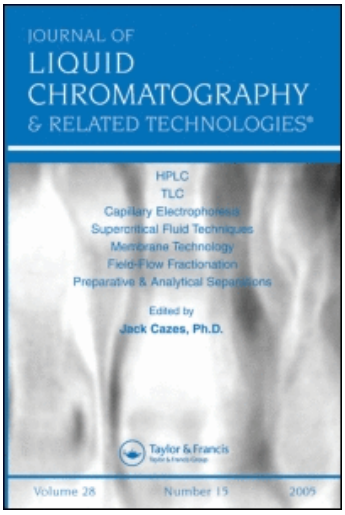

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ENANTIOMERIC SEPARATION OF AN AMPA ANTAGONIST USING A CHIROBIOTIC T™ COLUMN WITH HPLC AND EVAPORATIVE LIGHT-SCATTERING DETECTION

Andrea L. Guisbert[a]; V. Scott Sharp[a]; Jeffrey A. Peterson[a]; Donald S. Risley[a]
[a] Lilly Research Laboratories, Pharmaceutical Sciences Division, Lilly Corporate Center, Eli Lilly and Company, Indianapolis, IN, U.S.A.

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ENANTIOMERIC SEPARATION OF AN AMPA ANTAGONIST USING A CHIROBIOTIC T™ COLUMN WITH HPLC AND EVAPORATIVE LIGHT-SCATTERING DETECTION

Andrea L. Guisbert, V. Scott Sharp, Jeffrey A. Peterson, Donald S. Risley*

Eli Lilly and Company
Lilly Research Laboratories
Pharmaceutical Sciences Division
Lilly Corporate Center
Indianapolis, IN 46285, USA

ABSTRACT

A Chirobiotic T™ column was used for the direct separation of AMPA receptor antagonist LY293558 and the undesired enantiomer LY293559 in bulk drug substance. High performance liquid chromatography (HPLC) separation of the enantiomers was optimized using reversed phase and hydrophilic interaction chromatography (HILIC) by varying the organic composition of the mobile phase. Baseline resolution was achieved allowing accurate, trace level quantitation of the undesired enantiomer in the optically pure bulk material. Because the analytes lack a sufficient ultraviolet chromophore, an evaporative light-scattering detector (ELSD) was used to enhance detection. The ELSD was capable of obtaining detection limits as low as 0.1% of the undesired enantiomer. Additional experiments were conducted to assess the linearity, precision, and accuracy of the HPLC-ELSD system.

INTRODUCTION

LY293558 is an AMPA (2-amino-3(3-hydroxy-5 methylisoxazol-4-yl)propionic acid) receptor antagonist that has been shown to provide potent neuroprotective effects.[1-4] The structure of LY293558 is shown in Figure 1. The bulk material was synthesized as a single enantiomer, and the potential existed for the formation of the undesired enantiomer during the synthesis. A test for chiral purity is needed to ensure the purity of the bulk drug substance.

Numerous macrocyclic antibiotics have been successfully used as chiral selectors by capillary electrophoresis and high performance liquid chromatography (HPLC).[5-8] Among these, vancomycin and teicoplanin have emerged as commercially bonded stationary phases for HPLC columns. The teicoplanin chiral stationary phase, available as the Chirobiotic T™ HPLC column, has been very successful for the enantioresolution of racemic carboxylic acid compounds. The structure of teicoplanin has been previously described.[9-10]

Prior to the introduction of the Chirobiotic T™ column, numerous attempts for a direct HPLC separation of LY293558 and its enantiomer were unsuccessful. Current methodology calls for a chemical derivatization step followed by the subsequent separation on a Chiracel® OD column with ultraviolet (UV) detection. This process is labor intensive and introduces unnecessary error from the derivatization procedure. We addressed the direct HPLC separation detection problem by using an evaporative light-scattering detector (ELSD) which is ideal for detecting non-volatile compounds lacking a UV chromophore. Dreux et. al. have discussed the concept and operation of commercially available evaporative light-scattering detectors as sensitive universal detectors.[11] The ELSD has been shown to successfully detect phospholipids,[12]

