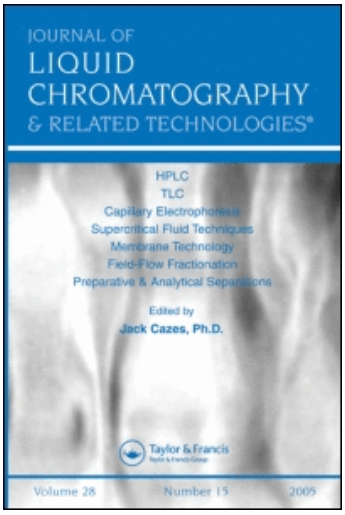

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Comparison, Applications, Advantages, and Limitations of Immobilized and Coated Amylose Tris-(3,5-Dimethylphenylcarbamate) Chiral Stationary Phases in HPLC

Ashraf Ghanem[a]; Hassan Y. Aboul-Enein[a]
[a] Centre for Clinical Research (MBC-03-65), King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

To cite this Article Ghanem, Ashraf and Aboul-Enein, Hassan Y.(2005) 'Comparison, Applications, Advantages, and Limitations of Immobilized and Coated Amylose Tris-(3,5-Dimethylphenylcarbamate) Chiral Stationary Phases in HPLC', Journal of Liquid Chromatography & Related Technologies, 28: 18, 2863 — 2874
To link to this Article: DOI: 10.1080/10826070500269919
URL: http://dx.doi.org/10.1080/10826070500269919

Journal of Liquid Chromatography & Related Technologies®, 28: 2863–2874, 2005

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070500269919

Comparison, Applications, Advantages, and Limitations of Immobilized and Coated Amylose Tris-(3,5-Dimethylphenylcarbamate) Chiral Stationary Phases in HPLC

Ashraf Ghanem and Hassan Y. Aboul-Enein
Centre for Clinical Research (MBC-03-65), King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

Abstract: A direct high performance liquid chromatographic enantioselective separation of a set of racemic acidic drugs on the new immobilized and conventional coated amylose tris-(3,5-dimethylphenylcarbamate) chiral stationary phases (Chiralpak IA and Chiralpak AD, respectively) was studied using *n*-hexane and 2-propanaol (80 : 20 v/v), containing TFA (0.1%) as mobile phase. The separation 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, as the coated phase (Chiralpak AD) 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 withstands the prohibited HPLC solvents such as dichloromethane, ethyl acetate, tetrahydrofuran, and others when used as eluents or as a dissolving agent for the analyte itself. 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 enantioselective esterification of flurbiprofen, with *n*-butanol in dichloromethane as organic solvent.

Keywords: Acidic drugs, Amylose tris-(3,5-dimethylphenylcarbamate), Chiralpak IA, Chiralpak AD, Enantioseparation, HPLC, Kinetic resolution

Address correspondence to Professor Hassan Y. Aboul-Enein, Centre for Clinical Research (MBC-03-65), King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh 11211, Saudi Arabia. E-mail: enein@kfshrc.edu.sa

INTRODUCTION

Enantiomers of racemic drugs with one or more chiral centers often have different pharmacological activity or toxicity. Thus, one enantiomer may be the effective agent and have a therapeutically useful action, while the second enantiomer does not, or may be less effective, totally ineffective, or in the worst case even toxic. Differences in the biological activity of enantiomers of a racemate arise from the different mode of protein binding and transport, mechanism of action, rate of metabolism, clearance, and persistence in the environment.[1] Accordingly, a great deal of effort has been developed over the years to make the asymmetric access to enantiomerically pure drugs and their enantioselective separation easier to handle.[2,3] Single enantiomers can be produced by chemical or chemo-enzymatic synthesis. The ways in which efficiency and practicality of these procedures are defined are dependent on a large number of factors. Among these factors are suitable equipment and reliable methods used in the determination of the enantiomeric excesses (ee).[4,5] The most common methods used in the assessment of the enantiomeric purity of the resulting products are: polarimetric methods, gas chromatographic methods, liquid chromatographic methods, and NMR spectroscopy.[6–10] 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 high performance liquid chromatography (HPLC) Enantioselective HPLC is one of the most powerful and widely used techniques for both analytical and preparative purposes.[11]

