Profiling the Isomerization of Biologically Relevant (E)-(Z) Isomers by Supercritical Fluid Chromatography (SFC)

Jacquelyn Cole(1), Rui Chen(1), Billy W. Day(2), and Vasiliy N. Korotochenko(2)
(1)TharSFC, a Waters Company and (2)Department of Pharmaceutical Sciences, University of Pittsburgh

In the past few decades, single enantiomers and stereoisomers have overtaken achiral molecules in the percentage of approved drugs in the market. Because isomers can have different biological/pharmacological/toxicological properties, authorities, such as the European Pharmacopoeia and the FDA, have asserted escalated emphasis on controlling isomer content in drug compounds and require that stereoisomers, "be treated as separate drugs and developed accordingly" with rare exception. The separation and quantification of stereoisomers is therefore of great importance, especially when considering pharmaceutical compounds (1).

Supercritical fluid chromatography (SFC) has become the choice of chromatography for separating stereoisomers owing to its speed, efficiency, and cost-effectiveness (2). However, SFC has been primarily used to separate optical isomers, including enantiomers and diastereoisomers. Its applications on (E)-(Z) isomers are limited in scope (3).

We present herein a case study employing SFC to profile the (E)-(Z) transformation of (*E*)-2-benzylidene-3-(cyclohexylamino)-2,3-dihydro-1*H*-inden-1-one (BCI), a small molecule inhibitor of dual specificity phosphatase 6 (Dusp6). BCI is a synthetic molecule which contains an asymmetrical carbon and a sp^2-hybridized C-C double bond resulting in both optical and (E)-(Z) isomerism. A six-min SFC method was developed to simultaneously separate all four isomers. The method was subsequently employed to profile the (E)-(Z) isomerization of the BCI molecule over the course of 40 days.

Experimental

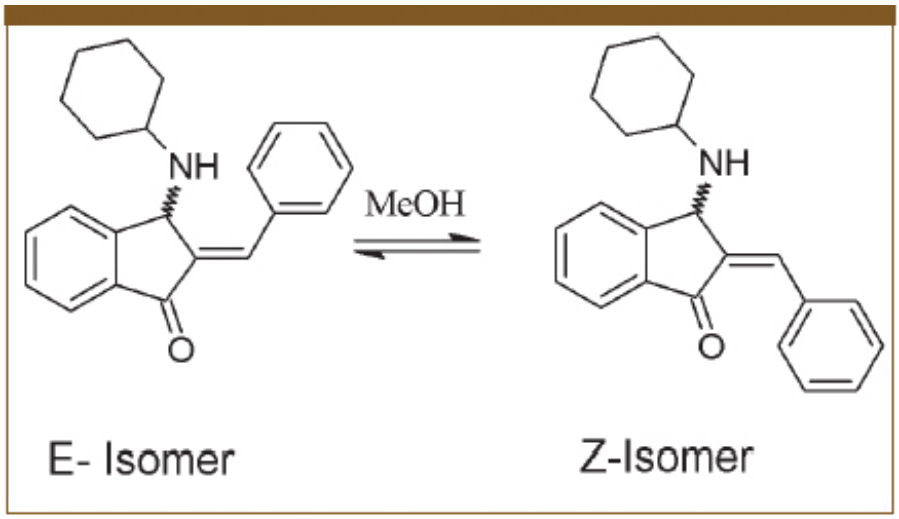

Figure 1: The chemical structures of the E-Z Isomers of BCI.

Figure 1

Materials: The (*E*)-2-benzylidene-3-(cyclohexylamino)-2,3-dihydro-1*H*-inden-1-one (BCI) was a gift from Professor Billy Day at the Department of Pharmaceutical Science and Chemistry, University of Pittsburgh. The chemical structures of the BCI (E)-(Z) isomers are shown in Figure 1. BCI was dissolved in HPLC grade methanol with an estimated concentration of 1 mg/ml for method development. A diluted sample (0.05 mg/ml) was used to profile the isomerization. The chiral stationary phases used in this study were Chiralpak AD-H, Chiralpak AS-H, Chiralcel OD-H, and Chiralcel OJ-H. All columns were 4.6 × 250 mm in dimension.

