APPLICATION NOTE

The Chiral Resolution of Fluoxetine

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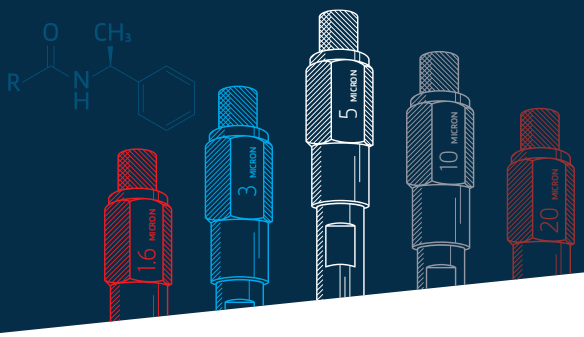

DAICEL Chiral Technologies

INTRODUCTION

Fluoxetine, known commercially as Prozac, is a selective serotonin reuptake inhibitor (SSRI) antidepressant. It is on the World Health Organization's List of Essential Medicines, and is currently in the top 20 of most prescribed medications in the United States. Several studies have been performed showing the enantiomers have similar anti-depressive effects however, they are metabolized by the body at different rates. Furthermore, of the two enantiomers of the main metabolite, norfluoextine, only the S enantiomer shows anti-depressive activity. It has also been shown that the R enantiomer is less likely to have a negative effect on cardiac function.

At present, there are no reported separations in the literature for the separation of this enantiomeric pair on polysaccharide-based chiral columns. Because of the differences in enantiomer and enantiomer metabolite activity, and the potential effects on cardiac function, a robust chiral separation would be of great benefit.

Presented in this note is a normal phase separation of Fluoxetine on Daicel's CHIRALPAK® IK (5 µm), which is an immobilized cellulosic-based polysaccharide chiral stationary phase functionalized with tris(3-chloro,5-methylphenylcarbamate) moieties. With its unique separation performance, IK yields a better-than-baseline separation not previously reported in the literature.

EXPERIMENTAL

Chromatographic Conditions for the Separation of Fluoxetine

Column	CHIRALPAK® IK (250 mm x 4.6 mm i.d., 5 µm) Part #: 91325	
Mobile Phase	95-5-0.1 = Hex-EtOH-DEA	90-10-0.1 = Hex-IPA-DEA
Flow Rate	1.0 ml/min	
Detection	UV 270 nm ref. 450 nm	
Temperature	25°C	
Sample	1.0 mg/ml in EtOH	
Injection Volume	5.0 µl	

Fluoxetine and Diethylamine (DEA) were purchased from Sigma Aldrich and used as-is. The solvents used were all purchased from Pharmco, were HPLC-grade or higher, and were used as-is. Specifically the hexanes (Hex) contained 95% n-hexane. The ethanol (EtOH) was reagent alcohol, which contains 90% EtOH, 5% methanol, and 5% isopropanol (v/v/v). Initial screening and optimization were performed on an Agilent 1200 equipped with a quaternary mixing pump and utilized a DAD.

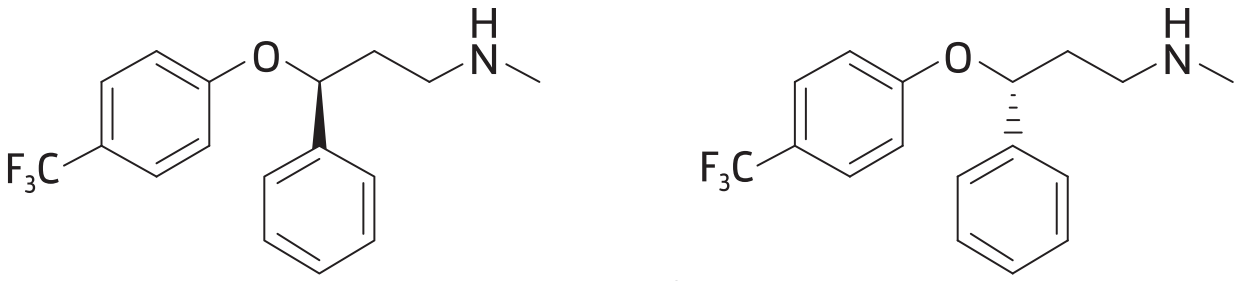

Figure 1: Enantiomers of Fluoxetine

RESULTS AND CONCLUSIONS

Fluoxetine was screened on Daicel's immobilized library of 3 µm polysaccharide-based chiral stationary phases (CSPs) plus CHIRALPAK® IK (5 µm) with starting conditions of 90-10-0.1 = Hex-EtOH-DEA and 80-20-0.1 = Hex-IPA-DEA. From this, partial separations were observed on CHIRALPAK® IG-3 with EtOH, and CHIRALPAK® IC-3 and ID-3 with IPA (Figure 2). However with both alcohols, baseline or near-baseline resolution was observed on CHIRALPAK® IK (Figure 3).

