Abstract

AIT Bioscience was commissioned to develop a LC-MS/MS bioanalytical method for eflornithine in human plasma. More specifically, the sponsor wanted a method capable of quantifying the D and L enantiomers. There is a method reported in the literature describing a method used for quantifying the enantiomers of eflornithine in human plasma using a Chirobiotic TAG column but the run time was 30 minutes and the buffer used for their chromatographic conditions cannot be used under LC-MS/MS conditions. Our approach was to investigate the derivatization of eflornithine with a chiral derivatizing agent used in this laboratory for previous studies, (R)-(-)-o-methoxy-o-(trifluoromethyl)-phenylacetyl chloride. This approach would permit separation of the derivatized eflornithine diastereomers under reversed phase chromatography conditions that are more amenable to LC-MS/MS conditions. Here we describe conditions used to collect fractions obtained from the chiral separation of eflornithine that were enriched in the D or L isomer. Aliquots of these fractions were derivatized with the chiral derivatizing agent and chromatographic separation of the solutions containing the enriched D or L diastereomer were used to assign the elution order of derivatized D and L eflornithine.

Methods

- Inject 10 µg/mL D,L-eflornithine solution using Chirobiotic column and published conditions (1)
- Collect fractions of each enantiomeric peak (D or L, identified by retention time)
- Derivatize the separate enantiomeric fractions (10 µL D,L-eflornithine solution, 100 µL 50 mM sodium bicarbonate, 890 µL 0.1% (R)-(-)-o-methoxy-o-(trifluoromethyl)phenylacetyl chloride in acetonitrile)
- Determine D and L peak assignments by injecting the derivatized samples into a C18 column using the desired LC-MS/MS conditions

Results

- Fraction collection of a racemic eflornithine solution using a published method provided reference solutions enriched in each enantiomer which were not commercially available.
- The reference solutions were reacted with (R)-(-)-o-methoxy-o-(trifluoromethyl)phenylacetyl chloride to produce di-derivatized eflornithine diastereomers which were detected at 615 m/z as expected.
- Several chiral derivatizing reagents were explored. (R)-(-)-o-methoxy-o-(trifluoromethyl)-phenylacetyl chloride was selected because it provided a simple, fast (5 min), and rugged approach.
- Chromatographic and mass spectrometric conditions were optimized to achieve baseline separation of the diastereomers. Column cooling was necessary for optimal separation.

Conclusion

A chiral derivatizing agent was successfully used to identify individual enantiomeric peaks from an eflornithine racemic mixture. An assay was then developed, optimized and fully validated to regulatory standards using reversed phase LC-MS/MS.