

## LC/MS/MS Method with HILIC Separation for Quantitation of Fosfomycin in Human Plasma

Zhe Sun, Jie Xing and Zhaoqi Zhan

### Introduction

Fosfomycin (FOS) is an old and broad-spectrum antibiotic drug manufactured since 1970s. It is mainly used for treatment of urinary tract infections (UTIs). However, the development of bacterial resistance occurs frequently, making fosfomycin unsuitable for treatment of severe infections in the past. Recently, use of fosfomycin formulations was approved in several countries, because it was found to be active against many multidrug-resistant (MDR) pathogens. Quantification of fosfomycin in human plasma may provide insight into its pharmacokinetics characteristics, which is crucial for current therapy modification [1,2]. Therefore, a reliable analytical method is needed for determination of fosfomycin in biological samples. In this study, a LC/MS/MS method with HILIC chromatography was developed and used for quantification of fosfomycin, a small and highly-hydrophilic antibiotic, in human plasma.

### Experimental

#### Analytical conditions and sample preparation

The standard of fosfomycin was obtained and used in this study. Two pooled human plasma samples were obtained from a commercial supplier. A stock solution of 1000 mg/L fosfomycin in Milli-Q® water was used to prepare calibration standards in blank plasma samples. Racemic fosfomycin-<sup>13</sup>C<sub>3</sub> benzylamine was used as the internal standard. A stock solution of 100 mg/L racemic fosfomycin-<sup>13</sup>C<sub>3</sub> benzylamine was prepared in ammonium acetate (5 mM). Quality control samples (QC) were prepared in the same manner as the calibration standards. Sample pre-treatment was carried out by protein crashing, adding mixed organic solvent (ACN/MeOH, 1:1). The ratio of plasma and solvent mixture was 1:4 (v/v). The crashed plasma was centrifuged for 10min and then filtered using a 0.22-micron nylon filter. The filtered solution was diluted with 5 mM ammonium acetate to obtain standards of various concentrations. This procedure was applied in both pre-spiked and post-spiked samples. A LCMS™-8060 triplequadrupole system and Shim-pack™ GIS HILIC col-umn (150 mm x 3.0 mmI.D., 3 µm) were employed in the study.

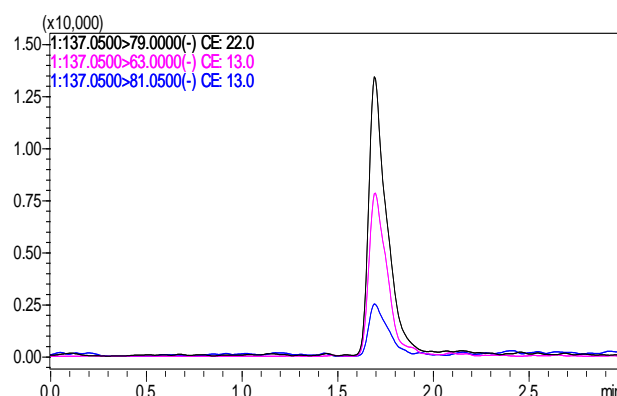
**Table 1.** Analytical conditions of fosfomycin analysis

Column	Shimadzu Shim-pack GIS HILIC Column (150 mm x 3.0 mmI.D., 3µm)
Flow rate	0.3 mL/min
Mobile phase	A: 5 mM ammonium acetate in water B: Acetonitrile
Elution mode	Isocratic, 10% B
Oven temp.	40 °C
Injection vol.	5.0 µL
Interface & temp.	Heated ESI, 300°C
MS mode	MRM (-)
Block temp.	350 °C
DL temp.	250 °C
CID gas	Ar (230 kPa)
Nebulizing gas flow	N <sub>2</sub> , 3 L/min
Drying gas flow	N <sub>2</sub> , 10 L/min
Heating gas flow	Zero air, 10 L/min

