

Overview

A series of XBL compounds with two chiral centers have been systematically studied with three types of chiral columns.

In order to achieve chiral separation of XBL100705 in a LC-MS quantitative method, non-volatile buffer or non-polar solvent (such as hexane) was eliminated from the HPLC mobile phase used.

All four stereoisomers could be separated with a chiral AGP column, while C2-XBL100705 and C3-XBL100705 were separated with a Chirobiotic T2 column. No separation was observed with a ChiralPak AD-RH column. With Chirobiotic T2, a lower limit of quantitation (LLOQ) of 0.5 pg/mL in plasma was achieved. Diastereomer pairs were separated with a regular C18 column.

Introduction

Racemic drugs pose unique challenges not only in their biological effects but in the pharmacokinetics of the stereoisomers/enantiomers. Since 1990, the proportion of single-enantiomer drugs among approved new chemical entities worldwide has been increasing. To support clinical trials of single-enantiomer drugs in human plasma and urine, sensitive chiral separation LC/MS methods with short run times are needed. Many chiral separation HPLC methods published so far were coupled with conventional detectors (UV, fluorescence or electrochemical detection). In these methods, strong non-volatile buffer or special organic solvents (such as Hexane) were used as the mobile phases. These LC conditions are not suitable to couple the HPLC with mass spectrometers. In addition, the chiral separation pattern depends on the compound structure, and on the type and manufacturer of the chiral column.

In this work we systematically studied the function of different types of chiral columns, the specificity of these columns with different manufacturers, and their application in chiral HPLC/MS methods.

Experimental

Sample Preparation

The analytes and internal standards were extracted from human plasma and urine using solid phase extraction. The organic solvent extract was evaporated to dryness under a nitrogen stream and the residue was reconstituted with reconstitute solution.

Liquid chromatography:

LC System:	Pump Autosampler System Controller	Shimadzu LC-10AD Shimadzu SIL-HT Shimadzu SCL-10A
Analytical Column:		
Types	Size	Manufacturer
Chirobiotic T2	2.0 x 250 mm	Advanced Separation Technologies Inc.
Chiral AGP	4.0 x 150 mm	ChiroM Tech, Inc.
ChiralPak AD-RH	2.1 x 150 mm	Chiral Technologies Inc.
Zorbax Extend-C18	2.1 x 50 mm	Agilent Technologies
Gradient		
Flow rate:	0.2-0.7 mL/min	
Injection Volume:	10-30 μ L	

Mass Spectrometry

MS System:	AB Sciex API-4000
Condition:	LC/(+)-ESI-MS/MS (MRM)

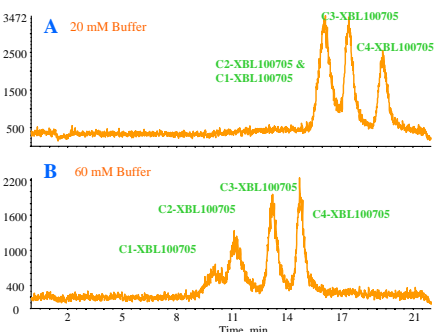


Figure 1. The Ion Chromatograms of XBL100705 with AGP Chiral column. (A) 20 mM buffer concentration in HPLC mobile phase, (B) 60 mM buffer concentration.

Results and Discussion

The composition of the mobile phase is critical for the chiral separation:

Chirobiotic T2 column: 80 or higher percent methanol in the mobile phase gave a short retention time (R_t) and reasonable separation. The addition of 10 to 20% acetonitrile increased the separation but also increased R_t . Less than 2% water narrowed the peak width but sacrificed a little separation. Acid would shorten R_t , while basic would increase R_t .

AGP column: behavior similar to a reverse phase column. A higher buffer concentration increased the chiral separation and the MS detection limit as shown in Figure 1. Chiral separation is very sensitive to mobile phase pH (Figure 2). At pH 7.4 or higher, the separation of C1- and C2-XBL100705 decreased, while at pH equal to or less than 6.5, the separation of C3- and C4-XBL100705 decreased. pH 6.9 gave all four stereoisomers good separation. The optimal pH value will vary from column to column.