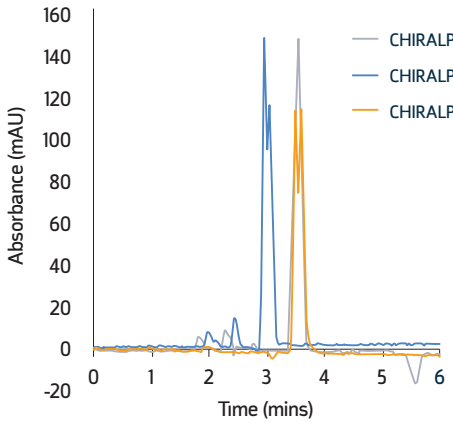

Figure 2: Partial Separations of Fluoxetine on CHIRALPAK® IC-3, ID-3, and IG-3

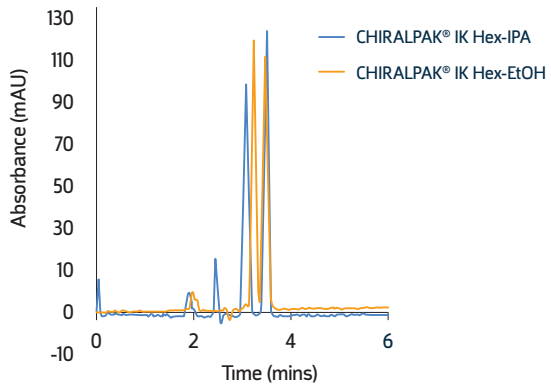

Figure 3: Screening Separations of Fluoxetine on CHIRALPAK® IK

To optimize the separation using EtOH, the mobile phase strength was reduced to 95-5-0.1 = Hex-EtOH-DEA to improve the compound's retention. A 250 mm length column was also used to improve the resolution. This was sufficient to produce a baseline resolution of the two enantiomers using EtOH (Figure 4).

Similar to the optimization using EtOH, to optimize the separation with IPA, the mobile phase strength was reduced to 90-10-0.1 = Hex-IPA-DEA to improve the retention. A 250 mm length column was used as well. This was sufficient to produce a better-than-baseline resolution of the two enantiomers using IPA (Figure 5).

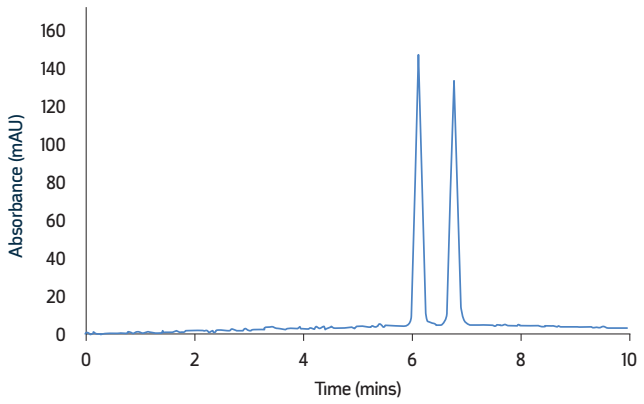

Figure 4: Optimized Separation of Fluoxetine on CHIRALPAK® IK with EtOH

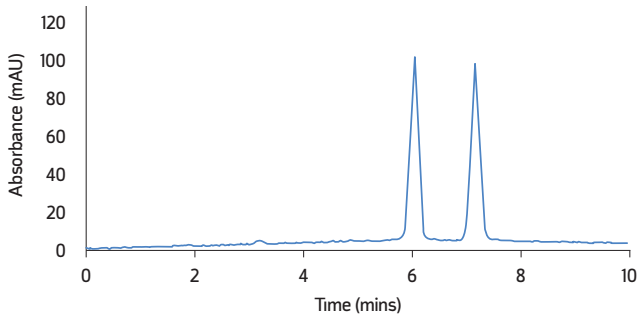

Figure 5: Optimized Separation of Fluoxetine on CHIRALPAK® IK with IPA

This new chiral separation emphasizes the importance of new chiral stationary phase development. The best way to affect selectivity and improve upon a chiral separation is to make changes to the phase system, i.e. the mobile phase or chiral stationary phase. Mobile phase combinations are well established and restricted to some degree (must be compatible with an HPLC, must be miscible with other solvents, column limitations, etc.), so the greatest expansion or potential to find a new separation comes with a new chiral stationary phase. In this case, a new chiral separation for a WHO Essential Medicine, allowing for repeated and robust analysis.