APPLICATION NOTE

The Chiral Analytical and Preparative Resolution of Sotorasib Atropisomers

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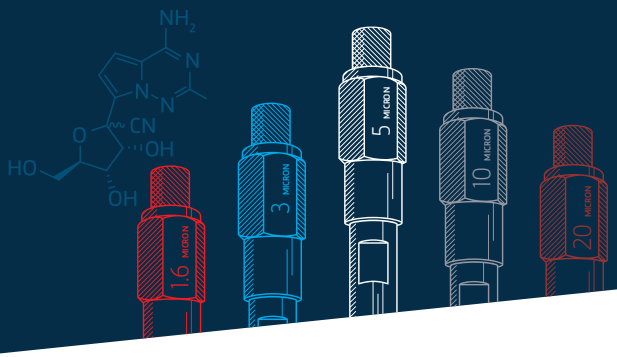

DAICEL Chiral Technologies

INTRODUCTION

In 2021, Sotorasib became the first FDA-approved KRAS inhibitor, marketed under the name Lumakras® in the United States. Sotorasib targets the protein K-Ras, which is encoded by the KRAS gene. A KRAS gene mutation is linked to several different forms of cancer, including non-small cell lung cancer, where the KRAS mutation is found in roughly 25% of cases.

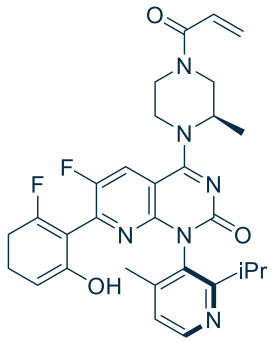

Figure 1: Sotorasib

Structurally Sotorasib contains a chiral center located alpha to the six-membered dinitrogen ring (Figure 1). While this chiral center is generally resolved via classical resolution, a specific feature of this compound creates a challenge – separating the atropisomers arising from the rotational restriction of the C-N single bond connecting the isopropyl-pyridine ring to the molecular core.

It is difficult-to-impossible to synthesize one of these configurations cleanly, but chiral chromatography can easily resolve the pair. With numerous other KRAS inhibitors currently under evaluation with similar elements of chirality, chromatography is likely to play an important role moving forward in the production of such compounds.

As the global leader in polysaccharide-based chiral stationary phases (CSPs), Daicel Chiral Technologies offers unique solutions to challenging problems like the separation of Sotorasib atropisomers. Several separations were found and optimized for both analytical and preparative method development after a thorough screening on Daicel's entire immobilized CSP library. Shared herein are those results and representative chromatograms to demonstrate said separations.

EXPERIMENTAL

Sotorasib was purchased from MedChemExpress (Cat. No. HY-114277) and used as-is. The solvents used were all purchased from Scientific Equipment Company (SECO), were HPLC-grade, and were used as-is. Screening and optimization were performed on an Agilent 1200 configured with high-pressure mixing quaternary mobile phase delivery system, vacuum degasser, autosampler, temperature controlled column compartment, and photodiode array UV detector. The instrument was controlled by an Agilent ChemStation Version RevB.04.03.

Chromatographic Conditions for the Separation of Sotorasib	
Column	CHIRALPAK® IK (250 mm x 4.6 mm i.d.) Part # 91325
Mobile Phase	Acetonitrile
Flow Rate	1.0 ml/min
Detection	305 nm
Temperature	25°C
Sample	1.5 mg/ml in Acetonitrile
Injection Volume	5 µl

DISCUSSION

Sotorasib was prepared as a 1.5 mg/ml solution in a 2:1 mixture of acetonitrile (ACN) and methanol (MeOH), and screened on Daicel's immobilized CSP library, which included CHIRALPAK® IA, IB N-5, IC, ID, IE, IF, IG, IH, IJ, IK, and IM (5 µm, 4.6 mm i.d. x 250 mm length). Because of the compound's poor solubility in traditional normal phase mixtures (hexane with alcohols), screening was performed using polar organic mode solvents only, which included ACN, MeOH, and ethanol (EtOH). Two separations of note were found with ACN, one on CHIRALPAK® IK and the other on CHIRALPAK® IE (Figure 2, A and B respectively). These two were noted specifically as they