Chromatography: All experiments were carried out using a TharSFC Investigator (TharSFC, a Waters Company, Pittsburgh, Pennsylvania). The system was equipped with a Waters 2998 PDA detector (Milford, Massachusetts). For all experiments, the key experimental parameters were as follows: the flow rate was 4 ml/min, the back pressure

was 100 bar, the temperature was 40 °C, the PDA scan was 220–300 nm, and the injection volume was 10 µL. The initial screening was done using a linear gradient: 2% to 25% methanol in 6 min, holding at 25% for 4 min, and returning to 2% in 1 min. The optimal method for isomerization study was: 5% to 25% in 5 min, holding at 25% for 3 min and returning to 5% in 1 min.

Results and Discussion

Figure 2 shows the method screening for the chiral separation of the E-isomer. Both AD-H and OJ-H columns yielded baseline resolution, whereas AS-H and OD-H offered no resolution of the enantiomeric pair of the E-isomer. OJ-H was selected for the ensuing isomerization study due to the shorter analysis time. At day 5, a pair of peaks, immediately adjacent to the enantiomeric pair of the E-isomer, started to emerge (Figure 3). The peaks were collected, analyzed by NMR (results not shown), and confirmed to be the enantiomers of the Z-isomer. Over the course of 40 days, a gradual transformation from E- to Z-isomer was observed. Representative chromatograms are shown in Figure 3. Since both E- and Z- isomers are racemic, the peak area% of one E-isomer and its derivatives was plotted against time to gauge the rate of the observed isomerization. Shown in Figure 4 is the plot of 1/peak area% *vs.* time, with a R^2 of 0.9623, suggesting second-order reaction kinetics. It is noted such data maneuvers can be misleading and more careful experiments are required to fully understand the reaction

