reliable assessment of the enantiomeric purity of the resulting products [6]. Among these methods are: polarimetric methods, gas chromatographic methods, liquid chromatographic methods and NMR spectroscopy. The modern and most sensitive methods used in the determination of enantiomeric purity of the outcome of asymmetric reactions, allowing a detection as little as 0.1% of one enantiomer in the presence of another, are enantioselective gas chromatography (GC) and liquid chromatography (LC) methods [7]. Enantioselective LC is the most popular technique used for the separation and quantification of the enantiomers especially for non-volatile substances [8].

The correct choice of the chiral stationary phase (CSP) in LC determines the success or failure of a chromatographic enantioselective separation. Since their introduction to chiral separation, derivatized cellulose- and amylose-based CSPs have proved their usefulness as chiral selectors in LC. A wide range of enantiomeric compounds, including racemic aromatic alcohols [9], enantiomeric amides [10], pyriproxyfen [11], amino alcohols [12] diols [13], β-blockers [14–16], racemic carboxylic acids [17] and other miscellaneous compounds [18], have been separated on these CSPs. Of the derivatives of amylose, the tris-(3,5-dimethylphenylcarbamate) was the best derivative used in chiral recognition and the most efficient for many racemates [19]. However, the amylose tris-(3,5-dimethylphenylcarbamate) (Chiralpak AD) is not compatible to all solvents as eluents. Some solvents known as prohibited LC solvents, such as ethyl acetate (EtOAc), tetrahydrofuran (THF), methyl *tert*-butyl ether (MtBE), dichloromethane (DCM) and chloroform, in which the polysaccharide derivatives themselves are dissolved or swollen, are unable to be used as eluents. Consequently, a reaction performed in any of the prohibited LC solvents cannot be directly or online monitored by LC unless the harmful solvent is removed and the analyte itself is dissolved in traditional mobile phase solvents. To improve the defect, the polysaccharide derivatives have been immobilized on a silica matrix and have been used extensively as chiral stationary phase [19]. Such immobilization of the polymeric chiral selectors on the silica support is considered as an efficient approach to confer a universal solvent compatibility to this kind of CSP, thereby broading the choice of solvents able to be used as mobile phases [20].

A new generation of CSPs for LC using a novel immobilization technology has been recently launched. Thus, Chiralpak IA, a 3,5-dimethylphenylcarbamate derivative of amylose, immobilized onto silica (the immobilized version of Chiralpak AD) has been recently commercialized [20].

β-Blockers also known as beta-adrenergic blockers, are medicines that affect the body's response to certain nerve impulses. This, in turn, decreases the force and rate of the heart's contractions, which lowers blood pressure and reduces the heart's demand for oxygen. β-Blockers have at least one chiral center in their side chain and most of them are marketed as racemic mixtures except *S*-timolol and *S*-penbutolol [21]. Differences in activities among enantiomers of the β-blockers are well known. For example, the *S* enantiomer of propranolol is hundred times more potent as a β-blocking agent than the *R* enantiomer. Therefore, their synthesis and enantioselective separations are of a great interest [1]. In this context, we wish to study the effects of the immobilization of the amylose tris-(3,5-dimethylphenylcarbamate) on silica support (Chiralpak IA) on the chiral recognition ability in LC. The study consists of a comparison of the immobilized and coated (free) polysaccharide-based chiral stationary phases (Chiralpak IA and Chiralpak AD, respectively) for the enantioselective separation of enantiomers of β-blockers. The solvent versatility of the immobilized Chiralpak IA is demonstrated in a representative example consisting of the lipase-catalyzed kinetic resolution of a β-blocker in dichloromethane.

2. Experimental

2.1. Instrumentation

The mobile phase for LC was filtered through a Millipore membrane filter (0.2 μm) from Nihon Millipore (Yonezawa, Japan) and degassed before use. The LC system consisted of a Waters binary pump, Model 1525 (Milford, MA, USA), equipped with a dual λ absorbance detector model 2487, an autosampler model 717plus and an optical rotation detector (Chiralyzer, JM Science Inc., Grand Island, NY, USA)) operating at room temperature. The UV-detector was set at different wavelength depending on the analyte. The Chiralpak AD column (4.6 mm × 250 mm i.d. coated on 5 μm silica-gel) was purchased from Chiral Technologies Europe (France) and the Chiralpak IA (4.6 mm × 250 mm i.d. immobilized onto 5 μm silica-gel) was obtained from Chiral Technologies (West Chester, PA, USA). Collection of data was performed using Breeze Software9® from Waters.