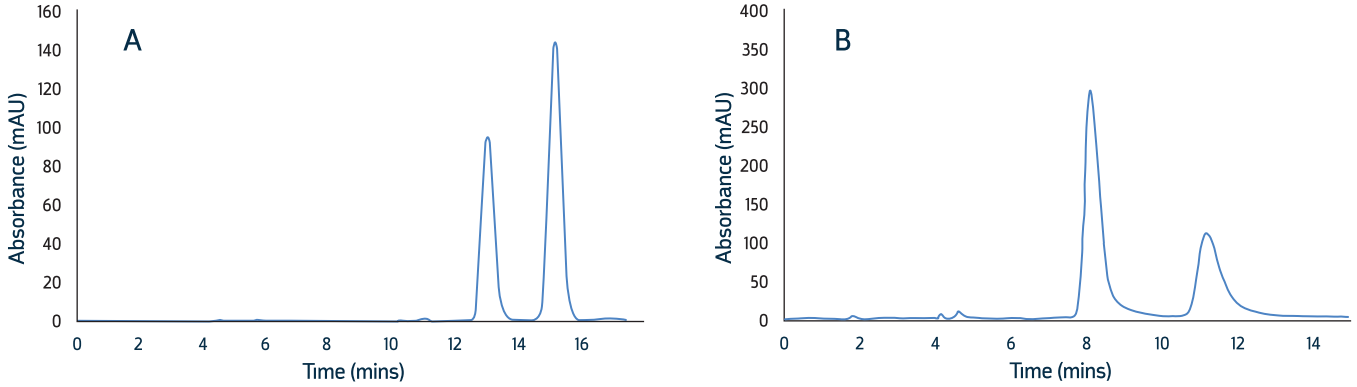

Figure 2: Separation of Sotorasib on CHIRALPAK® IK (A) and CHIRALPAK® IE (B)

appeared to result in an elution order reversal, as indicated by the minor enantiomer peak eluting second on IE and first on IK.

The separation on IE was optimized by adding 5% by volume of MeOH, and adding 0.1% by volume of diethylamine (DEA). This helped to both sharpen the peaks and decrease the overall elution time. Interestingly the separation on IK required no further optimization, including the addition of DEA. Because Sotorasib is a basic molecule, a basic additive like DEA would generally be required. A standard of the final Sotorasib API was then injected with both optimized methods. It was found that the desired API eluted first on IE method, and second on the IK method, indicating indeed the suspected elution order reversal was occurring.

The elution order of the IK method makes it preferable as an analysis method – the undesired impurity in the front can be more easily quantified improving the overall limit-of-detection (LOD). While the peak shape of the separation on IK is more preferable, the IE method elutes the desired API first, meaning this method can produce higher purity material for a preparative application.

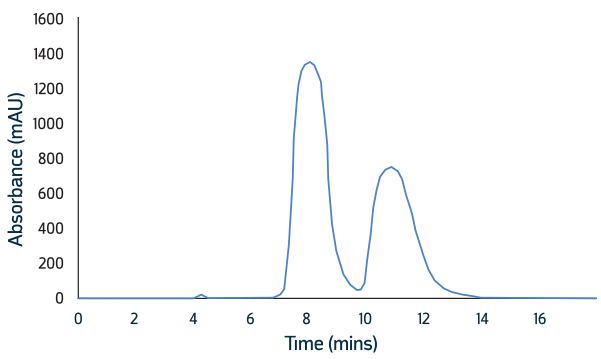

Figure 3: Preparative Separation of Sotorasib on CHIRALPAK® IE (200 µl loading)

Using the same solution for screening, the IE method was overloaded until the back of peak 1 touched the front of peak 2 (Figure 3). This was found to be around 200 µl, and could potentially be even higher depending on where fraction collection is set, and how much yield loss for the desired API is acceptable in a single pass. Because atropisomers can often be interconverted with the application of heat, this yield loss could be diminished by racemizing the recovered peak 2 fractions and reprocessing to isolate more desired peak 1, assuming this thermal treatment does not cause degradation. Ultimately, the conditions shared here yielded an estimated productivity of 30 grams of racemate processed per day on an 11 cm preparative column.

CONCLUSIONS

Sotorasib was the first FDA-approved KRAS inhibitor, which has brought about an intense focus on this class of compounds. Like Sotorasib, many of these compounds contain a unique form of chirality called atropisomerism. The desired configuration of these molecules often cannot be synthesized cleanly, so chiral chromatography is set up to play a major role in bringing these compounds to the market. As demonstrated in this note, the diversity of Daicel's chiral columns are uniquely positioned to help solve both the analytical and preparative challenges associated with atropisomers like Sotorasib.