### Results and Discussion

#### A. Development of MRM method for fosfomycin in human plasma

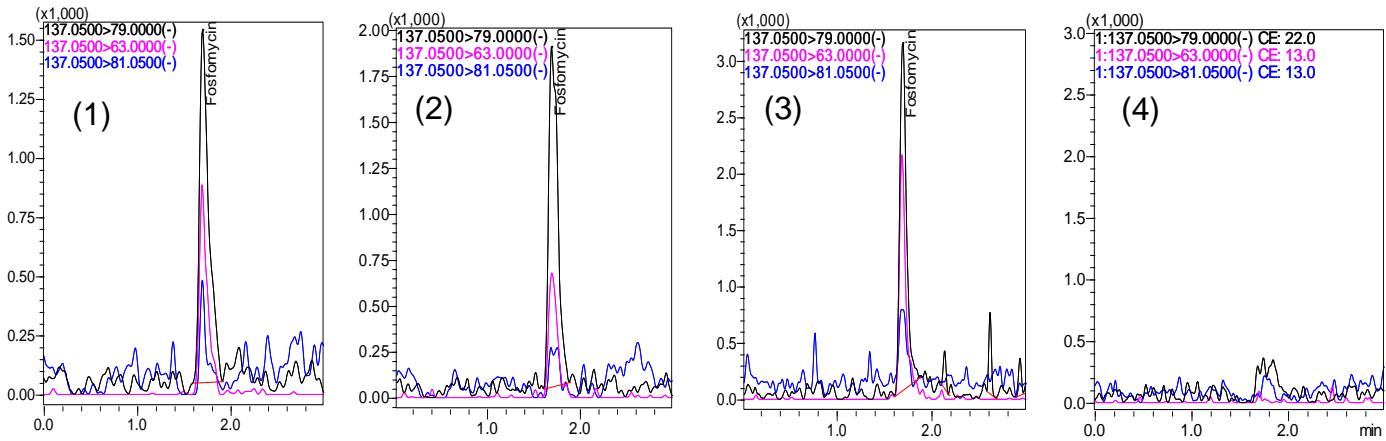
An MRM method in negative mode was developed for quantitative analysis of fosfomycin in human plasma samples. The MRM chromatogram of the fosfomycin pre-spiked plasma standard is shown in the Figure 1.



**Figure 1.** MRM chromatograms of pre-spiked standard of fosfomycin in the human plasma, fosfomycin of 0.2 ppm

**Table 2.** Summary of MRM quantification method for analysis of fosfomycin in plasma on LCMS-8060: calibration range, linearity, accuracy and repeatability (%RSD, area) in two pooled plasmas

Name	RT (min)	MRM (negative)	Calib. range (ppm)	R <sup>2</sup>	Accuracy (%)	%RSD (n=6)		
						Low conc. (0.1 ppm)	Medium conc. (0.4 ppm)	High conc. (2 ppm)
FOS (Plasma 1)	1.70	137.05>79.0	0.02 ~ 6	0.999	93.4	4.2	4.7	2.1
FOS (Plasma 2)	1.72	137.05>79.0	0.02 ~ 6	0.999	97.0	4.7	2.0	0.9



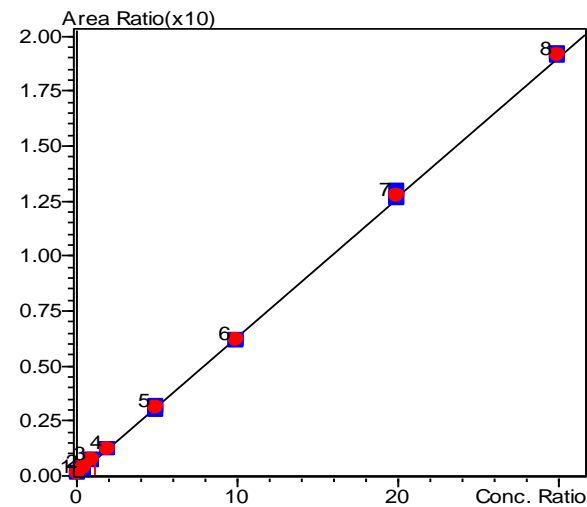
**Figure 2.** MRM chromatograms of fosfomycin in human plasma of 0.02 ppm in (1) pre-spiked, (2) post-spiked and (3) in neat solution. (4) MRM chromatogram of blank plasma sample.