The correct choice of the stationary phase in HPLC determines the success or failure of a chromatographic separation. Since their introduction to chiral separation, derivatized cellulose- and amylose-based CSPs have proven their usefulness as chiral selectors in both HPLC and capillary electrophoresis (CE). A wide range of racemic compounds, including aromatic alcohols,[12] amides,[13] pyriproxyfen,[14] amino alcohols,[15] diols,[16] β-blockers,[17–19] carboxylic acids,[20] and other miscellaneous compounds,[21] have been separated on these CSPs. Among the amylose derivatives, the tris-(3,5-dimethylphenylcarbamate) derivative was the best chiral selector used in chiral recognition and the most efficient for many racemates.[22] However, the amylose tris-(3,5-dimethylphenylcarbamate) (Chiralpak AD) is not compatible to all solvents when used as eluents in mobile phase. Some solvents known as prohibited HPLC 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 HPLC solvents cannot be directly, or online, monitored by HPLC unless the harmful solvent is removed and the analyte itself is dissolved in traditional mobile phase

solvents. To improve this defect, the polysaccharide derivatives have been immobilized on a silica matrix and have been used extensively as chiral stationary phase.[22] 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 broadening the choice of solvents able to be used as mobile phases.

A new generation of CSPs for HPLC 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.[23]

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 HPLC. The study consists of a comparison of the immobilized (Chiralpak IA) with the coated (Chiralpak AD) polysaccharide-based chiral stationary phases for the enantioselective separation of enantiomers of a set of clinically used acidic drugs (cf. Fig. 1). The solvent versatility of the immobilized Chiralpak IA is demonstrated in a representative example consisting of the lipase-catalyzed kinetic resolution of flurbiprofen in dichloromethane, using *n*-butanol in the esterification mode.

EXPERIMENTAL

Instrumentation

The mobile phase for HPLC was filtered through a Millipore membrane filter (0.2 μm) from Nihon Millipore (Yonezawa, Japan) and degassed before use. The HPLC 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 (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 × 250 mm ID coated on 5 μm silica-gel) was purchased from Chiral Technologies Europe (France) and the Chiralpak IA (4.6 × 250 mm ID 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.

Materials

The HPLC-grade *n*-hexane, 2-propanol, *n*-butanol, and dichloromethane were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The trifluoroacetic acid (TFA) was purchased from Aldrich (Milwaukee, USA). Fenoprofen, flurbiprofen, ibuprofen, ketoprofen, warfarin, *O*-methoxy mandelic acid were

