Separation of Atropisomers of a Pharmaceutical Compound by Chiral Liquid Chromatography and Thermodynamic Analysis of Separation Mechanism
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Purpose
In the pharmaceutical industry, the analysis of atropisomers is of considerable interest from a scientific and regulatory perspective. However, achieving good quantification of atropisomers using HPLC can be very challenging in some cases due to inter-conversion of the isomers during the analysis process. The compound of interest contains two stereogenic axes due to the hindered rotation about the single bonds connecting the aryl groups. These structural elements result in the formation of four configurational isomers (atropisomers). The separation of the four atropisomers is very challenging due to the low energy barrier between the configurations. The purpose of this study is to separate the four atropisomers, and investigate the factors that minimize the inter-conversion and affect the separation. Finally, a thermodynamic study of the retention behavior of the atropisomers will provide a better understanding of the separation mechanism.

Methods
In this study, an HPLC method was developed to separate the four atropisomers using a secondary hydroxyl derivatized β-cyclodextrin (CD) bonded stationary phase, Astec Cyclobond I 2000 HP-RSP. The mobile phase was optimized which provided excellent resolution of the 4 isomers. The influence of column temperature on retention and enantioselectivity was investigated.

Results
DryLab™ was used to optimize the mobile phase composition. Column temperature has a significant effect on both separation and inter-conversion of the atropisomers. Lower column temperature was selected for the separation. A satisfactory separation of the four atropisomers of a pharmaceutical compound was achieved using chiral liquid chromatography. In addition, sample preparation conditions were identified which provide 4 hours of stability preventing inter-conversion of the atropisomers.

Conclusion
The thermodynamic study of the retention provided a more complete understanding of the driving forces for enantioselectivity. The results indicate that the separation of the two pairs of enantiomers is primarily enthalpy controlled within the temperature range studied. The mechanistic aspects of the separation will also be discussed in the presentation.