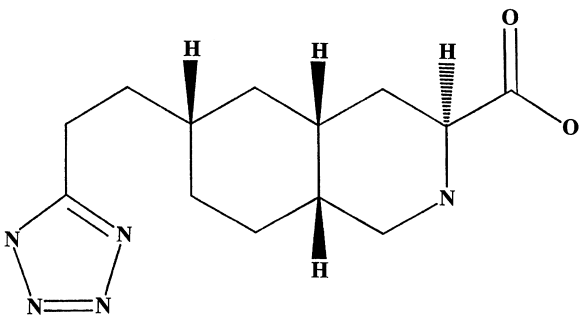

Figure 1. Structure of LY293558.

triglycerides, fats and fatty acid esters,[13] carbohydrates,[14] synthetic polymers,[15] amino acids,[16-17] steroids,[18] surfactants, and cyclodextrins.[19] The ELSD has also been used in pharmaceutical applications for the determination of small organic molecules, impurities, swab samples, raw materials, and inorganic counterions.[20-28]

In this study we evaluated the Chirobiotic T™ column for the enantioresolution of LY293558 from LY293559. Optimized chromatographic conditions were established by varying organic composition of the mobile phase. Additionally, the HPLC-ELSD system was evaluated for its ability to accurately determine the chiral purity of LY293558.

EXPERIMENTAL

LY293558 and LY293559 were synthesized at Eli Lilly and Company (Indianapolis, IN). Chempure Brand acetonitrile (ACN) was obtained from Curtin Matheson Scientific (Houston, TX). The water was deionized and filtered through a Millipore Milli-Q™ water purification system (New Bradford, MA). National Formulary-grade nitrogen (>97%) was used for the ELSD.

The HPLC system consisted of a Shimadzu SCL-10A controller, LC-10AS pumps, SIL-10A autoinjector, and a DGU-3A membrane degasser (Shimadzu, Kyoto, Japan). The UV detector was a Shimadzu SCL-10A. A Varex MK III evaporative light- scattering detector from Alltech Associates, Inc. (Deerfield, IL) was used. The Chirobiotic T™ column (25 cm x 4.6 mm) was manufactured by Advanced Separation Technologies, Inc. (Whippany, NJ).

Optimized chromatographic conditions consisted of mobile phase comprised 65% ACN/35% water with flow rate of 1.0 mL/min. and 10 μL injections. The detector drift tube temperature was optimized at 80°C and the detector nitrogen gas flow rate was optimized at 2.40 standard liters per minutes (SLPM). The analytes were dissolved in water.

RESULTS AND DISCUSSION

The effect of the organic modifier was examined by increasing the acetonitrile from 10% to 80% in water and evaluating the separation of LY293558 from LY2935959. The resolution of these enantiomers was maintained at approximately 3.0 with ACN compositions from 5% to 50%, with resolution increasing to 7.3 when ACN concentrations were increased from 50% to 80%. The capacity factor (k') for LY293558 decreased with increasing acetonitrile from 10% to 40% but then increased with increasing ACN composition from 40% to 80%. This horseshoe effect (Figure 2) was surprising, however a simi-