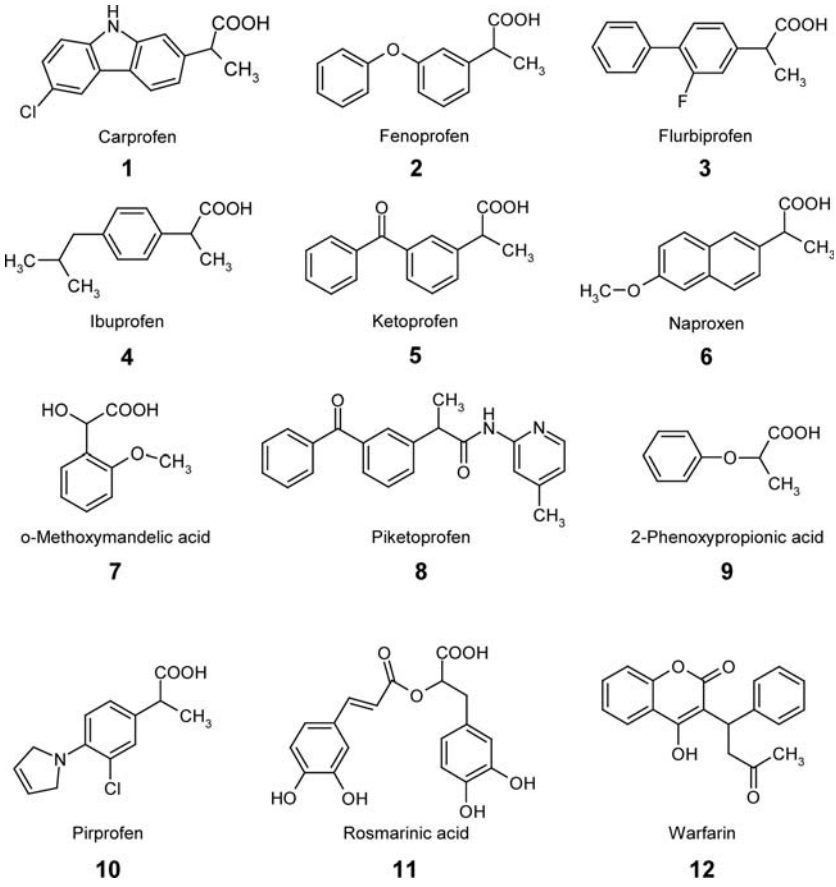

Figure 1. Structures of selected acidic drugs used for the enantioselective separation on both Chiralpak IA and Chiralpak AD.

purchased from Sigma (St. Louis, USA). *O*-phenoxypropionic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Naproxen was purchased from Synthex Research, UK. Carprofen, piketoprofen, pirprofen, and rosmarinic acid were gifts from different sources. Immobilized lipase from *Candida antarctica* (Novozym 435) was a gift from Novo Nordisk (Denmark).

Chromatographic Conditions

The mobile phase consisted of HPLC-grade *n*-hexane and 2-propanol (80/20 v/v) with trifluoroacetic acid (0.1%). The flow rate was fixed at 1 mL/min except in certain cases. The column was at room temperature (24°C). UV detection was set at different wavelengths depending on the analyte.

Lipase Catalyzed Reactions

The enantioselective esterification of flurbiprofen mediated by lipase in dichloromethane was carried out at room temperature in 5 mL glass vials. The magnetic stirrer speed was kept at 400 rpm. In a typical experiment, 150 mg immobilized lipase from *Candida antarctica* (Novozym 435) and 3 mL of a stock solution consisting of 0.6 mM racemic flurbiprofen was dissolved in dichloromethane. *n*-Butanol (1.5 mL) was added and the reaction mixture was shaken at room temperature. An aliquot of the supernatant is withdrawn at several time intervals and analyzed by HPLC without removal of the dichloromethane nor further derivatization or workup.

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 HPLC, therefore, broadening the choice of solvents used as mobile phases.[23] Prohibited HPLC solvents like ethyl acetate (AcOEt), dichloromethane (DCM), tetrahydrofuran (THF), and other solvents in which the polysaccharide derivatives themselves are dissolved or swollen, cannot be used as eluents in conventional coated HPLC columns, however, it can be successfully used in HPLC columns containing immobilized stationary phases. Based on that, a set of acidic drugs (cf. 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 this new column, a mixture of *n*-hexane and ethylacetate in different ratios (70 : 30, 80 : 20, 90 : 10 v/v) with TFA (0.1%) were used at 1 mL/min flow rate and different UV detection, depending on the analyte. However, a large negative peak appears to interfere with the peak of most analytes investigated in this study which prevent their separation, except *o*-methoxy mandelic acid, which has been partially resolved using *n*-hexane/ethyl acetate/TFA (70 : 30 : 0.1 v/v/v) at flow rate 0.5 mL/min (cf Fig. 2). 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 tetrahydrofuran (THF) (UV absorption 215 nm) as mobile phase, the chromatograms did improve but without any influence on the enhancement of the separation of most compounds.

Only the enantiomers of fenoprofen, flurbiprofen, and ketoprofen could be partially resolved upon using *n*-hexane/THF/TFA (90:10:0.1 v/v/v) with a flow rate of 0.5 mL/min.

