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Short communication

Direct chiral separation with Chiralpak AD converted to the reversed-phase mode

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Abstract

A Chiralpak AD column is converted from originally designed normal-phase to reversed-phase mode. Separations of pharmaceutical chiral intermediates in the reversed-phase are illustrated. Some characteristics of this new reversed-phase application are compared with those of the normal-phase mode. © 1998 Elsevier Science B.V.

Keywords: Enantiomer separation; Chiralpak AD; Chiral stationary phases, LC

1. Introduction

Chiralpak AD and Chiralcel OD columns from Chiral Technology Inc. have recently gained more recognition in the separation of enantiomers of pharmaceutically important compounds [1–9]. Both columns have the same dimethylphenyl carbamate functionality, but the AD is of amylose derivatives and OD is of cellulose. The Chiralcel OD column is marketed in both normal-phase mode (OD) and reversed-phase mode (OD-R) [10]. However, because the Chiralpak AD column is marketed as the normal-phase application by the manufacturer, there is very little literature on reversed-phase applications of the Chiralpak AD column. The pioneering work on the reversed-phase application by McCarthy showed that the Chiralpak AD column in the reversed-phase mode did not resolve all the stereoisomers of nadolol even at the inconveniently low column temperature of 0°C, thus the normal-phase application is still that author's choice [4]. The work presented here demonstrates some advantages of utilizing Chiralpak AD in reversed-phase mode compared to the normal-phase mode. Three examples of enantioseparations of pharmaceutical intermediates in the reversed-phase mode are given.

2. Experimental

HPLC	Waters 996 PDA-Millennium 2010 Waters 600E pump with column heater Waters 717plus autosampler
Column	Chiralpak AD 25 cm×4.6 mm Chiral Technology Inc., PO Box 564, Exton, PA 19341, USA
Reagents	Methanol (Fisher optima) Hexane (Fisher optima) Anhydrous ethanol (Pharmco 200 proof) Deionized water (MilliQ RO plus 10 and MilliQ UV plus)

To convert the Chiralpak AD from normal-phase

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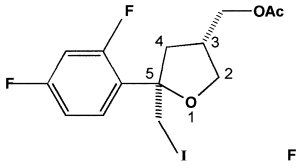
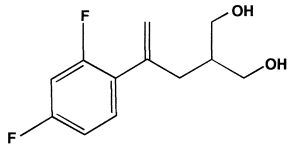
solvent to reversed-phase solvent, it is recommended to wash the column with anhydrous ethanol at 0.5 ml/min overnight. Make sure that the column pressure does not exceed the maximum limit. Then equilibrate the column with the reversed mobile phase.

3. Results and discussion

The molecular structures of the first reversed-phase mode separation of pharmaceutical intermediates are as follows.



compound 1
Acetate of 5-(2,4-difluorophenyl) tetrahydro-5-(S)-(iodomethyl)-3-(R)-furanmethanol



compound 2
4-(2,4-difluorophenyl)-2-(hyroxymethyl)-4-penten-1-ol

The compound **1** has two chiral centers, and therefore two pair of enantiomers, i.e. *cis* (*SR*) and (*RS*), *trans* (*SS*) and (*RR*), the desired enantiomer has the *cis* (*SR*) configuration shown at above left. Because of the asymmetric synthesis chemistry utilized, *trans RR* is practically nonexistent. The compound **2** is a potential achiral impurity. Thus a method which separates *cis* (*SR*), (*RS*) and *trans* (*SS*) as well as compound **2** is adequate. A solution of the mixture of compound **1** and **2** at a concentration of about 0.5 mg/ml was prepared and chromatographed first using normal-phase mode, then reversed-phase mode. The chromatograms and HPLC parameters are shown below and in Fig. 1.

HPLC parameters:

Column	Chiralpak AD 25 cm×0.46 cm at 30°C
Mobile phase	Hexane–anhydrous ethanol (200 proof), (99:1, v/v)
Flow-rate	1 ml/min
Wavelength	215 nm

Note that the elution order of four compounds are compound **1** *trans* (*SS*), cis (*SR*) coeluting with compound **2**, and compound **1** *cis* (*RS*). The desired enantiomer compound **1** *cis* (*SR*) was not separated from compound **2** in normal-phase mode.

