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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_2EDTA plasma is described.

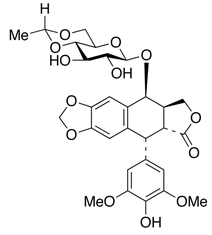
In this method, the drug was extracted from a 0.1 mL of human plasma using simple liquid-liquid extraction method. Separation was performed on a reverse phase C18 column. Detection was achieved using a AB/SCIEX API-4000 system in the positive ion mode along with multiple reaction monitoring (MRM). The lower limit of quantitation 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_2EDTA plasma with higher specificity and lower background noise.

Structure



Etoposide

Methods

Sample Preparation:

Plasma samples (0.1 mL) were extracted using liquid-liquid extraction with MTBE. The extracts were centrifuged, frozen and the supernatant was dried under N_2. 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-20AC $_{HT}$
System Controller:	Shimadzu CBM-20A
Analytical Column:	C18 column, 50 x 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:	AB/Sciex API-4000
Condition:	LC/(+)ESI-MS/MS

MRM Transition:

Etoposide: 606.2 → 229.3

Etoposide-d_3 (IS): 609.4 → 228.7

Results and Discussion

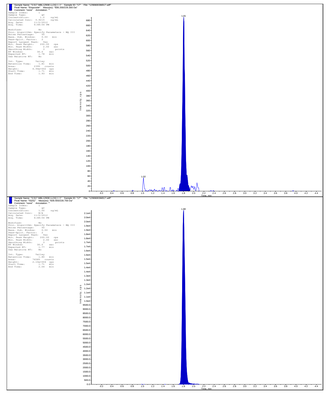


Figure 1. Representative chromatogram of Etoposide LLOQ. The upper chromatogram is for Etoposide, the lower chromatogram is for Etoposide-d_3 (IS).

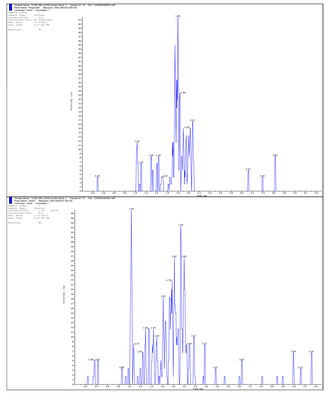


Figure 2. Representative chromatogram of Etoposide control plasma sample (double blank). The upper chromatogram is for Etoposide, the lower chromatogram is for Etoposide-d_3 (IS).

Table 1. Validation Data Summary

Calibration Range			1 to 500 ng/mL	
Correlation coefficient(r^2, mean)			0.9941	
Accuracy & Precision			Accuracy	Precision
	QC	Conc. (ng/mL)	RE%	CV%
Inter-Batch (n=24)	LLOQ	1	-9.0	8.9
	Low	3	-7.0	5.8
	Medium	25	-2.0	4.2
	High	400	-8.3	2.6
Method Recovery	Compared with Nominal Value (%)			
	Low	86.5		
	Medium	81.4		
	High	81.9		
Matrix Effect	IS-Normalized Matrix Factor (MF)			
	Low	1.06		
	Medium	1.03		
	High	1.00		
		Condition		Accuracy RE%
Freeze/Thaw		3 Cycles, <-20 ºC		<9.0
Freeze/Thaw		3 Cycles, <-70 ºC		<13.0
Bench-Top		6 hrs, Room Temperature		<11.3
Autosampler Stability		2 Days, Room Temperature		<7.0
Extract Sample Stability		3 Days, 4ºC		<11.3
Long-Term Storage Stability		138 Days, <-20 ºC		<14.0
Long-Term Storage Stability		138 Days, <-70 ºC		<12.7

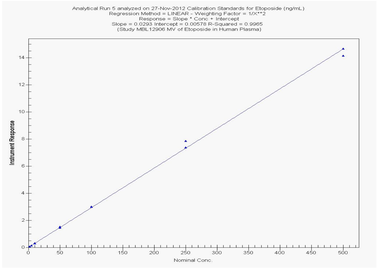


Figure 3. Typical Calibration Curve of Etoposide in Human Plasma

• **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 (r^2) of 0.9941.

•**Reproducibility:** The inter-batch accuracy (RE%) and precision (CV%) for all QC plasma 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 ºC and -70 ºC), 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.