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

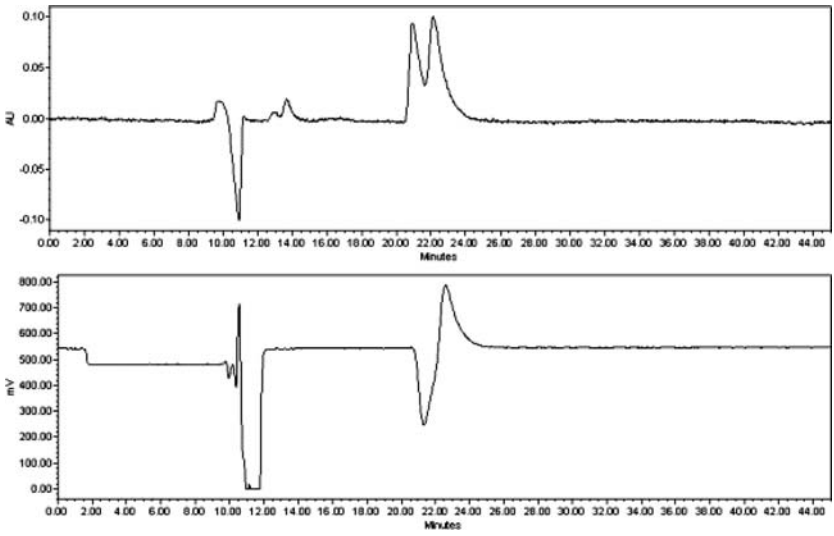

Figure 2. Typical HPLC chromatogram and the optical detection of the enantioselective separation of *O*-methoxy mandelic acid; mobile phase: *n*-hexane/ethyl acetate/TFA (70 : 30 : 0.1 v/v/v) at flow rate 0.5 mL/min and UV detection at 240 nm.

tris-(3,5-dimethylphenylcarbamate) chiral stationary phases (Chiralpak IA and Chiralpak AD, respectively) for the enantioselective separation of enantiomers of a set of acidic drugs by HPLC using conventional HPLC organic solvents. Thus, the enantioselective analysis of acidic drugs was investigated using *n*-hexane and 2-propanaol (80 : 20 v/v) as mobile phase containing TFA (0.1%) at 1 mL/min flow rate, except for 2-phenoxypropionic acid at which the flow rate was set at 0.1 mL/min to ensure a baseline separation. Chromatograms including the separation factor (α) and the resolution (R_s) are shown in Fig. 3.

Of the chiral racemic acidic drugs investigated in this study, carprofen, flurbiprofen, 2-phenoxypropionic acid, and piketoprofen have been resolved on both columns. Only, carprofen was resolved better on Chiralpak AD ($\alpha = 1.1$ and $R_s = 2$) in comparison with Chiralpak IA ($\alpha = 1.2$ and $R_s = 3.1$), the rest were better resolved on Chiralpak AD, albeit the same conditions were used in both columns. Ketoprofen and pirprofen were not resolved on Chiralpak IA, but baseline separated on Chiralpak AD under the same condition. Ibuprofen was not separated at all on both columns under the conditions used. The highest separation factor ($\alpha = 1.25$) and resolution ($R_s = 6.6$) was achieved in the enantioselective separation of 2-phenoxypropionic acid enantiomers on Chiralpak AD.

Although the chemical structure of the stationary phase is similar in both columns, the chiral recognition in the case of immobilized Chiralpak IA is different from that in the coated Chiralpak AD. Indeed, the immobilization

