Quantification of Etoposide in Human Plasma Using API-4000 LC-MS/MS Systems with Higher Specificity and Lower Background Noise

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Overview

A sensitive and specific liquid chromatographic–tandem mass spectrometric (LC-MS/MS) method capable of quantifying etoposide in human K,E,DTA plasma is described. In this method, the drug was extracted from a 0.1 mL of human plasma using simple liquid–liquid extraction method. Separation was performed on a reverse phase C18 column. Detection was achieved using a ABSciEX API-4000 system in the positive ion mode along with multiple reaction monitoring (MRM). The lower limit of quantification was 1 ng/mL.

This method has been successfully applied to preclinical pharmacokinetic studies.

Introduction

Etoposide is one of the most commonly used antineoplastic agents. Recently, some analytical methods have been developed for pharmacokinetic studies, but most LC-MS/MS methods have issues due to lower specificity, higher background, and poor chromatograms. In this study, the positive ESI MRM mode of API-4000 LC-MS/MS Systems was used to measure etoposide in human K,E,DTA plasma with higher specificity and lower background noise.

Structure

![Etoposide](Image)

Etoposide

Methods

Sample Preparation

Plasma samples (0.1 mL) were extracted using liquid–liquid extraction with MTBE. The extracts were centrifuged, and the supernatant was dried under N2. The reconstituted solution was then transferred into 96-well plate for LC-MS/MS analysis.

Liquid Chromatography:

- **Pump:** Shimadzu UFLC LC-20AD
- **Autosampler:** Shimadzu UFLC SIL-20A
- **System Controller:** Shimadzu CRB-20A
- **Analytical Column:** C18 column, 50 μm, 2.0 mm, 5 μm
- **Gradient:** The analyte was eluted using a gradient of mobile phase A (10 mM ammonium acetate in methanol water (5:95 v/v)) and mobile phase B (0.1% formic acid in methanol) from 25% to 95% mobile phase B in 1.8 min (total run is 4.5 min).

Injection Volume: 5 μL

Mass Spectrometry:

- **MS System:** ABSciEX API-4000
- **Condition:** LC(+)/ESI(+)-MS/MS
- **MRM Transition:** Etoposide: 606.2 → 229.1, Etoposide-d4 (IS): 609.4 → 228.7

Results and Discussion

![Representative chromatogram of Etoposide LLOQ](Image)

Figure 1. Representative chromatogram of Etoposide LLOQ. The upper chromatogram is for Etoposide, the lower chromatogram is for Etoposide-d4 (IS).

![Representative chromatogram of Etoposide control plasma sample (stable stock). The upper chromatogram is for Etoposide, the lower chromatogram is for Etoposide-d4 (IS).](Image)

Figure 2. Representative chromatogram of Etoposide control plasma sample (stable stock). The upper chromatogram is for Etoposide, the lower chromatogram is for Etoposide-d4 (IS).

![Typical Calibration Curve of Etoposide in Human Plasma](Image)

Figure 3. Typical Calibration Curve of Etoposide in Human Plasma

Table 1. Validation Data Summary

<table>
<thead>
<tr>
<th>Condition</th>
<th>Accuracy</th>
<th>RE%</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze/Thaw</td>
<td>3 Cycles, -20°C</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Freeze/Thaw</td>
<td>3 Cycles, -70°C</td>
<td>-13.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Freeze/Thaw</td>
<td>4 h, Room Temperature</td>
<td>-13.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Autosampler Stability</td>
<td>2 Days, Room Temperature</td>
<td>7.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Extract Sample Stability</td>
<td>3 Days, 4°C</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Long-Term Storage Stability</td>
<td>180 Days, ≤-20°C</td>
<td>15.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Long-Term Storage Stability</td>
<td>180 Days, ≤-70°C</td>
<td>15.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

- **Selectivity:** The chromatograms of the LLOQ human plasma samples are shown in Figure 1. Under the LC-MS/MS conditions that were used, it has higher specificity and lower background noise when compared with the control plasma samples (shown in Figure 2).

- **Linearity:** The linear calibration range for etoposide is 1 to 500 ng/mL, in human plasma. A typical calibration curve is shown in Figure 3 with the mean correlation coefficient (r²) of 0.9941.

- **Reproducibility:** The inter-batch accuracy (RE%) and precision (CV%) for all QC samples, including LLOQ, were from -2.0 to -9.0% and from 2.6 to 8.9%, respectively (Table 1). The recovery of etoposide from human plasma is consistent for all QC levels (Table 1) and has no significant matrix effect (Table 1), indicating the assay is very reliable and rugged.

- **Stability:** Etoposide was found to be stable under 3 freeze/thaw cycles (−20 °C and −70 °C), and has excellent short-term stability (bench-top/autosampler/extract sample stability) and long-term stability (138 days) (Table 1).

Conclusions

A simple, specific and sensitive LC-MS/MS assay has been developed and fully validated for etoposide from 0.1 mL human plasma samples with a lower limit of quantitation of 1 ng/mL. This method has been successfully applied to preclinical pharmacokinetic studies.