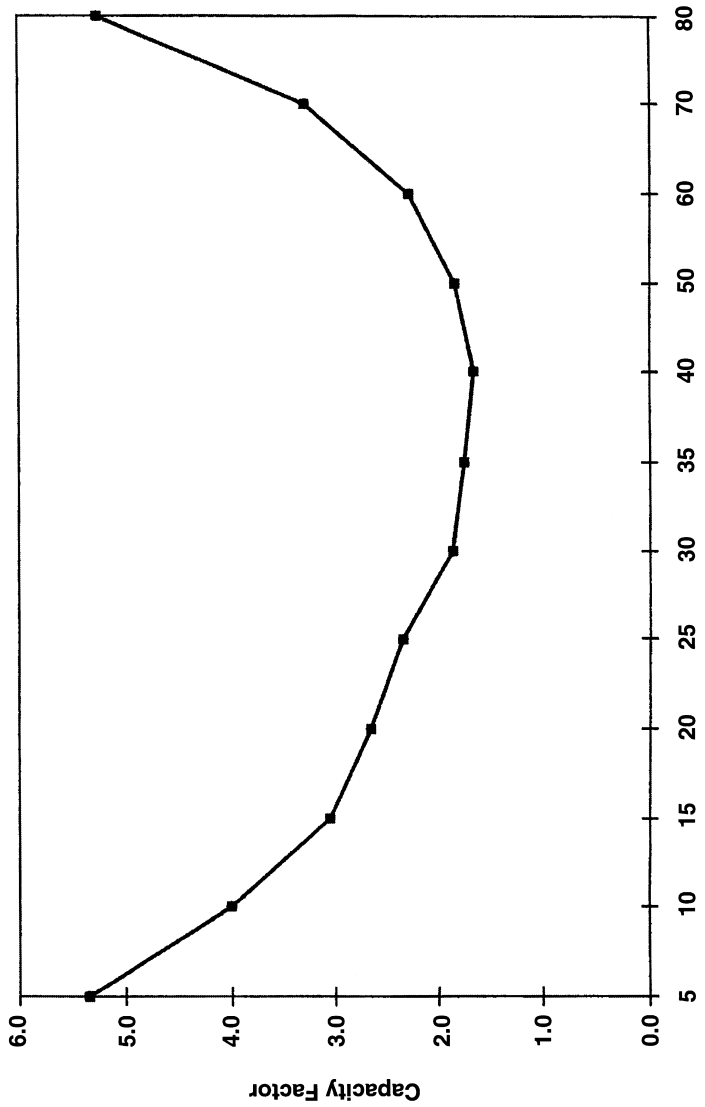

Figure 2. Effect of increasing the organic modifier on the capacity factor.

lar trend is reported in the product literature.[10] This effect has been more recently described as hydrophilic interaction chromatography (HILIC) for polar compounds.[29,30] The technique employs hydrophilic interactions in the presence of mixed aqueous/organic mobile phases for the establishment of a stagnant enriched water layer on the surface of the stationary phase, into which analytes may partition based upon their polarity. This mechanism has been very effective for retention and separation of highly polar compounds. The separation mechanism of HILIC is therefore opposite to that of reversed phase chromatography, and is also different from traditional normal phase and polar organic modes of chromatography. The HILIC mobile phases are relatively high in water content (10-50% aqueous), an environment that can provide significant advantages in regard to the solubility of many biologically active substances.

The effect of pH on resolution was examined by adjusting the aqueous portion of the mobile phase from pH 4.0 to 6.5. Resolution of the enantiomers decreased slightly as the pH was increased. The changes in pH had minimal effect on the k'. Based on the data from the effects of the ACN composition and pH on the mobile phase composition, the chromatographic conditions described in the experimental section were established. Figure 3 shows a chromatogram of the enantiomeric separation using the optimized chromatographic conditions.

Evaporative light-scattering detector response is relatively independent of molecular functional groups within a chemical entity. To illustrate this, a UV detector and an ELSD detector were connected in series allowing for the superimposition of the two detector responses on a single chromatogram corresponding to the same sample injection of LY293558. Because LY293558 lacks a sufficient chromophore, this situation resulted in the ELSD response being much larger than the corresponding UV response (Figure 4).

It is customary to perform linearity determinations over a wide range of sample concentrations to fully assess the dynamic range of the detection system.[31] The linearity of the system was initially evaluated by injecting eight samples prepared from a racemic standard stock solution. The samples encompassed a concentration range of 0.02 to 0.92 mg/mL for each enantiomer. The enantiomer peak area responses were not found to be linear over the entire range tested, but linearity was demonstrated over the narrower range from 0.02 to 0.22 mg/mL for each enantiomer. The correlation coefficients for LY293558 and LY293559 were determined to be 0.994 and 0.996, respectively, over this concentration range.