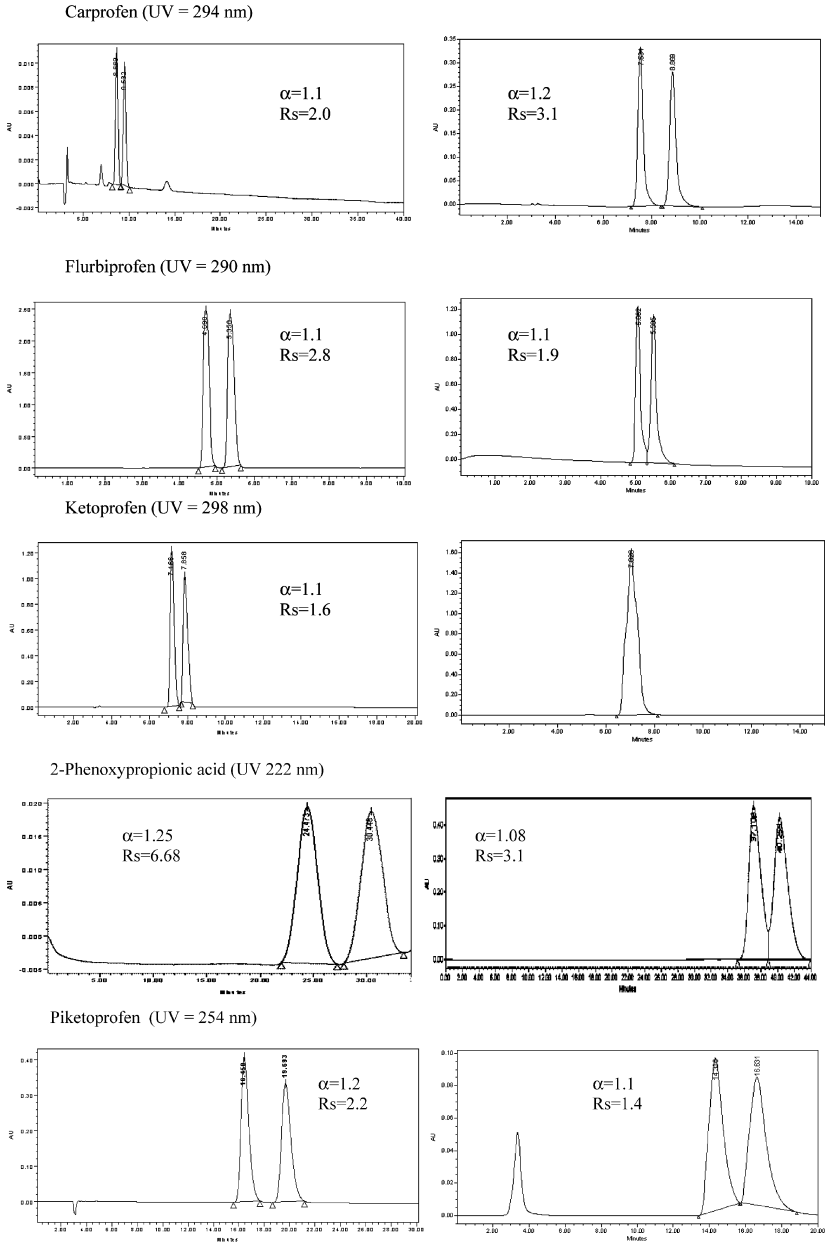

Figure 3. Chromatograms of the enantioselective HPLC analysis of racemic acidic drugs on Chiralpak AD (left) and Chiralpak IA (right) using *n*-hexane and 2-propanaol (80 : 20 v/v) as mobile phase containing TFA (0.1%) at 1 mL/min flow rate, except in case of 2-phenoxypropionic acid where the flow rate is 0.1 mL/min.

(*continued*)

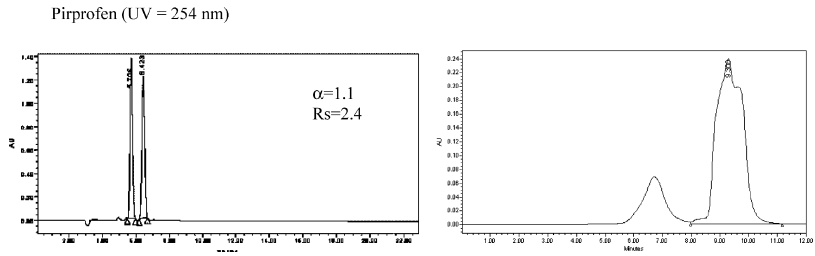

Figure 3. Continued.

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, except for the separation of carprofen which was better resolved on immobilized Chiralpak IA. 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. 3.

As all analytes resolved above have been dissolved in the mobile phase before injection, inspired by these results, the influence of a non-conventional solvent such as dichloromethane (DCM) on the chiral recognition ability is studied. This has been done by dissolving the analyte itself in DCM and carrying out the analysis using the mobile phase reported above (*n*-hexane/2-propanaol/TFA, 80 : 20/0.1 v/v/v) at 1 mL/min flow rate and different UV detection depending on the analyte. No influence on chiral recognition ability was observed when dissolving the analyte in DCM and performing the analysis as previously discussed above, since all the chromatograms were similar to those shown in Fig. 3. A representative example consisting of the enantioselective analysis of flurbiprofen dissolved in conventional mobile phase (A), in DCM (B), and only DCM as reference, is shown in Fig. 4.