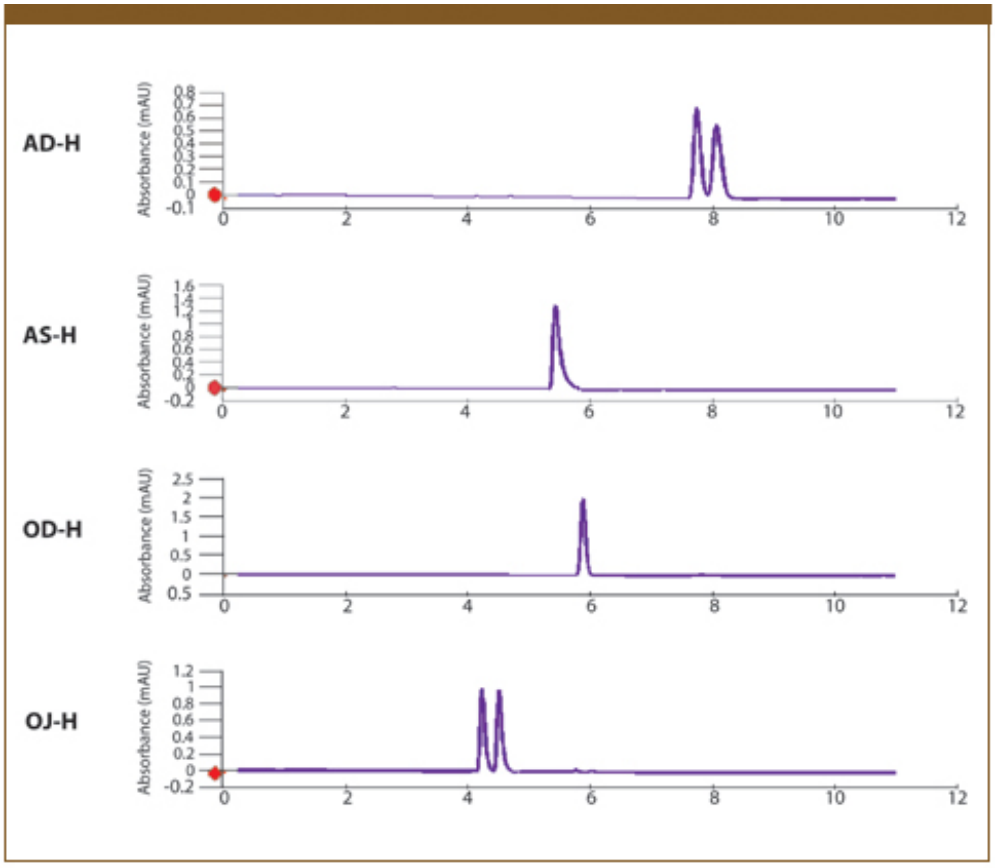

Figure 2: SFC chromatograms of the E-isomer of BCI using four chiral stationary phases. Key parameters are listed in the experimental section.

Figure 2

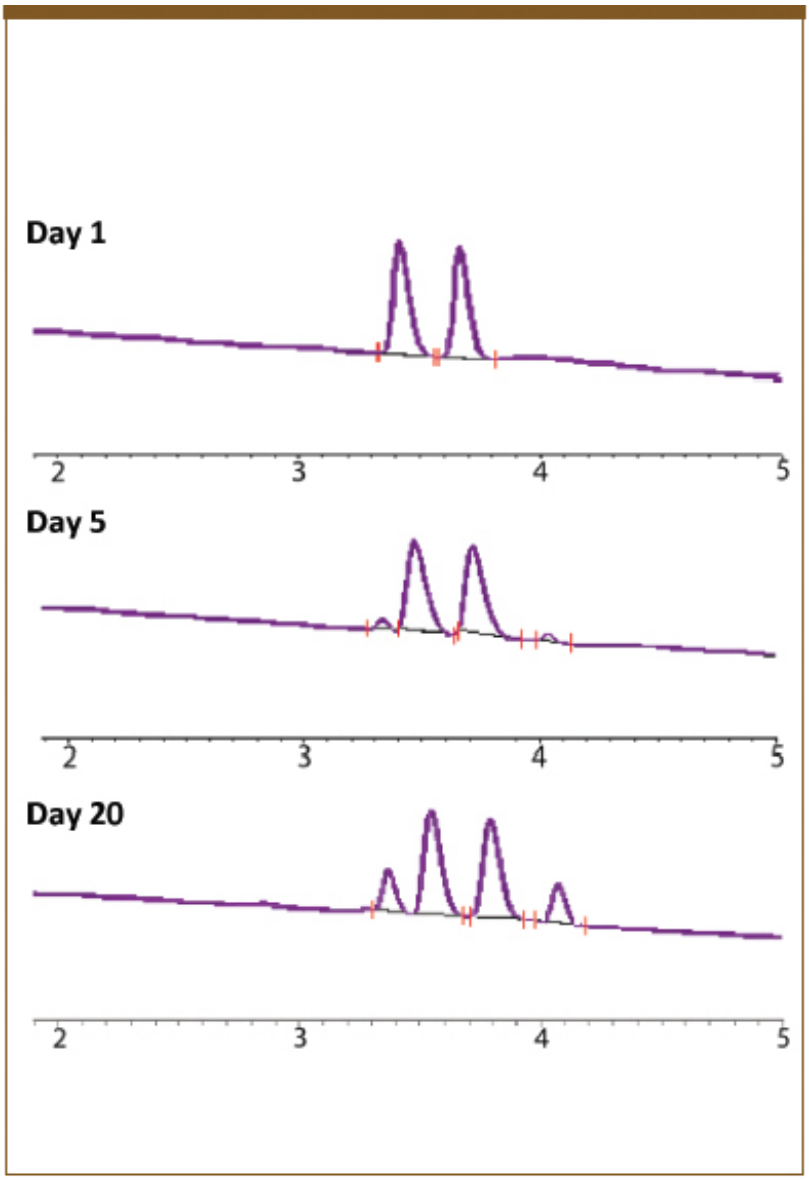

Figure 3: Representative SFC chromatograms showing the E-Z transformation of BCI.

Figure 3

kinetics. Nevertheless, simultaneous baseline resolution of all four isomers by SFC is undoubtedly an essential part of such a study.