Since the linear working range was narrow, a high-low chromatography approach was used to determine the undesired enantiomer. High-low chromatography is a sampling technique used to improve the detection limit of trace

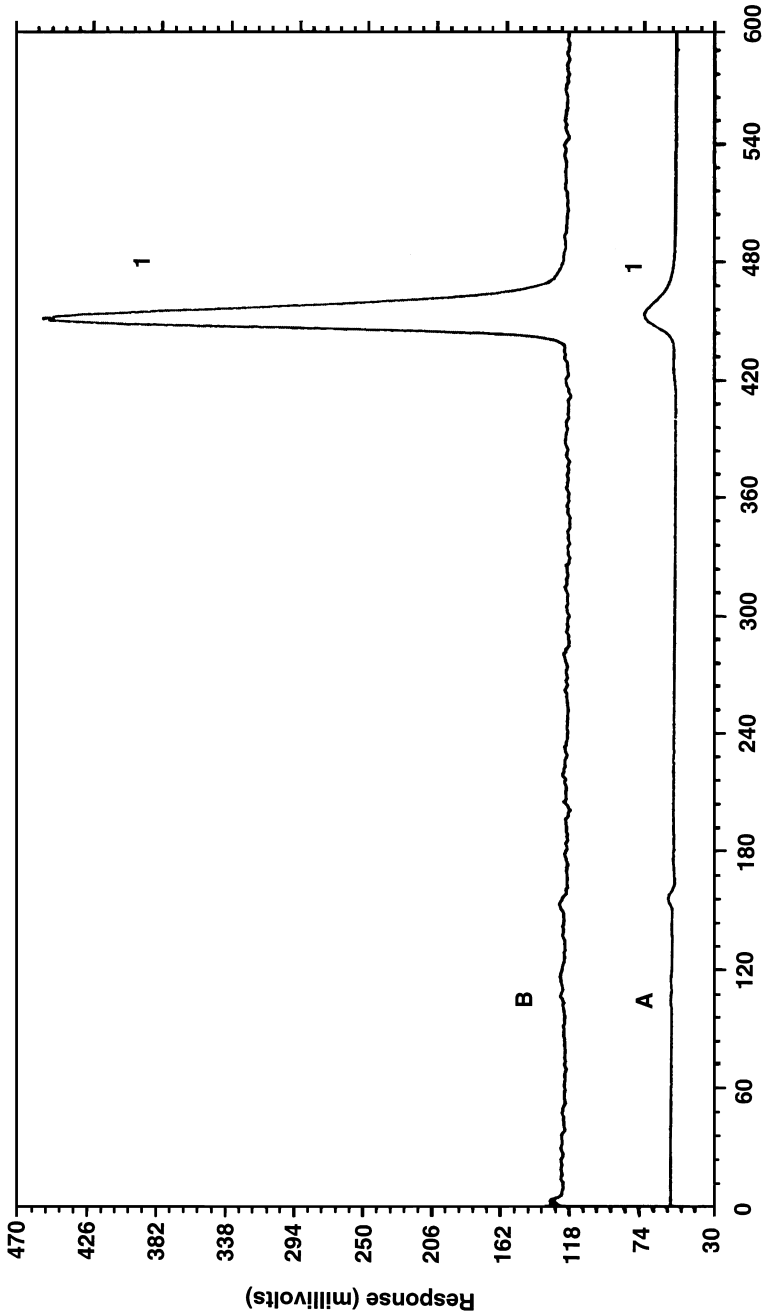

Figure 3. Sample injection of LY293558 as detected by UV (1A) and ELSD (1B).