Once a separation of a racemic acidic drug is established, the immobilized Chiralpak IA with its ability to withstand prohibited HPLC solvents like DCM can be used to monitor any reactions performed in DCM where the resolved compound is involved. To highlight the stability of such a column and its versatility in reaction monitoring performed in DCM, the lipase-catalyzed kinetic resolution of racemic flurbiprofen was investigated in DCM using the esterification mode (cf. Fig. 5).

In general, the enantioselective esterification of a racemic acid involves the reaction of the substrate (the acid) with an alcohol in the presence of the enzyme and an organic solvent. Thus, only one enantiomer of the racemic acid fits into the active site of the enzyme and undergoes the biochemical transformation to the product. In other words, the substrate is selectively esterified yielding the corresponding ester in high ee and leaving the second enantiomer in enantiomerically pure/enriched form. The resolution of the racemic flurbiprofen was performed by the enantioselective esterification with *n*-butanol in the presence of immobilized lipase from *Candida antarctica* (Novozym 435) in DCM. The

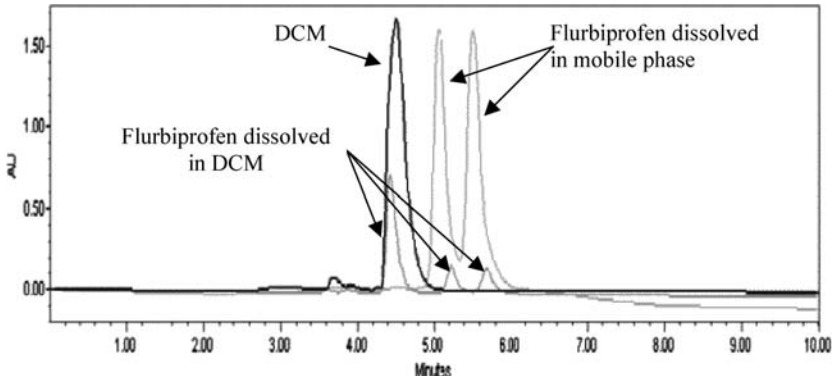

Figure 4. Overlapped chromatograms of the enantioselective HPLC analysis of flurbiprofen dissolved in mobile phase (blue) or in DCM (green) and only DCM (black) on the immobilized amylose tris(3,5-dimethylphenylcarbamate) on silica (Chiralpak IA); mobile phase: *n*-hexane/2-propanol/TFA (80 : 20 : 0.1 v/v/v) at flow rate 1 mL/min and UV detection at 290 nm.

liberated water as a by-product is scavenged by using molecular sieves 4Å. The reaction is monitored by withdrawing an aliquot of the supernatant, which consists of the substrate and product dissolved in DCM, for direct analysis on Chiralpak IA without any workup or further derivatization. However, using the same condition above with a flow rate 1 mL/min, the peaks of ester's enantiomer interfere with those of flurbiprofen. To ensure a baseline separation of both substrate and product in one analysis, the flow rate was decreased to 0.1 mL/min, which, indeed, was at the expense of the elution time (cf. Fig. 4 and 6 for noting the effect of flow rate on the retention time).

The results revealed, that when using immobilized lipase from *Candida antarctica* (Novozym 435) in enantioselective esterification of flurbiprofen with *n*-butanol in DCM, the (*S*)-flurbiprofen is selectively esterified affording the (*S*)-(+)-flurbiprofen butyl ester (64.3% ee), leaving the (*R*)-flurbiprofen in enantiomerically enriched form (45% ee) at conversion (Conv.) = 41% and enantiomeric ratio E = 7 (cf. Fig. 6). The absolute configuration of the resulting acid and ester was determined by comparison with authentic samples. Attempts to enhance the enantioselectivity

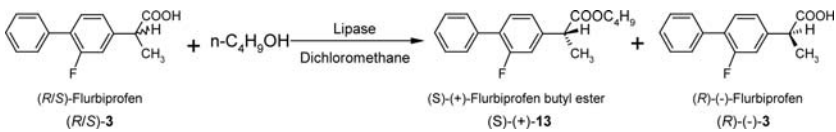

Figure 5. Lipase-catalyzed enantioselective esterification of flurbiprofen with *n*-butanol in dichloromethane.