Conclusions

In this report, we have demonstrated the simultaneous resolution of all four isomers of BCI, both chiral and (E)-(Z) isomeric, by SFC in less than 5 min. The developed SFC method also holds potential for the kinetic study of (E)-(Z) isomerization.

References

(1) H. Caner, E. Groner and L. Levy, *Drug Discovery Today*, **9(3)**, 105–110 (2004).

(2) D. Mangelings and Y. V. Heyden *J. Sep. Sci.* **31**, 1252–1273 (2008).

(3) F. Hasdenteufel, *Separation & Purification Reviews*, **35**, 193–221 (2006).

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TharSFC, a Waters Company
575 Epsilon Drive, Suite 100, Pittsburgh, PA 15238

tel. (412)967-5665, fax (412)967-9446
Email:

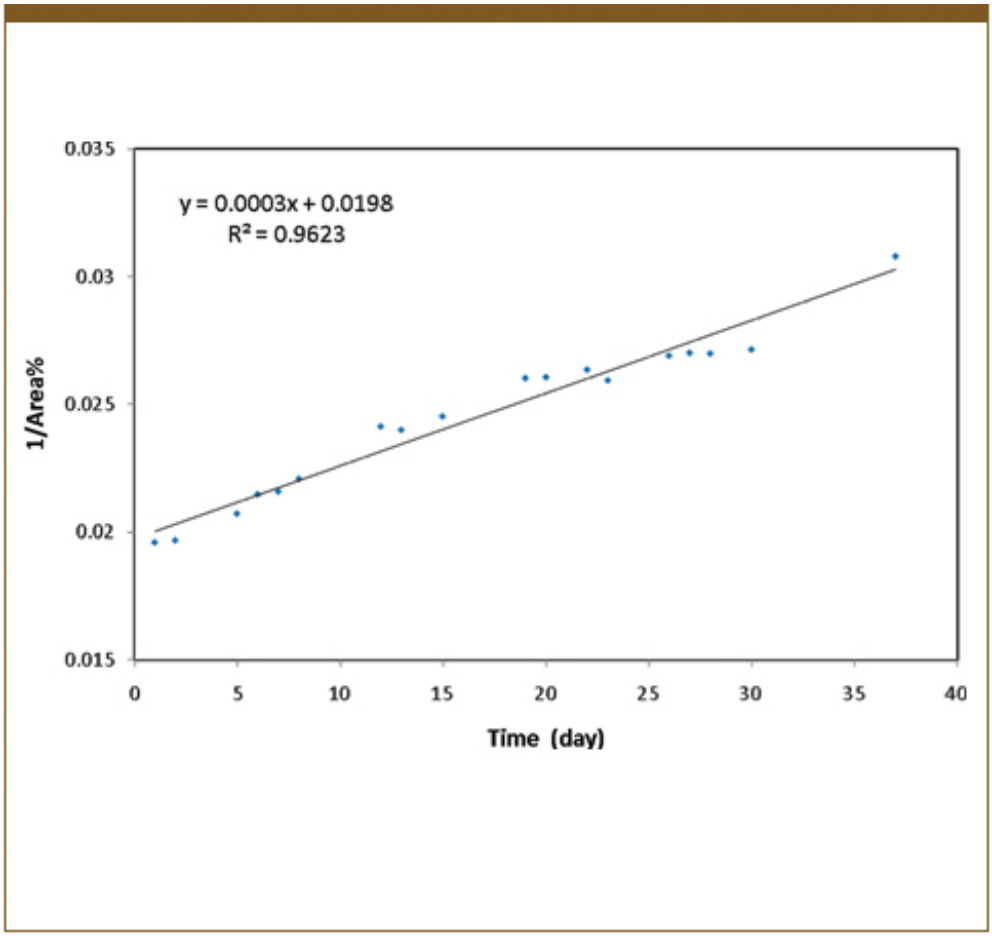

Figure 4: An example of kinetic plot: 1/peak area% *vs.* time.

Figure 4

info@tharsfc.com [1]
Website: www.waters.com/sfc [2]