Liposomal Encapsulated and Free Topotecan Analysis in Human Tissues, Plasma, Ultrafiltrate, and Urine by Liquid Chromatography/Tandem Mass Spectrometry

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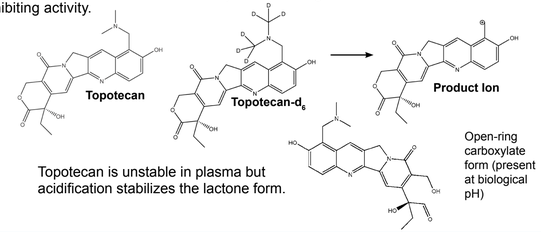
Overview

This work quantified free form topotecan in human plasma, plasma ultrafiltrate, urine, and tissue homogenate samples from patients dosed with liposomal formulated topotecan. Each matrix had individualized sensitivity, stability, and sample handling requirements to preserve the free and liposomal forms during collection and analysis.

An interdependent workflow approach was taken for each method to be optimized in tandem.

1. The plasma method was developed using a Sciex API 4000 mass spectrometer platform. Stability of topotecan in matrix was addressed alongside procedures for handling samples to preserve the stability of the liposomal encapsulated form.
2. A second method for ultrafiltrate was then developed into the clinically significant range using a Sciex API 6500 mass spectrometer.
3. Once each fraction was able to be quantified with methods from the two steps above, development was completed for the ultrafiltration fraction collection to be performed at clinical collection sites.
4. Sample splits for free topotecan analysis were filtered using a 10 kDa cutoff device and plasma ultrafiltrate collected. Samples were analyzed for total drug concentration in plasma and free drug in ultrafiltrate to assess the collection procedures.
5. Plasma was tested for use as a surrogate matrix for the tissue method in the lowest dynamic range method required (lower limit of quantitation of 0.025 ng/mL) using the API 6500.
6. Once the limit of quantitation (LOQ) in plasma surrogate was established, the homogenization procedure of four representative tissue types using plasma was determined. Optimization of the sample processing of tissues was completed.
7. Tissue biopsy samples that had been collected and frozen at the clinical site were transferred to the analytical lab for homogenization 4:1 v/w in acidified human plasma to stabilize the free topotecan for analysis.
8. Using the API 6500, with its extended dynamic range, for ultrafiltrate and tissue analysis allowed the higher-range urine method to be completed in tandem with the lower-range methods using the same LC/MS/MS system.
9. Plasma, ultrafiltrate, and urine methods were validated after development in accordance with procedures established to comply with GLP criteria.
10. The qualified tissue method was able to use a plasma surrogate matrix for analysis of multiple tissue types.

Structures: Under physiological conditions the lactone moiety of topotecan undergoes pH-dependent conversion to a carboxylate open-ring form, which lacks topoisomerase I inhibiting activity.



Topotecan is unstable in plasma but acidification stabilizes the lactone form.

Acknowledgement: Joy E. Crocker for help with poster preparation.

LC/MS/MS analysis of total and free topotecan in multiple matrices across a broad concentration range following liposomal topotecan administration provides a complete picture of drug delivery.

Results

Figure 1: Chromatographic Development in Diluted Plasma (50 to 50000 ng/mL)

Comparison of (from left) a plasma blank, lower limit of quantitation sample, upper limit of quantitation sample, and linearity. This comparison shows use of an API 4000 (top row) and API 6500 (bottom row) mass spectrometer and extended dynamic range linearity.

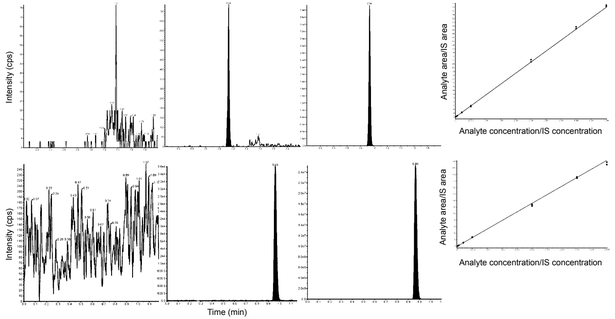


Figure 2: Plasma Ultrafiltrate Method (0.25 to 250 ng/mL)

Same comparison, showing baseline and chromatographic differences plus concentration of the extracts. Analyzed using API 6500. Ultrafiltrate samples required an amended gradient to separate analyte peaks from interferences at the 0.25 ng/mL LOQ. This extended retention time to 1.8 minutes.

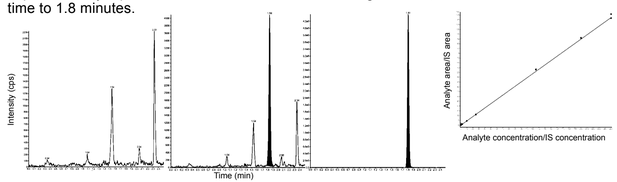


Figure 3: Tissue Homogenate Method (0.025 to 25 ng/mL [0.125 to 12.5 ng/g])

Same comparison, showing baseline and chromatographic differences plus concentration of the extracts. Analyzed using API 6500. Gradient changes were required to separate analyte peaks from baseline interferences in plasma used to homogenize samples at 0.025 ng/mL LOQ. This extended retention time to 2.2 minutes.