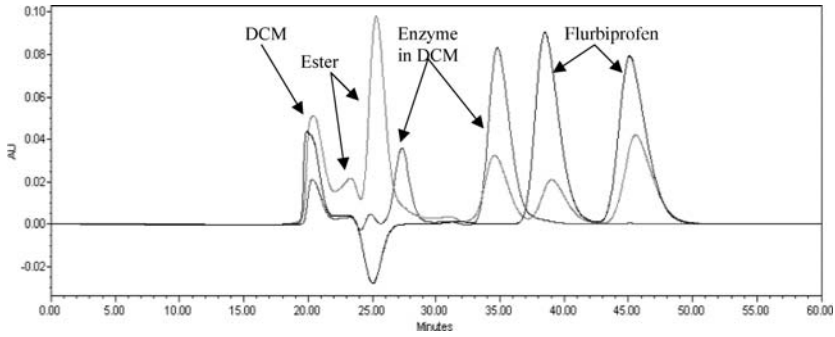

Figure 6. Overlapped chromatograms of the enantioselective HPLC analysis of the outcome of the lipase-catalyzed enantioselective esterification of racemic flurbiprofen with *n*-butanol in dichloromethane (red), only flurbiprofen in DCM (black) and only lipase dissolved in DCM (blue).

of the reaction are still under investigation in different prohibited HPLC solvents.

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 *n*-hexane and 2-propanaol (80 : 20 v/v) containing TFA (0.1%) as the mobile phase for the enantioselective analysis of some acidic drugs in HPLC. The separation factor, resolution, and retention time of the enantiomers on both columns, under the same conditions, were different albeit the similarity in structure of the stationary phase. 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 the reaction performed in prohibited HPLC solvents like DCM is demonstrated in a selected example, consisting of the lipase-catalyzed kinetic resolution of flurbiprofen in DCM as organic solvent, which reflects the useful application of this newly developed phase in chiral analysis.

ACKNOWLEDGMENTS

Thanks are due to the administration of King Faisal Specialist Hospital and Research Center for their support to the Pharmaceutical Analysis

Laboratory Research Program and to Mr. Hubert Hoenen for his technical assistance. The authors would like to thank Mr. Thomas Lewis, Chiral Technologies, West Chester, PA, USA, for supplying the Chiralpak IA column used in this study.