2.2. Materials

The LC-grade *n*-hexane, methanol and dichloromethane were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The ethanolamine, vinylacetate and isopropenyl acatate were purchased from Aldrich (Milwaukee, USA). Alprenolol

is obtained from Haessle (Sweden). Atenolol, metoprolol, propranolol, pindolol and phenoxypropionic acid are from Aldrich (Milwaukee, USA). Bisoprolol was obtained from Merck (Darmstad, Germany). Oxprenolol was obtained from Ciba Geigy AG (Switzerland), Timolol was obtained from Merck, Sharpe & Dohme (Rahway, NJ, USA). Penbutolol was obtained from Hoechst (Frankfurt, Germany). *O*-Methoxymandelic acid, ketoprofen and warfarin were purchased from Sigma (St. Louis, USA). Lipase from *Pseudomonas cepacia* (PSL) was a gift from Amano (Nagoya, Japan).

2.3. Chromatographic conditions

The mobile phase consisted of LC-grade methanol (100%) with ethanolamine modifier (0.1%). The flow rate was fixed at 0.2 ml/min. The column was at room temperature (24 °C). UV detection was set at different wavelength depending on the analyte and reported in Figs. 2 and 3.

2.4. Lipase-catalyzed reactions

Transesterifiactions were carried out at room temperature in 5 ml glass vial. The magnetic stirrer speed was kept at 400 rpm. In a typical experiment, 77 mg (0.3 mmol) racemic propranolol, 100 μl vinyl acetate or 66 μl isopropenyl acetate (0.6 mmol), 50 mg *P. cepacia* and 3 ml dichloromethane were added. An aliquot of the supernatant is withdrawn at several time intervals and analyzed by LC without further derivatization or workup.

3. Results and discussion

The immobilization of the polysaccharide-based chiral selectors on a silica support is considered as an effective approach to confer a universal solvent compatibility to this kind of CSP in LC. Therefore, broadening the choice of solvents used as mobile phases [20]. Prohibited LC solvents like ethyl acetate, dichloromethane, ethyl acetate (AcOEt), tetrahydrofuran (THF) and other solvents in which the polysaccharide derivatives themselves are dissolved or swollen, cannot be used as eluents in conventional coated LC columns, however, it can be successfully used in LC columns containing immobilized stationary phases. Based on that, a set of β-blocker drugs (Fig. 1) were selected and investigated for the enantioselective separation on the new immobilized amylose tris-(3,5-dimethylphenylcarbamate) on silica commercially known as Chiralpak IA. To study the solvents versatility of the new column, a mixture of *n*-hexane and ethylacetate in different ratios (70:30, 80:20, 90:10, v/v) were used a mobile phases. However, a large negative peak appears to interfere with the peak of analyte and none of the reported β-blockers has been separated. The large negative peak is probably due to the high UV absorption of ethylacetate (260 nm) in comparison to the UV absorption of the analyte itself. Moving from ethylacetate (UV absorption 260 nm) to dichloromethane (UV absorption 233 nm) as mobile phase, the chromatograms did improve, albeit the existence of a small negative peak, but without any influence on the enhancement of the separation. Trying to change the analyte itself from β-blockers having a secondary hydroxyl group to acidic drugs having a carboxylic function group, such as ketoprofen, *O*-methoxymandelic acid and warfarin a separation could be established (unpublished results). It concluded to us that it is difficult to establish a baseline separation of β-blockers using the above reported LC prohibited solvents upon using Chiralpak IA as stationary phase in LC albeit the ability of this stationary phase to withstand the non-conventional and prohibited LC solvents.

To study in details the effect of the immobilization of the amylose tris-(3,5-dimethylphenylcarbamate) on silica on the chiral recognition ability, a comparison was performed between the immobilized and coated amylose tris-(3,5-dimethylphenylcarbamate) chiral stationary phases (Chiralpak IA and Chiralpak AD, respectively) for the enantioselective separation of enantiomers of β-blockers by LC using conventional LC organic solvents. Thus, the enantioselective analysis of β-blockers was investigated using methanol and ethanolamine 100:0.1 (v/v) ratio. Chromatograms including the separation factor (α) and the resolution (R_s) are shown in Fig. 2 and Table 1.

