APPLICATION NOTE

The Chiral Resolution of (±)-Catechin on Several Daicel Immobilized Chiral Columns

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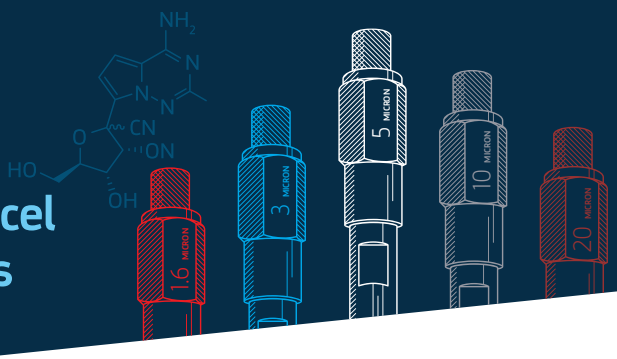

DAICEL Chiral Technologies

INTRODUCTION

Flavonoid is the name given to a class of naturally occurring chemical compounds often found in plants. They contain a characteristic 15-carbon skeleton, which consists of two phenyl rings and a heterocycle, and can be further functionalized with other chemical groups. In the instance when the functionalization are hydroxyl groups, this gives rise to a family of phenols and polyphenols known as flavanol.

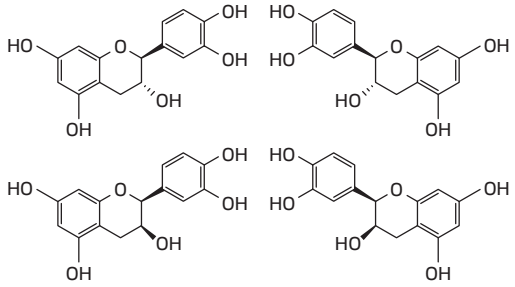

Figure 1: (-)-catechin (top left), (+)-catechin (top right), (+)-epicatechin (bottom left), (-)-epicatechin (bottom right)

Flavonoids and flavanols are known to have powerful antioxidant properties, and have been shown to exhibit other beneficial health indications. One specific example is catechin. Containing two chiral centers, catechin has four possible isomeric configurations, (+)-catechin, (-)-catechin, (+)-epicatechin, and (-)-epicatechin. This application note is focused specifically on the chiral separation of (+)-catechin and (-)-catechin.

Several key intermolecular interactions can take place on a chiral column during a chiral separation, the most influential being π-π stacking, hydrogen bonding, and dipole-dipole forces. These interactions are well known, but not easy to model and predict how they might influence a particular chiral separation. They could lead to no separation, a separation, or when comparing two chiral columns, a reversal of elution order. For the separation of (±)-catechin, the latter is true for several Daicel columns.

To date, no chiral separations have been reported on Daicel's polysaccharide-based chiral stationary phases. This application note is the first to share four such separations, under normal phase conditions. And although not a part of the original intent of this application note, reversals of elution order that take place between CHIRALPAK® IB N-3/IF-3 and CHIRALPAK® IE-3/IH-3 are also highlighted.

EXPERIMENTAL

The samples of racemic (±)-catechin and (+)-catechin were purchased from Sigma Aldrich (St. Louis, MO). The Trifluoroacetic acid (TFA) was purchased from Spectrum Chemicals. The solvents used were all purchased from Pharmco, were HPLC-grade or higher. Specifically, the Hexanes (Hex) used contained 95% n-hexane, and the Ethanol (EtOH) was Reagent Alcohol (90% EtOH denatured with 5% Methanol (MeOH) and 5% 2-Propanol (IPA) v/v/v). All screening and optimization were performed on an Agilent 1200 equipped with a quaternary mixing pump, and utilized a DAD.

Chromatographic Conditions for the Separation of (±)-catechin

Column	CHIRALPAK® IB N-3 (250mm x 4.6 mm i.d.) Part #: 88525	CHIRALPAK® IE-3 (250mm x 4.6 mm i.d.) Part #: 85525	CHIRALPAK® IF-3 (150mm x 4.6 mm i.d.) Part #: 86524	CHIRALPAK® IH-3 (150mm x 4.6mm i.d.) Part #: 89524
Mobile Phase	80-20-0.1 = Hex-EtOH-TFA	80-20-0.1 = Hex-EtOH-TFA	80-20-0.1 = Hex-(50-50 = EtOH-MeOH)-TFA	80-20-0.1 = Hex-(50-50 = EtOH-MeOH)-TFA
Elution Order	(-),(+)	(+),(-)	(-),(+)	(+),(-)
Flow Rate	1.0 ml/min			
Detection	UV 220 nm ref. 450 nm			
Temperature	25°C			
Sample	(+)-catechin Enantiomer – 1.2 mg/ml in EtOH Racemic – 1.0 mg/ml in EtOH			
Injection Volume	2 µl			