After a careful conversion from normal-phase the reversed-phase method was developed. The chromatogram of the mixture is shown in Fig. 2.

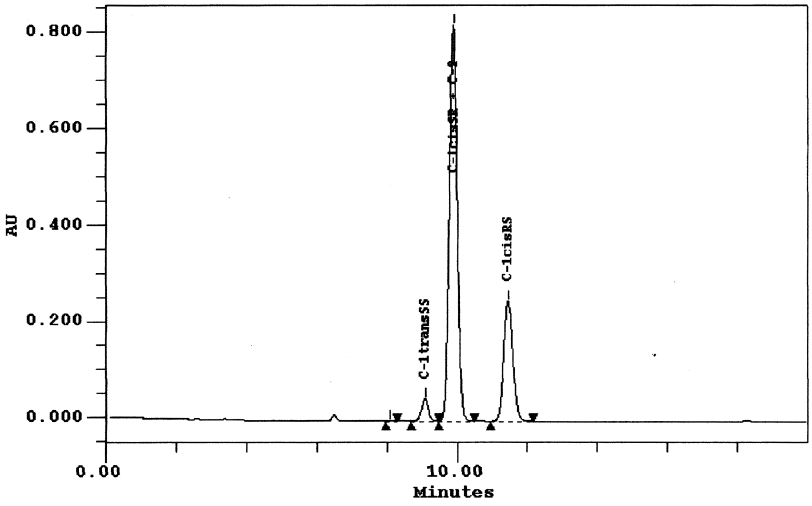


Fig. 1. Compound **1** stereoisomers and compound **2** in normal-phase Chiralpak AD.

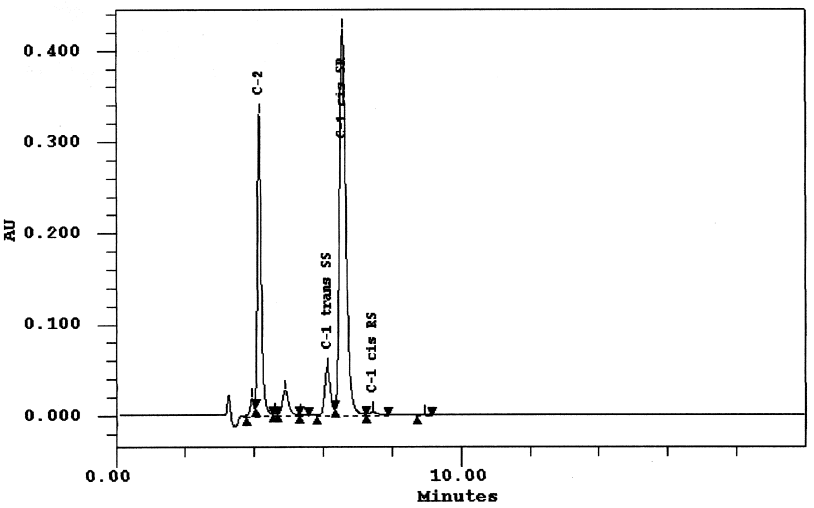


Fig. 2. Compound **1** stereoisomers and compound **2** in reversed-phase Chiralpak AD.

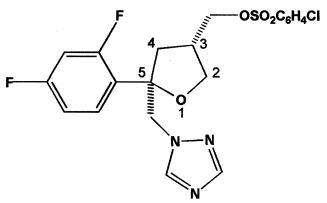
HPLC parameters:

Column	Chiralpak AD 25 cm×0.46 cm at 40°C
Mobile phase	Methanol–H_2O, (80:20, v/v)
Flow-rate	1 ml/min
Wavelength	215 nm

Although switching from normal-phase to reversed-phase did not change the elution order of the compound **1** stereoisomers, the compound **2** was completely separated from the desired *cis* (*SR*) enantiomer. And the reversed-phase method is sensitive enough to calculate the enantiomeric purity of the compound **1** *cis SR* enantiomer.

As indicated in the above example, the switching of the mobile phase mode may not necessarily change the elution order of the stereoisomers. But it could however have profound effects on achiral impurities which coelute with chiral isomers because the polarity of the mobile phase is changed from one extreme to the other. The polar reversed mobile phase is a much stronger eluent than the normal mobile phase to the polar compound **2**. This results in a much earlier elution of the achiral impurity, therefore completely resolving from the compound **1** enantiomers.

