ULTRA-FAST SFC SEPARATIONS WITH **DAICEL SUB-2 µm CHIRAL COLUMNS**

APPLICATION NOTE

INTRODUCTION

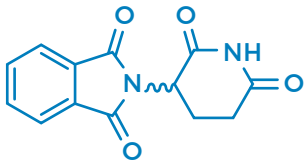
As the pressure to find new treatments for emerging diseases continues to increase, the need to perform fast, reliable analytical analyses has also increased. Significant innovation has taken place to allow chemists to synthesize large libraries of compounds in micro-well plates, which often leads to a bottleneck forming in being able to assess critical reaction parameters such as chemical and enantiomeric purity, before proceeding to the next steps of development.

First introduced in 2016, Daicel Corporation has launched a line of (6) sub-2 µm immobilized chiral stationary phases (CSPs) to address these needs. Although originally designed for use in Ultra-High Performance Liquid Chromatography (UHPLC), these sub-2 µm columns have more recently found new applications in Supercritical Fluid Chromatography (SFC). The improvements in resolution that can be achieved by utilizing smaller particles, coupled with the low viscosity mobile phases employed in SFC, allow for ultra-fast method development screening, further maximizing the power of these columns.

EXPERIMENTAL

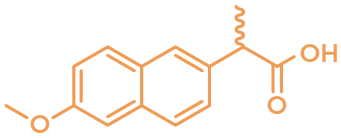
Two test compounds, Thalidomide and Naproxen, were chosen to assess their separation under SFC conditions (HPLC separations have been previously reported). Initial screening was performed on 150 mm x 4.6 mm i.d. analytical columns, with methanol, ethanol, isopropanol, and acetonitrile as modifiers, utilizing a Waters ACQUITY UPC^2 SFC. The sub-2 µm columns are only available in 3.0 mm i.d., so 50 mm x 3.0 mm i.d. columns were utilized for optimization, which was performed on an Agilent Aurora Fusion A5 SFC.

FIGURE 1: THALIDOMIDE



CHROMATOGRAPHIC CONDITIONS FOR THE SEPARATION OF THALIDOMIDE	
COLUMN	CHIRALPAK® IC-U and IC-3 (50 mm x 3.0 mm i.d.)
MOBILE PHASE	MeOH/CO_2 = 40/60
FLOW RATE	3.0 ml/min
DETECTION	UV, 210 nm, ref 450 nm
TEMPERATURE	25°C
SAMPLE	1.0 mg/ml MeOH
INJECT. VOL.	5.0 µl

FIGURE 2: NAPROXEN



CHROMATOGRAPHIC CONDITIONS FOR THE SEPARATION OF NAPROXEN	
COLUMN	CHIRALPAK® IG-U and IG-3 (50 mm x 3.0 mm i.d.)
MOBILE PHASE	MeOH/CO_2 = 10/90
FLOW RATE	5.0 ml/min
DETECTION	UV, 210 nm, ref 450 nm
TEMPERATURE	25°C
SAMPLE	2.0 mg/ml MeOH
INJECT. VOL.	5.0 µl

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DISCUSSION

After initial screening, several partial separations for both compounds were observed. Because of the simplicity and versatility of methanol as a modifier, these separations were chosen for optimization. For Thalidomide, CHIRALPAK® IC yielded the best separation (largest alpha with the shortest retention); CHIRALPAK® IG yielded the best separation for Naproxen.

Based on the results of the screening, the methods were repeated on the smaller 50 mm x 3.0 mm i.d. columns, and the flow rate of each method gradually increased (from 1.0 ml/min) until the two enantiomer peaks touched.

In the case of Thalidomide, the 3 µm separation began to collapse around 40 sec. (flow rate approximately 2 ml/min; chromatogram not shown), whereas the sub-2 µm separation allowed for an additional flow rate increase to 3 ml/min, cutting the analysis time in half (peak 1 eluted around 21 sec.; chromatogram shown in Figure 3).

Comparatively, the separation of Naproxen on 3 µm began to collapse around 90 sec. (flow rate approximately 2 ml/min; chromatogram not shown), whereas the sub-2 µm separation could be pushed to 5 ml/min (peak 1 eluted around 17 sec.; chromatogram shown in Figure 4). In both scenarios, some resolution was still observed on the larger 3 µm columns at the increased flow rate, however the baseline separation achieved on the sub-2 µm methods allowed for a more accurate quantification, in a short time.

FIGURE 3:

SEPARATION OF THALIDOMIDE
ON CHIRALPAK® IC-3 VS. CHIRALPAK® IC-U

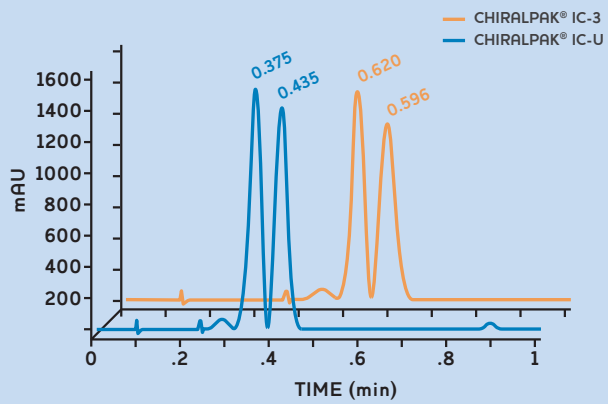
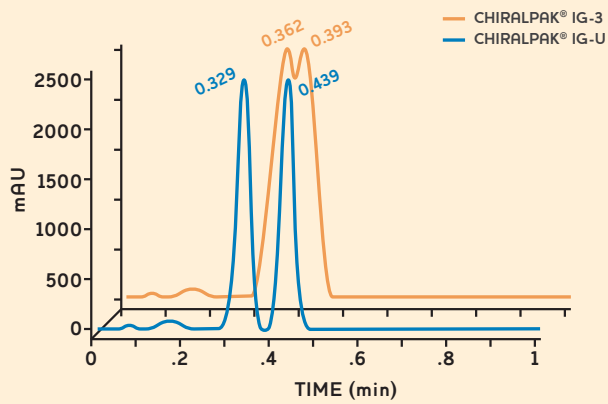


FIGURE 4:

SEPARATION OF NAPROXEN
ON CHIRALPAK® IG-3 VS. CHIRALPAK® IG-U



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