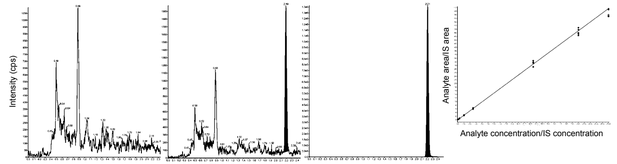
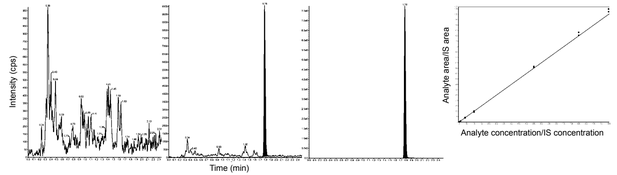


Figure 4: Urine Method (1 to 1000 ng/mL)

Same comparison, showing baseline and chromatographic differences and linearity in the mid-range assay. Analyzed using API 6500. Gradient changes were needed to separate analyte peaks from baseline interferences in urine at 1 ng/mL LOQ.



Conclusions

Interconnected studies have diverse analytical needs. Considering the bigger picture during development of bioanalytical assays allows for a more streamlined approach to the validation (or qualification) and analysis of samples in each method.

Abstract

Introduction

- Liposomal encapsulations for drug compounds preserve compound stability and aid in delivering the drug to a specific target without metabolic alteration.
- Analysis of liposome formulations via liquid chromatography-mass spectrometry has inherent challenges of broad dynamic range requirements coupled with high sensitivity requirements to quantify both total and free drug in samples.
- This work encompasses the analysis of encapsulated and free-form topotecan in human plasma, plasma ultrafiltrate, urine, and human tissue homogenates. Each matrix has individual sensitivity, stability, and sample handling requirements to preserve the stability of both the free and liposomal forms during collection and analysis.
- The use of a plasma surrogate matrix for tissue analysis as well as quantification of liposomal-form samples against free-form drug quality controls was also addressed.

Method

- Sample preparation protocols for collection of blood plasma containing the liposomal formulation and then sub-sampling for either ultrafiltration to quantify free topotecan or acidification to stabilize topotecan in plasma were generated during method development.
- Human tissue samples were collected at clinics and frozen for shipment, then homogenized at the analytical lab prior to extraction.
- Separate protein precipitation extractions were performed on 20 µL of plasma or ultrafiltrate or 50 µL of human tissue homogenate. Urine samples were diluted with acidified buffer for analysis.
- Extracts were submitted independently for liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis with gradient elution on a Waters C_{18} BEH UHPLC column, using a Nexera integrated LC system coupled to either a Sciex API 4000 or API 6500 triple quadrupole mass spectrometer, depending on analytical range requirements.

Results

- A suitable method for LC/MS/MS analysis was developed for each matrix type, and sample handling and extraction conditions were optimized for each matrix as required by their individual stability and analytical range requirements.
- Plasma samples were collected from whole blood and sub-aliquots were taken for ultrafiltrate collection using an Amicon 10 kDa centrifugal filter. Total topotecan in plasma was validated according to GLP standards across a range of 20 to 20000 ng/mL using an API 4000 mass spectrometer.
- Ultrafiltrate was validated to GLP standards for free topotecan in a range of 0.250 to 250 ng/mL using similar precipitation extraction and LC separation conditions but employing an API 6500 mass spectrometer to meet sensitivity requirements.
- The urine assay was quickly developed in a middle range of 1 to 1000 ng/mL by using a high dilution of samples in acidified buffer and was analyzed on the API 6500 mass spectrometer in tandem with the ultrafiltrate assay. Using the more sensitive platform allowed for the most dilute samples to be injected, thereby decreasing matrix effects with a simplified extraction method.
- Human tissue biopsy samples were collected, frozen, and shipped to be homogenized (4:1 v/w) in acidified plasma prior to protein precipitation to stabilize the hydroxy acid form of topotecan. Each tissue type was then analyzed in a qualified method across a range of 0.125 to 12.5 ng/g using a plasma surrogate matrix.
- Quality control plasma samples were generated from liposomal and free-form topotecan and compared in three accuracy and precision runs for viability of quantification of the liposomal form using free drug controls in plasma. Stability assessments of both the free and liposomal forms were performed in blood and plasma. Challenges identified from analyte stability of the free form of topotecan in plasma were overcome by acidification of plasma and temperature control of the extraction conditions.
- Acidification was found to stabilize the free form; however, to minimize the impact on the integrity of the liposomal formulation, separate sample splits were taken and ultrafiltration and stabilization collection protocols were validated to ensure integrity of the liposomal form during ultrafiltration and analysis.

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