Rapid Analysis of Ceftriaxone in Human Intestinal Chyme, Human Plasma, and Dog Plasma by HPLC/MS/MS

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Overview

- The study was designed for rapid analysis of ceftriaxone in various matrices to support clinical and non-clinical studies of a novel oral β-lactamase enzyme.
- Ceftriaxone in a 26-enamiphtadine method was used to identify a variety of functions.
- Despite the highly polar nature and the complex atomic heterogeneity of ceftriaxone, extensive derivatization efforts are required to obtain a product fit for the LC/MS/MS platform.

Introduction

- Ceftriaxone has been analyzed using high-performance liquid chromatography with ultraviolet detection (HPLC/UV) however, there is a lack of robust methods employing HPLC/MS/MS for the determination of this compound in various biological fluids across time to fully understand the scope of action of ceftriaxone in vivo.
- Ceftriaxone has been analyzed using high-performance liquid chromatography with ultraviolet detection (HPLC/UV) however, there is a lack of robust methods employing HPLC/MS/MS for the determination of this compound in various biological fluids across time to fully understand the scope of action of ceftriaxone in vivo.
- This method was developed to quantitate ceftriaxone in various matrices to support clinical and non-clinical studies of a novel oral β-lactamase enzyme.

Methods

- Dog Plasma Sample Preparation and Extraction Procedure
  - 500 µL of internal standard (IS) working solution was added, and, after vortex mixing, 50 µL of the diluted sample was transferred to a collection plate for further dilution in 150 µL of 0.1% formic acid in water.
  - All samples were diluted using 400 µL of 0.1% formic acid in water and 50 µL of the diluted control blank sample was transferred to a collection plate.
  - Calibration range was 1.0 to 2000 ng/mL.

- Human Intestinal Chyme Sample Preparation and Dilution
  - The sample was subjected to digestion with 200 µL of 1000 µg/mL of tazobactam (a β-lactamase inhibitor).
  - The standard curve was prepared in 100% blank FaSSIF buffer.
  - The chyme sample was diluted 1:1 with 8 M guanidine hydrochloride in acetonitrile/0.1% formic acid in water.
  - The complex heteroatomic character of ceftriaxone results in multiple ionic species across a range of pH values.
  - The standard curve was prepared in water.

- Calibration Ionic Microspecies Distribution (across pH)

- Challenges In Method Development
  - Due to the highly polar nature and the complex heteroatomic nature of ceftriaxone and the structural complexity of the molecule.
  - The complex heteroatomic character of ceftriaxone results in multiple ionic species across a range of pH values.
  - The standard curve was prepared in water.

- Challenges in Method Development
  - The action of the SYN-004 was not completely inhibited by using the tazobactam added to the sample.
  - Inhibition of ex vivo enzymatic activity is an important aspect of analyzing highly reactive β-lactam antibiotics.

Results

- Typical Chromatograms of Extracted Dog Plasma Samples
  - Typical Chromatograms of Diluted Human Intestinal Chyme Samples
  - Typical Chromatograms of Extracted Human Plasma Samples
  - Typical Chromatograms of Diluted Human Intestinal Chyme Samples

Conclusions

- This manuscript describes methodology for the quantification of ceftriaxone in various matrices that were successfully applied in support of clinical and non-clinical studies.
- The LC/MS/MS method is capable of supporting the LEI requirements associated with the human plasma, human intestinal chyme, and dog plasma matrices.
- These methodologies may be applied to other drugs in the future.

References