Of the chiral β-blocker drugs investigated in this study, acebutolol **1**, atenolol **3**, bunolol **5**, celiprolol **7**, indenolol **8**, penbutolol **12** and timolol **15** have not been separated on both columns when using methanol and ethanolamine 100:0.1 (v/v) ratio as eluents. Alprenolol **2**, bisoprolol **4**, carazolol **6**, metoprolol **9**, oxprenolol **11**, pindolol **13** and propranolol **14** were baseline separated on Chiralpak AD while nebivolol **10** was not. However, most of these compounds either have

Table 1
The resolution (R_s) and separation factor (α) of the simultaneous LC separation of racemic β-blockers on Chiralpak AD and Chiralpak IA using *n*-hexane and ethanolamine (100:0.1, v/v) as mobile phase

Compounds	Chiralpak AD		Chiralpak IA	
	R_s	α	R_s	α
1	n.s.	n.s.	n.s.	n.s.
2	1.0	1.5	n.s.	n.s.
3	n.s.	n.s.	n.s.	n.s.
4	0.9	1.3	1.6	6.5
5	n.s.	n.s.	n.s.	n.s.
6	1.2	3.4	1.0	0.6
7	n.s.	n.s.	n.s.	n.s.
8	n.s.	n.s.	n.s.	n.s.
9	1.1	2.4	1.0	0.5
10	n.s.	n.s.	1.3	2.4
11	1.1	1.7	1.0	0.5
12	n.s.	n.s.	n.s.	n.s.
13	1.08	1.0	n.s.	n.s.
14	1.2	2.9	1.0	0.8
15	n.s.	n.s.	n.s.	n.s.

n.s., Not separated.

