Chiral Derivatization of D- and L-Eflornithine for Enantiomer Identification from a Racemate and Chromatographic Separation

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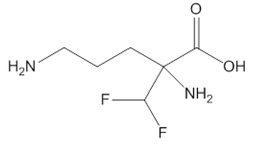
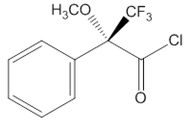
Abstract

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-(-)-α-methoxy-α-(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.

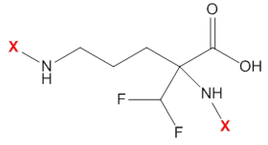
Structures



Eflornithine



(R)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride



X = (R)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl

Derivatized 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)-(-)-α-methoxy-α-(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

[1] M. Malm, Y. Bergqvist, J. Chromatogr. B 846 (2007) 98-104.

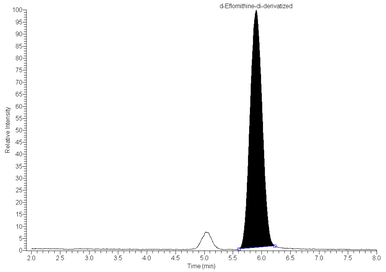
Instrumental Analysis – Measurement of derivatized eflornithine

Thermo Scientific Dionex UltiMate 3000		**Thermo Scientific TSQ Quantiva MS**	
Run Time:	8 min	Ion Source:	HESI
Column Temp.:	10 ^{0}C	Spray Voltage:	1750
Autosampler Temp.:	7.5 ^{0}C	Ion Transfer Tube Temp:	350 ^{0}C
Injection Volume:	2 µL	Vaporizer Temp:	350 ^{0}C
Flow Rate:	0.25 mL/min	Sheath Gas	80
Mobile Phase:	A: 20 mM acetic acid B: Methanol Isocratic 65% B	Aux Gas	9
Analytical Column:	Waters Acquity UPLC® BEH C18, 1.7 µm, 2.1 x 50 mm	Resolution	Unit/Unit

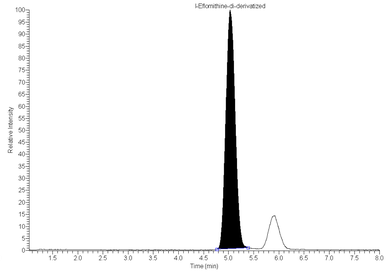
SRM Table

Compound	**Polarity**	**Precursor (m/z)**	**Product (m/z)**	**Collison Energy (V)**	**RF Lens (V)**
Derivatized Eflornithine	Positive	615.25	563.14	23	69

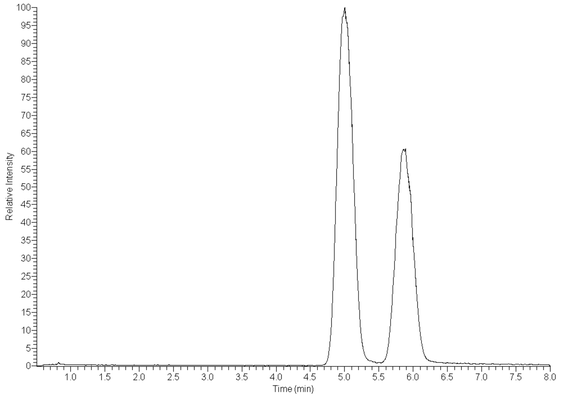
Data Analysis



Derivatized fraction 1: D-Eflornithine



Derivatized fraction 2: L-Eflornithine



Derivatized racemic mixture of eflornithine

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)-(-)-α-methoxy-α-(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)-(-)-α-methoxy-α-(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.