Switching mobile phase mode can sometimes even change the elution order of stereoisomers. The molecular structure of the compound is illustrated below.



Compound 3
[5-(2,4-difluorophenyl)tetrahydro-5-(1H-1,2,4-triazol-1-ylmethyl)furan-3-ylmethyl],4-chlorobenzenesulphonate

Compound **3** has two chiral centers, and the *cis* (3*S*5*R*) is the desired stereoisomer. The chromatogram of the compound **3** in normal-phase mode is shown in Fig. 3.

HPLC parameters:

Column	Chiralpak AD 25 cm×0.46 cm at 30°C
Mobile phase	Anhydrous ethanol (200 proof)
Flow-rate	1 ml/min
Wavelength	233 nm

The chromatogram on the top of the overlay is of the racemate, and the one at the bottom is of a typical sample.

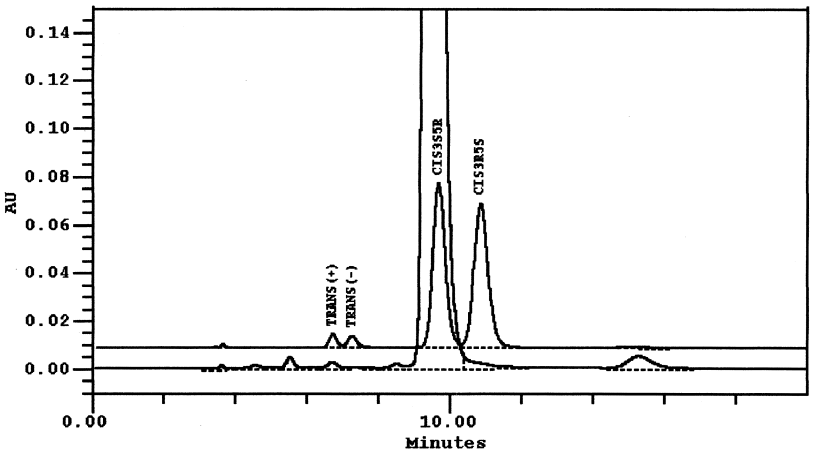


Fig. 3. The overlay plot of compound **3** racemate standard and compound **3** *cis SR* sample in the normal-phase mode.

Notice that the minor impurity *cis* (3*R*5*S*) rides on the tail of the major peak *cis* (3*S*5*R*). This peak is hardly resolved and would be very difficult to quantitate even though the resolution between two *cis* enantiomers in the racemate standard is fairly good (1.66).

In the reversed mobile phase mode not only the resolution but also the elution order of the stereoisomers has changed favorably, as shown below and in Fig. 4.

HPLC parameters:

Column	Chiralpak AD 25 cm×0.46 cm at 40°C
Mobile phase	Methanol–H_2O (90:10, v/v)
Flow-rate	1 ml/min
Wavelength	233 nm

As indicated from the above results, the reversed-phase mode not only has better resolution for each of the four isomers, but also elutes the impurity *cis* 3*R*5*S* ahead of the peak of desired enantiomer *cis* 3*S*5*R*. However, the elution order for the *trans* enantiomers remains unchanged. The reversed-phase

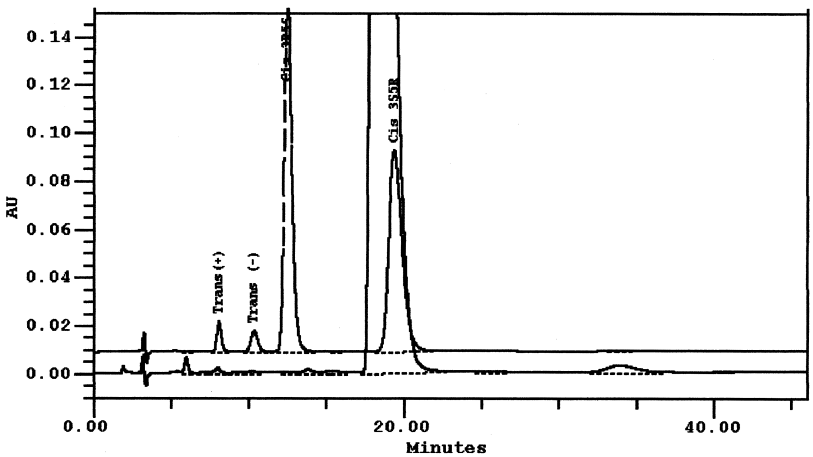
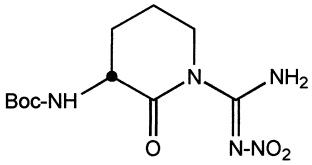