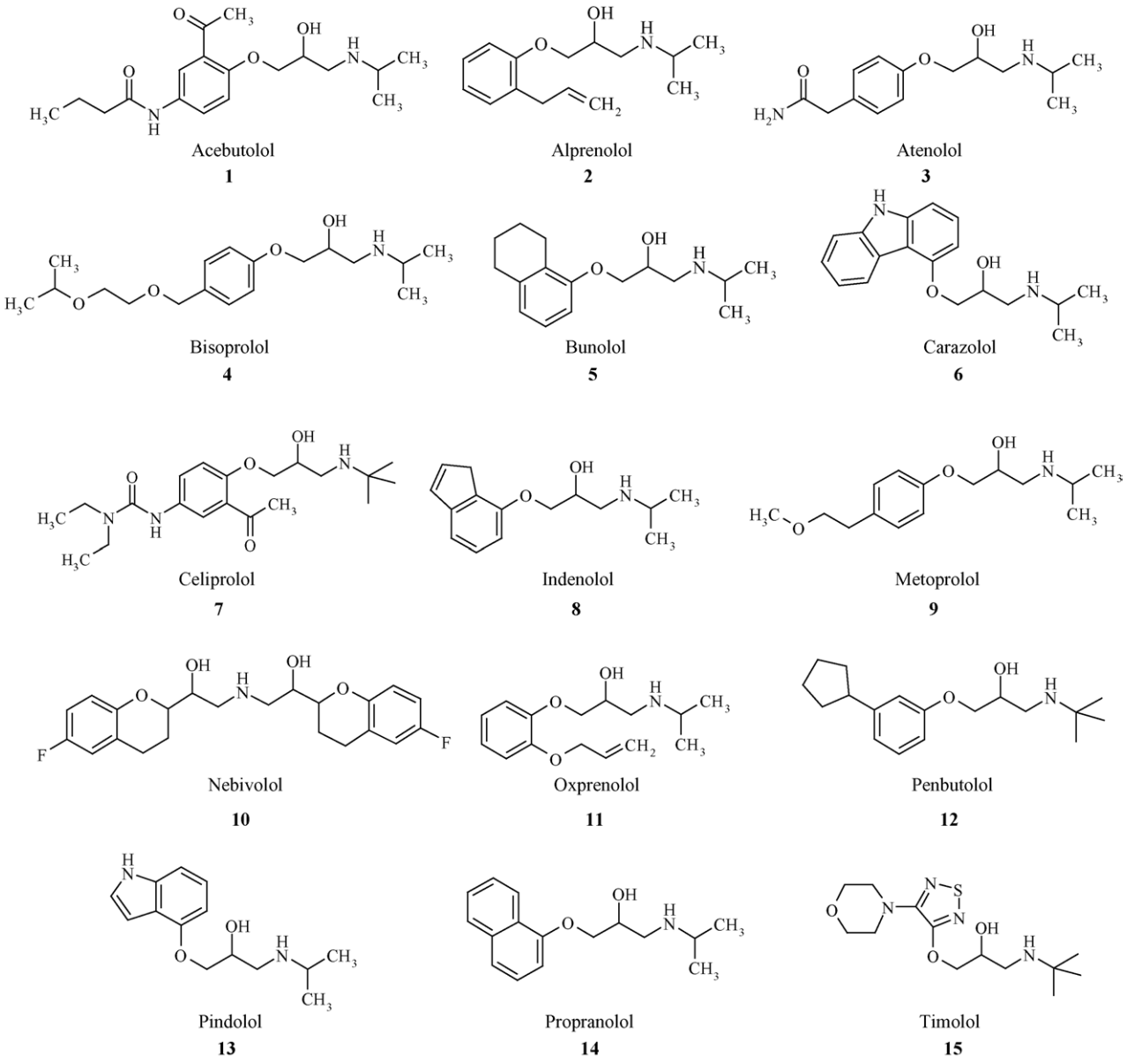

Fig. 1. Chemical structure of selected β-blocker drugs used in the investigation for the enantioselective separation on both Chiralpak IA and Chiralpak AD.

not been separated or poorly separated on Chiralpak IA, albeit the same conditions used in both cases. Alprenolol **2** and pindolol **13** have not been separated on Chiralpak IA while being baseline separated on Chiralpak AD. Carazolol **6**, metoprolol **9**, oxprenolol **11** and propranolol **14** have been partly or poorly separated on Chiralpak IA in comparison with Chiralpak AD. The separation factor (α) and resolution (R_S) of these compounds decreased when moving from Chiralpak AD to Chiralpak IA (cf. Table 1). An exceptional case is nebivolol **10** and bisoprolol **4**. The former which has not been separated on Chiralpak AD is baseline separated on Chiralpak IA with a separation factor $\alpha = 1.3$ and resolution $R_S = 2.4$. The latter has been separated on Chiralpak IA with a separation factor $\alpha = 1.6$ and resolution $R_S = 6.5$ compared to 0.9 and 1.3 in case of Chiralpak AD, respectively. Although the stationary phase is similar in both columns, the chiral recognition in case of Chiralpak IA is different from that in Chiralpak AD. Indeed, the immobilization of the amylose tris-(3,5-dimethylphenylcarbamate) on silica did affect the chiral recognition ability showing a lower resolving ability than the coated Chiralpak AD. This is probably due to the change in the polymer configuration and/or supramolecular structure due to the immobilization on silica. Results are summarized in Fig. 2.

As all analytes resolved above have been dissolved in the mobile phase before injection, we decided to study the influence of a non-conventional solvent, such as

dichloromethane on the chiral recognition ability by dissolving the analyte itself in DCM and carrying the analysis using the mobile phase reported above (methanol:ethanolamine 100:0.1, v/v). No influence on chiral recognition ability was observed when dissolving the analyte in DCM and performing the analysis as previously discussed above and all chromatograms were similar to those shown in Figs. 2 and 3. A representative example consisting of the