DISCUSSION

To begin the screening of the racemic material, a 1.0 mg/ml solution was prepared in EtOH, and a retention check on CHIRALPAK® IB N-3 (4.6x150mm) was run using 70-30-0.1 = Hex-EtOH-TFA. It was found from this check that sufficient retention was achieved under these mobile phase conditions, therefore the compound was screened with 70-30-0.1 = Hex-EtOH-TFA and Hex-IPA-TFA on Daicel's library of chiral columns.

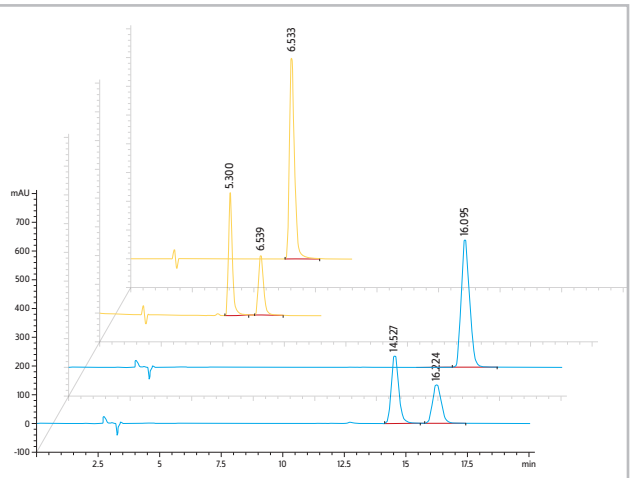

Figure 2 Separation of (±)-catechin on CHIRALPAK® IB N-3
Figure 3 Separation of (±)-catechin on CHIRALPAK® IF-3

*Single peak chromatogram corresponds to (+)-catechin

This screening demonstrated near baseline separations on many of Daicel's chiral columns. Most notably CHIRALPAK® IB N-3, IE-3, IF-3, and IH-3 were baseline or nearly baseline under Hex-EtOH conditions. It was also noted that the racemic material was not completely racemic (50:50), but rather closer to a 60:40 ratio. Because of this obvious difference, the reversal of elution order was spotted during the screening, and further explored during the optimization of the separations.

For CHIRALPAK® IB N-3, IE-3, and IF-3, simply increasing the retention of the compound by decreasing the elution strength of the mobile phase was sufficient to achieve baseline resolution. 80-20-0.1 = Hex-EtOH-TFA results in the elution of the (-)-catechin enantiomer first, and the (+)-catechin enantiomer second for CHIRALPAK® IB N-3 (Figure 2) and IF-3 (Figure 3). Under the same conditions for CHIRALPAK® IE-3, (+)-catechin is eluted first and the (-)-catechin is eluted second (Figure 4).

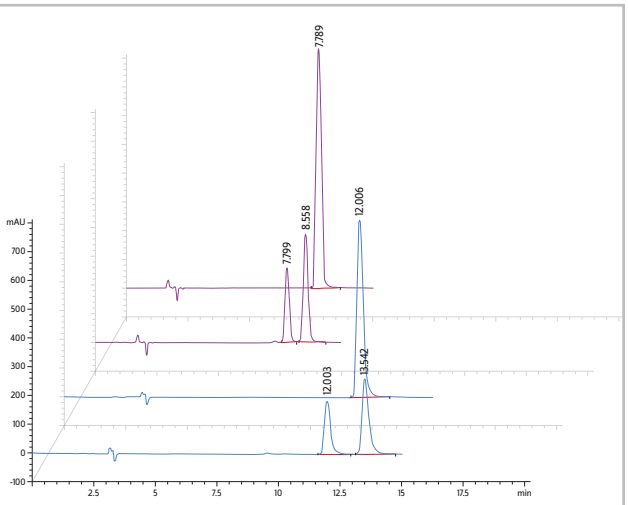

Figure 4 Separation of (±)-catechin on CHIRALPAK® IE-3
Figure 5 Separation of (±)-catechin on CHIRALPAK® IH-3

*Single peak chromatogram corresponds to (+)-catechin

While a separation is achieved with 80-20-0.1 = Hex-EtOH-TFA with CHIRALPAK® IH-3, the overall peak shape tails a bit more than desirable, resulting in a partial coelution. Increasing the retention further by going to 90-10-0.1 = Hex-EtOH-TFA only resulted in broader peaks. Therefore, a 50-50 mixture of EtOH and MeOH was used, to help sharpen the peaks to achieve a baseline resolution.