REFERENCES

1. Aboul-Enein, H.Y.; Abou-Basha, L.I. Chirality and drug hazards. In *The Impact of Stereochemistry on Drug Development and Use*; Aboul-Enein, H.Y., Wainer, I.W., Eds.; John Wiley & Sons: New York, 1997; Chap. 1, 1–19.
2. Muller, G.W. Thalidomide: From tragedy to new drug discovery. CHEMTECH **1997**, *27*, 21–25.
3. Rouhi, A.M. Chiral chemistry. Chem. Engn. News **2004**, (June 14), 47–62.
4. Ghanem, A.; Ginatta, C.; Jiang, Z.; Schurig, V. Chirasil-β-Dex with new C11-spacer for enantioselective gas chromatography. Application to the kinetic resolution of secondary alcohols catalyzed by lipase. Chromatographia **2003**, *57*, S275–S282.
5. Ghanem, A.; Schurig, V. Lipase-catalyzed irreversible transterification of 1-(2-furyl) ethanol using isopropenyl acetate. Chirality **2001**, *13*, 118–123.
6. Schurig, V.; Nowotny, H.P. Gas chromatographic separation of enantiomers on cyclodextrin derivatives. Angew. Chem. Int. Ed. Engl. **1990**, *29*, 939–957.
7. Schurig, V. Separation of enantiomers by gas chromatography. J. Chromatogr. A **2001**, *906*, 275–299.
8. Dietrich, A.; Maas, B.; Messer, W.; Bruche, G.; Karl, V.; Kaunzinger, A.; Mosandl, A. Stereoisomeric flavor compounds, Part LVIII: The use of heptakis (2,3-di-*O*-methyl-6-*O*-tert-butyldimethylsilyl)-β-cyclodextrin as a chiral stationary phase in flavor analysis. J. High Resolut. Chromatogr **1992**, *15*, 590–593.
9. Koenig, W.A.; Lutz, S.; Mischnick-Luebbecke, P.; Brassat, B.; Wenz, G. Cyclodextrins as chiral stationary phase in capillary chromatography I. Pentylated α-cyclodextrin. J. Chromatogr. **1988**, *447*, 193–197.
10. Armstrong, D.W.; Li, W.Y.; Pirha, J. Reversing enantioselectivity in capillary gas chromatography with polar and nonpolar cyclodextrin derivative phases. Anal. Chem. **1990**, *62*, 214–217.
11. Aboul-Enein, H.Y.; Ali, I. *Chiral Separation by Liquid Chromatography and Related Technologies*; Marcel Dekker, Inc.: New York, 2003.
12. Wainer, I.W.; Stiffin, R.M.; Shibata, T. Resolution of enantiomeric aromatic alcohols on cellulose tribenzoate high performance liquid chromatography chiral stationary phase: A proposed chiral recognition mechanism. J. Chromatogr. **1987**, *411*, 139–151.
13. Wainer, I.W.; Alembik, M.C. Resolution of enantiomeric amides on cellulose-based chiral stationary phase: Steric and electronic effects. J. Chromatogr. **1986**, *358*, 85–93.
14. Okamoto, M.; Nakazawa, H. Reversal of elution order during direct enantiomeric separation of pyriproxyfen on a cellulose-based chiral stationary phase. J. Chromatogr. **1991**, *588*, 177–180.
15. Balmer, K.; Lagerstrom, P.O.; Persson, B.A.; Schill, G. Reversed retention order and other stereoselective effects in the separation of amino alcohols on Chiralcel OD. J. Chromatogr. **1992**, *592*, 331–337.

16. O'Brien, T.; Crocker, L.; Thompson, R.; Thompson, K.; Toma, P.H.; Conlon, D.A.; Feibush, B.; Moeder, C.; Bicker, G.; Grinberg, N. Mechanistic aspects of chiral discrimination on modified cellulose. Anal. Chem. **1997**, *69*, 1999–2007.
17. Krstulovic, A.M.; Fouchet, M.H.; Burke, J.T.; Gillet, G.; Durand, A. Direct enantiomeric separation of betaxolol with application to analysis of bulk drug and biological samples. J. Chromatogr. **1988**, *452*, 477–483.
18. McCarthy, J.P. Direct enantiomeric separation of four stereoisomers of nadolol using normal-phase and reversed-phase high performance liquid chromatography with Chiralpak AD. J. Chromatogr. A **1994**, *685*, 349–355.
19. Okamoto, Y.; Kawashima, M.; Aburatani, R.; Hatada, K.; Nishiyama, T.; Masuda, M. Chromatographic resolution. XII. Optical resolution of β-blockers by HPLC on cellulose triphenylcarbamate derivatives. Chem. Lett. **1986**, 1237–1241.
20. Okamoto, Y.; Aburatani, R.; Fukumoto, T.; Hatada, K. Chromatographic resolution. XVII. Useful chiral stationary phases for HPLC. Amylose tris-(3,5-dimethylphenylcarbamate) and tris-(3,5-dichlorophenylcarbamate) supported on silica gel. Chem. Lett. **1997**, 1857–1860.
21. Okamoto, Y.; Yashima, E. Polysaccharide derivatives for chromatographic separation of enantiomers. Angew. Chem., Int. Ed. Engl. **1988**, *37*, 1020–1043.
22. Yashima, E.; Fukaya, H.; Okamoto, Y. 3,5-Dimethylphenylcarbamates of cellulose and amylose regioselectively bonded to silica gel as chiral stationary phases for high performance liquid chromatography. J. Chromatogr. A **1994**, *677*, 11–19.
23. Method development with Chiralpak IA: The new Daicel column with broad solvent versatility. Chiral Technol. Europe **2004** (April).

Received June 1, 2005
Accepted June 29, 2005
Manuscript 6668