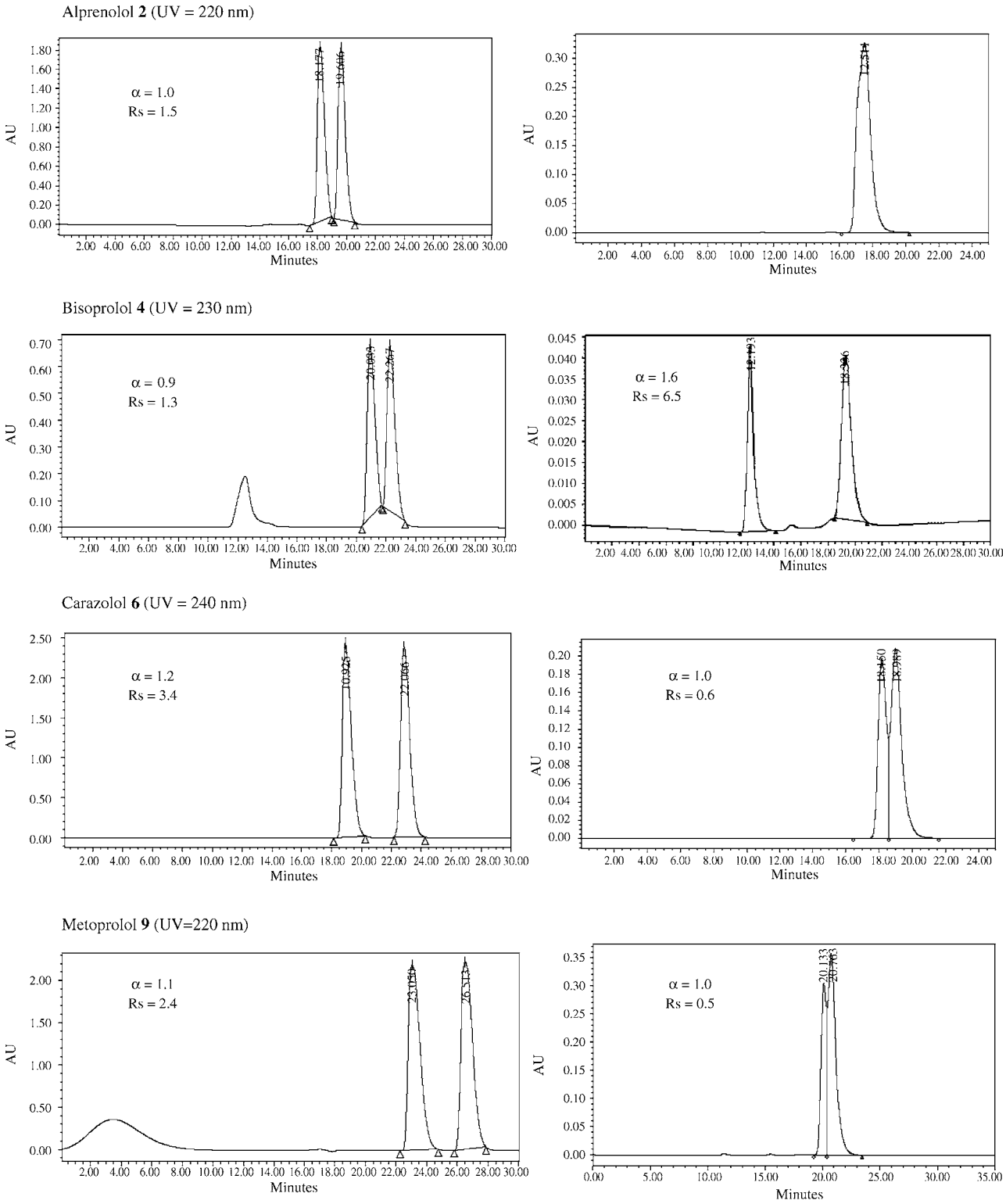

Fig. 2. Chromatograms of the enantioselective HPLC analysis of racemic β-blockers on Chiralpak AD (left) and Chiralpak IA (right) using *n*-hexane and ethanolamine (100:0.1, v/v).

