Improved Chiral Separation of Mephenesin on CHIRALPAK® IM

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INTRODUCTION

Mephenesin (Figure 1) is a cresol glyceryl ether that has been used for several indications including as a muscle relaxant, an antidote for strychnine poisoning, and as a treatment for Parkinson's disease and Multiple Sclerosis.

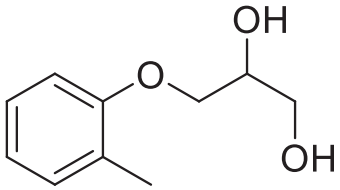

Figure 1: Mephenesin

The chiral separation of Mephenesin was previously reported on CHIRALCEL® OZ-H, which is a coated tris (3-chloro-4-methylphenylcarbamate) cellulose-based chiral stationary phase (CSP). The separation with a normal phase alkane/alcohol mobile phase afforded just baseline resolution of the two enantiomers.

Daicel's newest immobilized CSP, CHIRALPAK® IM, is the immobilized equivalent of CHIRALCEL® OZ-H. The added robustness coming from the immobilization process increases this selector's utility because of expanded solvent compatibility. This resulted in a significant improvement of the enantiomeric separation with the additional of ethyl acetate to the mobile phase.

Chromatographic Conditions for the Separation of Mephenesin

Column	CHIRALCEL® OZ-H (250 mm x 4.6 mm i.d.) Part #: 42325	CHIRALPAK® IM (250 mm x 4.6 mm i.d.) Part #: 92325	
Mobile Phase	Hex-EtOH = 80-20 (v/v)	Hex-EtOH = 80-20 (v/v)	Hex-EtOAc-EtOH = 70-30-0.1 (v/v/v)
Flow Rate	1.0 ml/min		
Detection	UV 280 nm ref. 450 nm		
Temperature	25°C		
Sample	1.9 mg/ml in EtOH		
Injection Volume	5 µl		

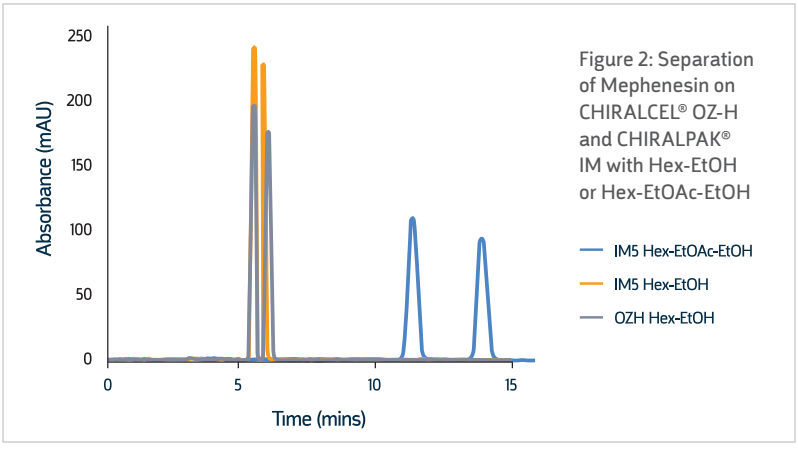

Figure 2: Separation of Mephenesin on CHIRALCEL® OZ-H and CHIRALPAK® IM with Hex-EtOH or Hex-EtOAc-EtOH

EXPERIMENTAL

Mephenesin was 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 separation check and optimization were performed on an Agilent 1200 equipped with a quaternary mixing pump and utilized a DAD.

DISCUSSION

Mephenesin was prepared as a 1.9 mg/ml solution in EtOH, and the separation from Daicel's application database (Hex-EtOH = 80-20 (v/v)) checked on both CHIRALCEL® OZ-H and CHIRALPAK® IM (Figure 2). This demonstrated almost baseline resolution on both columns. The separation on IM was optimized by the addition of EtOAc and removal of most EtOH (as indicated in the table above). This yielded a significant improvement in the chiral resolution, producing a greater-than-baseline resolution (Figure 2).

CONCLUSIONS

The increased robustness of immobilized chiral selector technology is critical for finding new chiral separations, and improving existing separations as demonstrated with the separation of Mephenesin. Under traditional normal phase conditions, the resolution was just baseline, and provided for minimal room for further optimization. The addition of EtOAc greatly influenced the analyte interactions with the CSP, and produced a greatly improved resolution on CHIRALPAK® IM.