After some optimization, 80-20-0.1 = Hex-(50-50 = EtOH-MeOH)-TFA resulted in a baseline resolution on CHIRALPAK® IH-3, with (+)-catechin eluted first, and (-)-catechin eluted second (Figure 5). It is important to note that the EtOH and MeOH must be premixed before mixing on the instrument to avoid any deleterious effects from the low miscibility of hexane and methanol.

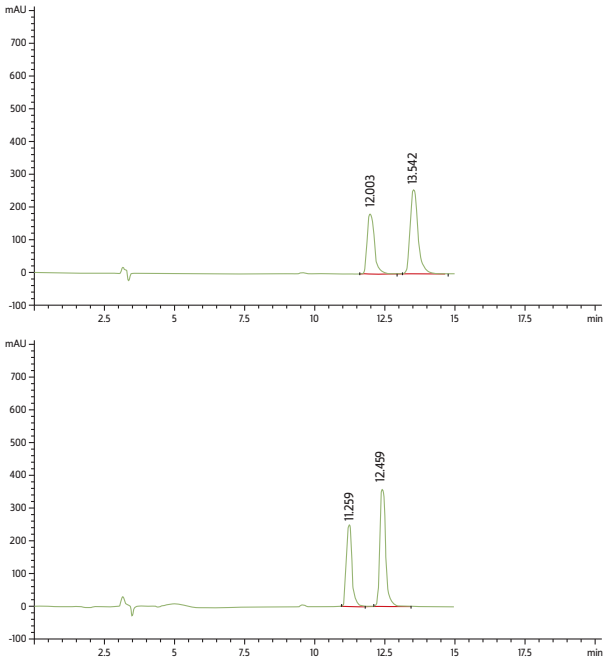

Figure 6 Comparison of the separation of (±)-catechin on CHIRALPAK® IE-3 without MeOH (top) and with MeOH (bottom)

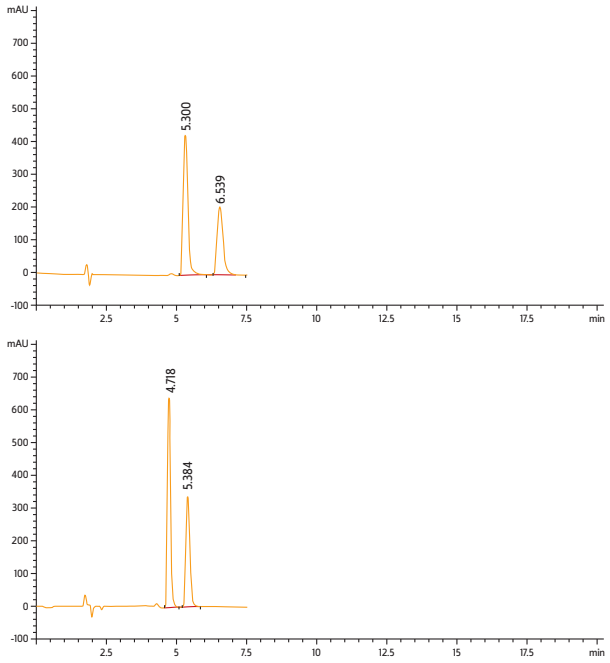

Figure 7 Comparison of the separation (±)-catechin on CHIRALPAK® IF-3 without MeOH (top) and with MeOH (bottom)

Methanol is helpful in sharpening the overall peak shape, for other columns as well. Although baseline resolution was achieved on both CHIRALPAK® IE-3 and IF-3 with 80-20-0.1 = Hex-EtOH-TFA, using the conditions of 80-20-0.1 = Hex-(50-50 = EtOH-MeOH)-TFA, the separation of the two enantiomers is more well resolved, and the overall analysis time is shortened (Figure 6 and 7).

CONCLUSIONS

The separation of (+)-catechin and (-)-catechin has been effectively demonstrated on several Daicel chiral columns. Specifically four columns were highlighted, demonstrating a reversal of elution order. Being able to reverse elution order can be very helpful when performing a preparative separation, but also when higher sensitivity or lower limit of detection is required. While not always achievable, for (±)-catechin, it can be accomplished on several different Daicel chiral columns, providing flexibility for the end-user to choose an application that best suits their needs.