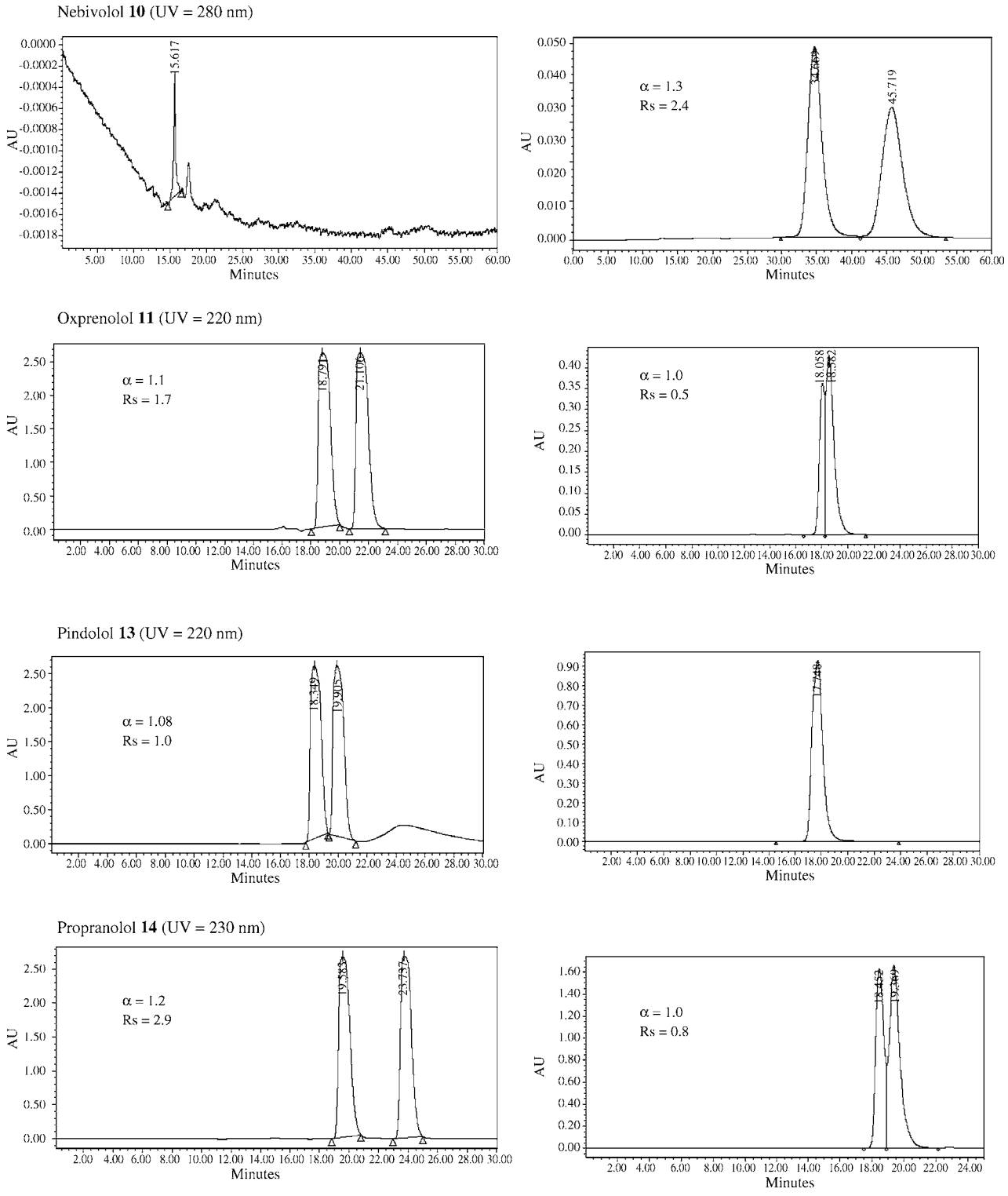

Fig. 2. (*Continued*).

enantioselective analysis of bisoprolol dissolved in conventional mobile phase (A), in DCM (B) and only DCM as reference is shown in Fig. 3.

Based on its ability to withstand prohibited LC solvents like DCM, the immobilized Chiralpak IA® can be used to monitor any reactions performed in DCM where the resolved compound is involved. To highlight the stability of such column and its versatility to be used in reaction monitoring performed in DCM, the lipase-catalyzed kinetic resolution of propranolol was investigated in DCM (Fig. 4). In general,