Fig. 4. The overlay plot of compound **3** racemate standard and compound **3** *cis SR* sample in the reversed-phase mode.

mode enables much more reliable quantitation of a trace amount of chiral impurity. The sensitivity of the detection is also improved considerably. The limit of detection of *cis* (3*R*5*S*) impurity in the system is obtainable to a level of 0.1% area.

Switching from normal-phase to the reversed-phase expands the scope of chiral chromatography of the Chiralpak AD column. Some chiral compounds that could not be separated in the normal-phase can now be resolved in the reversed-phase mode adequately. The D,L-Boc-*N*-Arg(NO_2)-lactams are the compounds that previously failed to resolve in the normal-phase mode but separate well in the reversed-phase mode. The molecular structure of the Boc-*N*-Arg(NO_2)-lactam is illustrated as follows.



Boc-N-Arg(NO_2)-Lactam

After many attempts failed to separate Boc-*N*-Arg(NO_2)-lactam in the normal-phase mode, the reversed-phase mode was finally tried. With a little development effort the compounds were separated with ample resolution. This is shown in Fig. 5.

HPLC parameters:

Column	Chiralpak AD 25 cm×0.46 cm at 40°C
Mobile phase	Methanol–H_2O (80:20, v/v)
Flow-rate	1 ml/min
Wavelength	275 nm

Note that the impurity D-enantiomer elutes before the desired peak of the L-enantiomer. This elution order is favored for quantitation as there is no interference from the tail of the major peak.

After three years operation in reversed-phase, there has been no evidence of deterioration of the column efficiency. Although the peak shape in the reversed-phase tends to be a little broader than that in the normal-phase, this is characteristic from the beginning for many compounds. The conversion does not impair the integrity of the column.

4. Conclusions

The application of Chiralpak AD in the reversed-phase mode gives more freedom to design new separation patterns for those chiral compounds difficult to resolve. As indicated above, the reversed mobile phases employed here are very simple, efficient and robust. The reversed-phase mode also breaks the inherent limitations of normal-phase chiral HPLC, thus the possibility of employing pH, buffer and other additives can be studied if necessary.

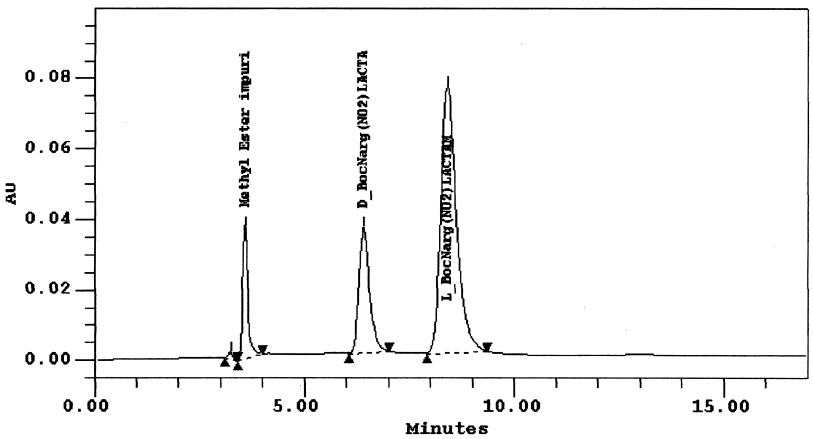


Fig. 5. The D,L-Boc-*N*-Arg(NO_2)-lactams in the reversed-phase mode.

There are many other chiral columns in the market originally intended to be used in the normal-phase, this work has offered chromatographers another dimension to tackle the onerous tasks of trial and error. *One word of caution, before converting your expensive chiral column to reversed-phase, study the column chemistry, contact the manufacturer and make sure the column will not collapse in the reversed-phase mode.*

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