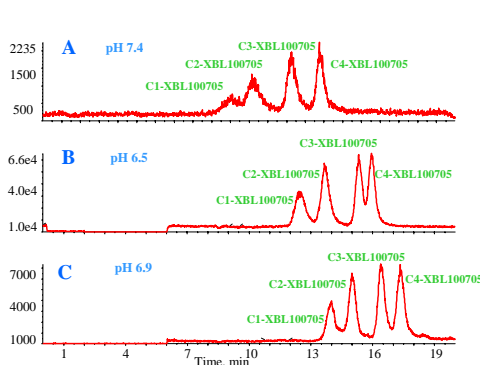


Figure 2. The Ion Chromatograms of XBL100705 with AGP Chiral column with different pH. (A) pH 7.4, (B) pH 6.5, (C) pH 6.9.

Columns from different manufacturers could give different separation patterns:

Chirobiotic T2 is antibiotic base column and AGP is protein base column. The mobile phase used for the AGP column is similar as that for reverse phase column, while the mobile phase for Chirobiotic T2 likely contains more than 95% organic solvent.

All four stereoisomers could be separated with AGP (Figs 1 & 2), but only C2- and C3-XBL100705 (enantiomers) could be separated with Chirobiotic T2 (Fig 3).

The HPLC flow rate affects the chiral separation:

For Chirobiotic T2, the separation efficiency varies with the HPLC flow rate. The lower the flow rate, the better the separation efficiency. As shown in Figure 3C, the best peak separation was obtained with 0.2 mL/min flow rate, but the peak width was broad and R_t very long. Increasing the flow rate to 0.45 mL/min reduced R_t and sharpened the peak, but separation decreased (Fig 3A). In this study, we changed the flow rate from 0.2 to 0.45 mL/min at about 13 min in the LC run, which improved the peak width and kept the better peak separation as shown in Figure 3B.

Chiral separation can sometimes be done with a non-chiral column:

C2-/C3-XBL100705 were separated from C1-/C4-XBL100705 with a general reverse phase C18 column since they are diastereomer pairs. If acetonitrile alone was used as HPLC mobile phase B, the chiral separation worsened after ten sample injections (Figure 4A). A special solvent was developed to replace acetonitrile, and the peak separation was almost constant after a couple hundred injections as shown in Figure 4B.

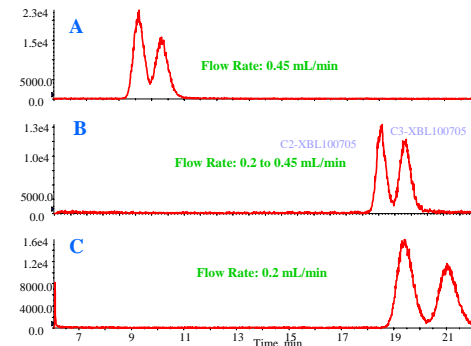


Figure 3. The Ion Chromatograms of XBL100705 with Chirobiotic T2 Column at different LC flow rate. (A) 0.45 mL/min, (B) 0.2 mL/min changed to 0.45 mL/min at 13 minutes, (C) 0.2 mL/min.

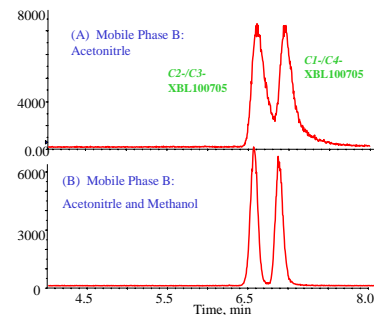


Figure 4. The Ion Chromatograms of XBL100705 with Reverse phase C18 column. (A) Mobile phase B is acetonitrile, (B) Mobile phase B is mixture of acetonitrile and methanol.

Conclusion

It was found that all four stereoisomers could be separated with Chiral AGP with 60 mM NH_4OOC buffer, while C2- and C3-XBL100705 could be separated with Chirobiotic T2 (enantiomers). No separation was observed with ChiralPak AD-RH. It was also found that C2- and C3-XBL100705 could be well separated from C1- and C4-XBL100705 with a C18 column (diastereomer pairs). In the method using the Chirobiotic T2 column, a lower limit of quantitation (LLOQ) of 0.5 pg/mL was achieved, while with the Chiral AGP, the LLOQ was 10 pg/mL.

Research in the field of chiral LC/MS continues at XenoBiotic Laboratories.