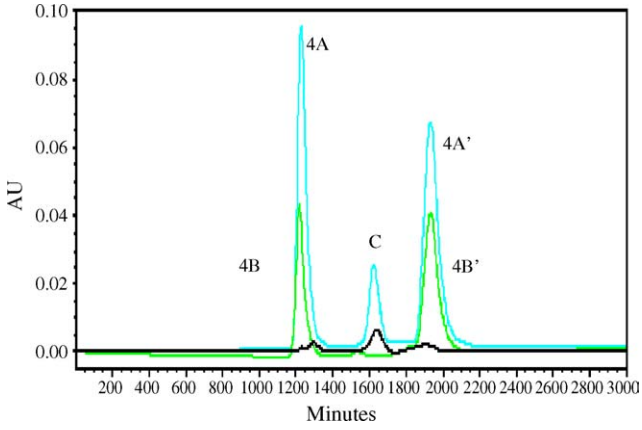

Fig. 3. Chromatograms of the enantioselective HPLC analysis of bisoprolol dissolved in mobile phase (4A and 4A′), DCM (4B and 4B′) and only DCM (C) on the immobilized amylose tris-(3,5-dimethylphenylcarbamate) on silica (Chiralpak IA) using *n*-hexane and ethanolamine (100:0.1, v/v) as mobile phase at 230 nm UV.

the lipase-catalyzed kinetic resolution of a racemate involves the presence of a suitable acyl donor and a lipase in an organic solvent. Thus, only one enantiomer of the racemic substrate is selectively esterified as the faster reacting enantiomers yielding the corresponding acetate in high ee and leaving the second enantiomer in enantiomerically pure/enriched form.

The resolution of the racemic propranolol was performed via the transesterification mode where the acyl donors of choice are enol esters, such as vinyl acetate or isopropenyl acetate. The vinyl alcohol formed as a byproduct when using vinyl acetate undergoes keto–enol tautomerization yielding the corresponding carbonyl compound (acetaldehyde), while the isopropenyl alcohol released when using isopropenyl acetate tautomerizes to acetone making the reaction practically irreversible in both cases. Both byproducts released in both cases are harmful to conventional LC chiral column; thus, a workup is needed before analyzing samples on conventional LC columns. Using Chiralpak IA, this workup is not required. The results revealed that using *P. cepacia* lipase and either vinyl acetate or isopropenyl acetate as acyl donors in DCM, the (*R*)-propranolol is selectively esterified leaving the (*S*)-propranolol in enantiomerically enriched form (Figs. 5 and 6). The reaction monitoring is performed by withdrawing an aliquot of the supernatant including DCM and injected directly to LC without any further purification, workup or removal of DCM. The absolute configuration of the resulting alcohol and ester was determined by comparison with authentic samples.

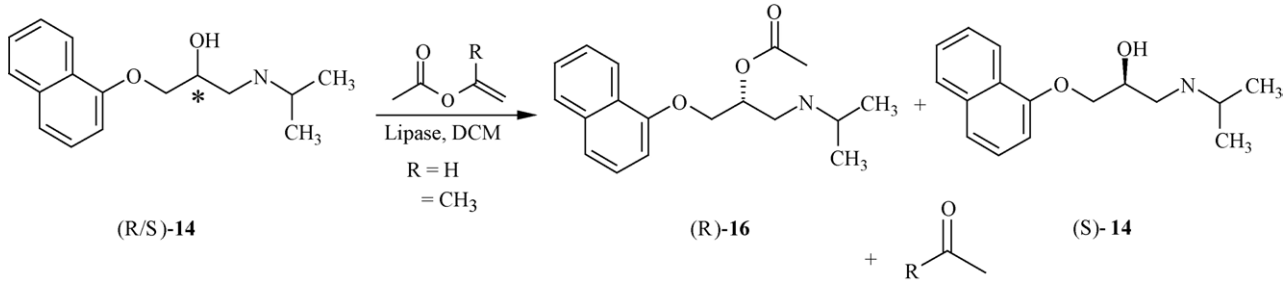

Fig. 4. Lipase-catalyzed irreversible transesterification of racemic propranolol using either vinyl acetate (R = H) or isopropenyl acetate (R = CH_3) in dichloromethane.