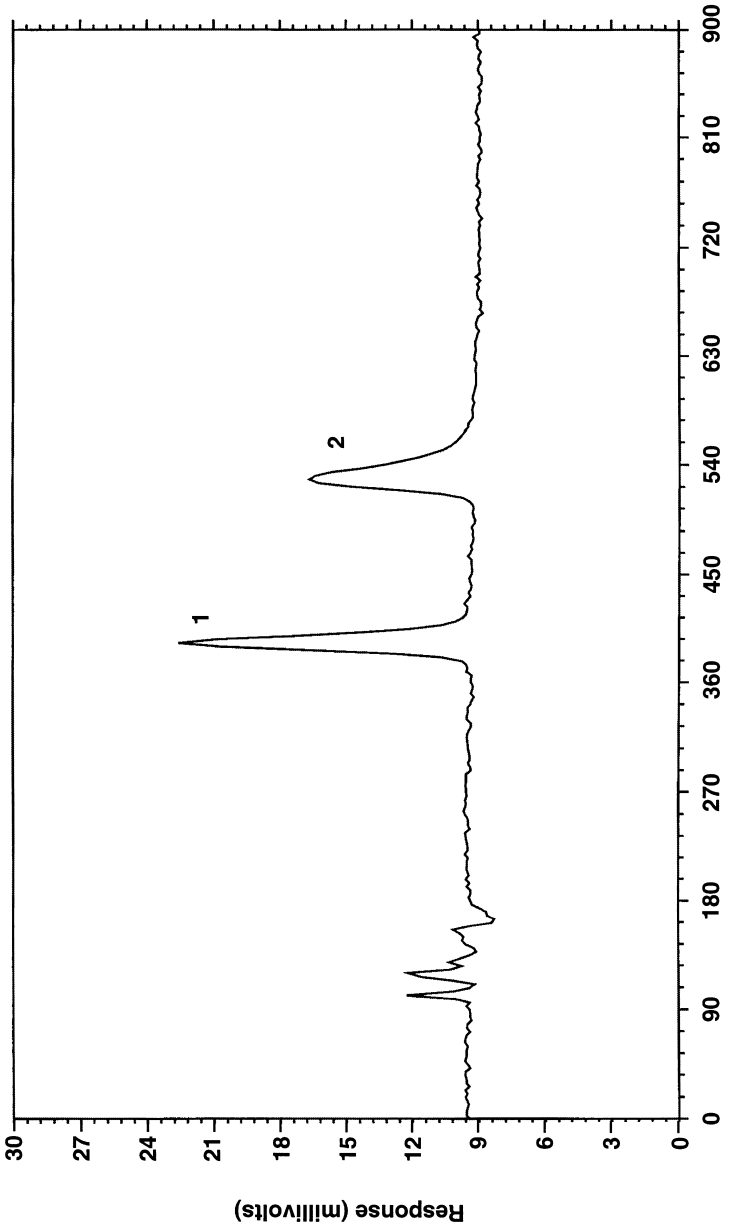

Figure 4. Sample chromatogram for the separation of LY293558 (1) and LY293559 (2).

components in a bulk drug substance by extending the dynamic range of the detection system.[31] The LY293558 sample concentration was prepared at 10 mg/mL for the high injection and a serial dilution was made to 0.15 mg/mL for the low injection to be within the linear working range.

The precision of the method was evaluated by injecting five individually prepared high concentration samples and five low concentration samples from a sample lot of LY293558. The five samples resulted in 1.1% of the unwanted enantiomer in the LY293558 drug substance lot. The RSD of the five sample preparations was 1.1%.

The accuracy of the method was determined by comparing the results from this HPLC-ELSD method to a previously validated method using pre-column derivatization prior to enantiomeric resolution with a Chiracel® OD column (HPLC-UV). The amount of undesired enantiomer was 1.1% and 1.2% by the HPLC-ELSD and HPLC-UV methods, respectively. These results indicate very good agreement between the two methods.

The recovery was determined by a standard addition technique whereby eight samples of LY293558 were prepared at a concentration of 10 mg/mL and were spiked with 0-5% of LY293559, the undesired enantiomer. The average percent recovery for these eight samples was 93.3%.

The limit of detection (LOD) can be defined as the lowest concentration of sample that can be clearly detected above baseline noise. The LOD for this method was experimentally determined to be 0.01 mg/mL. When using a nominal sample concentration of 10 mg/mL of LY293558, quantitation of LY293559 can be achieved at a level of 0.1%.

CONCLUSION

The Chirobiotic T™ HPLC column successfully separated LY293558 from its undesired enantiomer without the use of a chiral derivatizing agent. Accurate, low level quantitation was achieved using an HPLC-ELSD system. An ELSD is an effective alternative to standard UV detectors when substances containing weak UV chomophores are analyzed. A high-low chromatography approach was used to attain acceptable levels of precision, linearity, accuracy, recovery, and limit of detection with the HPLC-ELSD system.

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