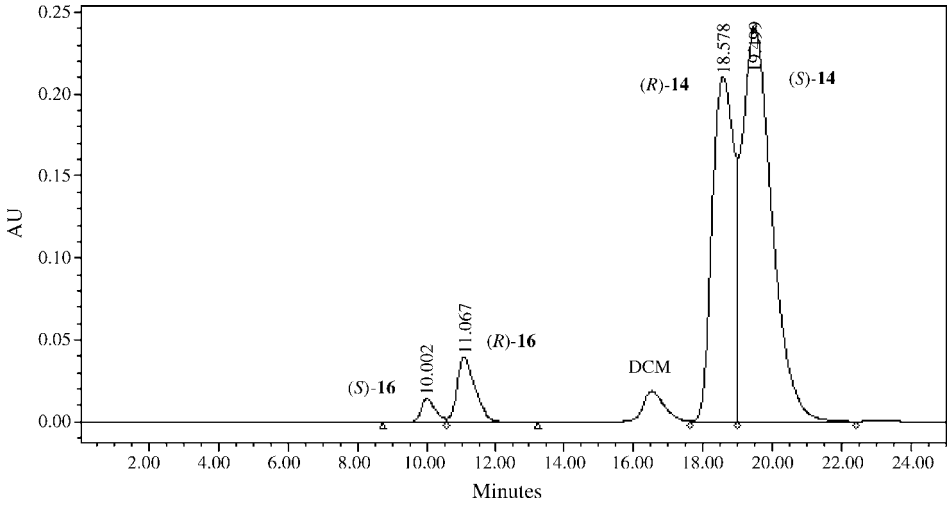

Fig. 5. Enantioselective HPLC analysis of the outcome of the lipase-catalyzed irreversible transesterification of racemic propranolol using vinyl acetate as acyl donor in DCM as organic solvent on Chiralpak IA after 5 h (ee_s = 22%; ee_p = 55%; conv. = 28.5 and E = 4.2).

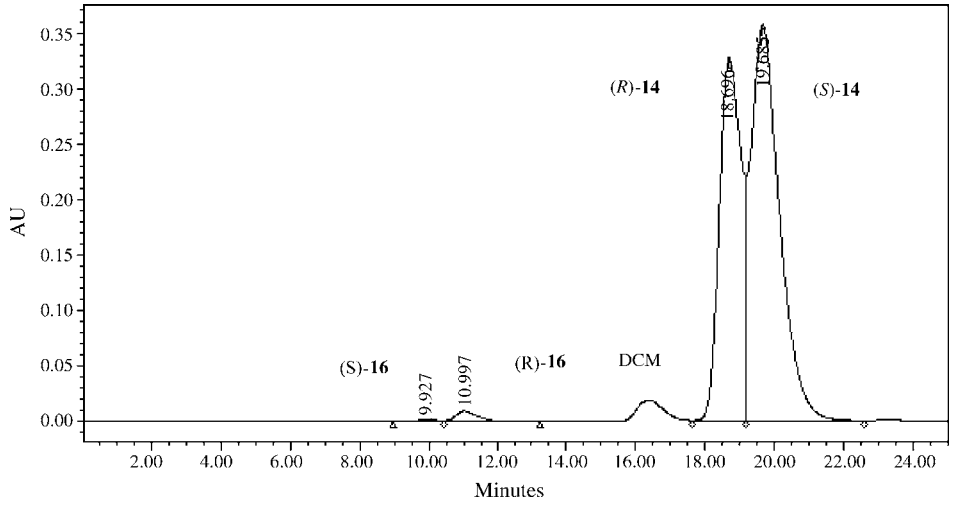

Fig. 6. Enantioselective HPLC analysis of the outcome of the lipase-catalyzed irreversible transesterification of racemic propranolol using isopropenyl acetate as acyl donor in DCM as organic solvent on Chiralpak IA after 5 h (ee_s = 17.7%; ee_p = 70.7%; Conv. = 20.0 and E = 7).

4. Conclusions

A comparison was performed on both immobilized and conventional coated amylose tris-(3,5-dimethylphenylcarbamate) chiral stationary phases (Chiralpak IA and Chiralpak AD, respectively) using methanol as mobile phase and ethanolamine as an organic modifier (100:0.1, v/v) for the enantioselective analysis of some β-blockers in LC. The separation factor and retention time of the enantiomers on both columns under the same conditions were different albeit the similarity in structure of the stationary phase in both columns. The immobilization of the amylose tris-(3,5-dimethylphenylcarbamate) on silica did affect the chiral recognition ability showing a lower resolving ability than the coated Chiralpak AD. However, the versatility of the Chiralpak IA in monitoring reaction performed in prohibited LC solvents like DCM is demonstrated in a selected example consisting of the lipase-catalyzed kinetic resolution of propranolol in DCM as organic solvent which reflect the useful application of this newly developed phase in chiral analysis.

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