Racemic fosfomycin-<sup>13</sup>C<sub>3</sub> benzylamine was used as internal standard. MRM transition of 137.05>79.0 was selected as the quantifier ion for fosfomycin, and transitions of 137.05>63.0 and 137.05>81.05 were used as reference ions. For the internal standard fosfomycin-<sup>13</sup>C<sub>3</sub>, the MRM transition of 140.0>79.0 was used as the quantifier. A calibrant series of eight concentration levels was prepared by pre-spiked fosfomycin standards in blank human plasma. The concentrations were at 0.02,0.1,0.2,0.4,1,2,4 to 6 ppm, which

correspond to concentrations of fosfomycin of 1,5,10,20,50,100,200 and 300 ppm in the plasma (Table 2). The established calibration curve (Figure 3) was applied for determination of fosfomycin in post-spiked human plasma and the neat fosfomycin standards. QC samples were prepared in the same way as calibration standards.

**B. Performance evaluation for quantitation method of fosfomycin**

**Linearity and LLOQ of MRM quantitation:** The linearity of the plotted calibration curve (R<sup>2</sup>) with IS method was 0.999 for the range from 0.02 ppm to 6.0 ppm. The LLOQ was determined with 0.02 ppm pre-spiked sample, obtaining S/N ≥ 10 and RSD% (n=6) < 8% (Figure 2). A blank plasma prepared following the similar sample preparation procedure without addition of fosfomycin showed no interference peaks for fosfomycin and the internal standard (IS). The repeatability of the method was checked with low, medium and high conc. standards. The %RSD for the peak area (n=6) were calculated to be at 0.9~4.7% (Table 2). In order to investigate the accuracy of the method, QC samples of varying concentrations (0.5 ppm and 3 ppm) were prepared in the same manner as the calibration standards for both sets of plasma samples. The accuracy, deviation and also precision were calculated. The results are summarized in Table 4. All calculated values were within the acceptance criteria of ±15% of the mean concentrations.



**Figure 3.** IS calibration curve of fosfomycin pre-spiked in blank human plasma, eight concentration levels ranging from 0.02 ppm to 6 ppm on LCMS-8060 (IS: 0.2 ppm).

**Table 3.** Evaluation of recovery (%) and matrix effect (%) using various fosfomycin standards spiked in the plasma and neat solution

Sample	Conc. of FOS standard (ppm)	Recovery (%)	Matrix effect (%)
Plasma 1	0.02	81.2	74.8
	0.1	71.0	67.5
	0.2	68.8	65.0
	0.4	60.7	56.2
	1.0	64.9	52.2
	4.0	68.3	59.6
	6.0	68.8	60.7
Plasma 2	0.1	67.1	76.5
	0.4	77.5	70.6
	1.0	64.5	64.4
	6.0	63.8	73.7

### C. Recovery and matrix effect

Three sets of standard samples of fosfomycin, i.e., pre-spiked, post-spiked and neat solution were prepared for investigation of recovery and matrix effect. Two plasma matrixes were used to prepare these samples. For calculation of the recovery (%), the response of the post-spiked standard was compared to that of the pre-spiked standard to obtain the percentage. The results were summarized in Table 3. It can be seen that the recovery for plasma 1 was in the range of 60.7~81.2%, and for plasma 2, it was in the range of 63.8~77.5%. Matrix effect (%) was calculated by comparing the response of the post-spiked standards and that of the neat standards. The results of the matrix effect were shown in the same table. For plasma sample 1, matrix effect was calculated to be 52.2~74.8%, and for plasma 2, the matrix effect was in the range of 64.4~76.5%.

**Table 4.** Accuracy and precision results of QC samples obtained from the two human plasma

Sample	Prepared QC conc. (ppm)	Measured QC conc. (ppm)	Accuracy (%)	%RSD (n=6) (peak area)
QC plasma 1	0.5	0.49	98.4	1.5
	3.0	2.98	99.3	2.5
QC plasma 2	0.5	0.50	100.8	2.4
	3.0	3.07	102.3	3.3

### Conclusions

A fast and sensitive LC/MS/MS method was developed for determination of fosfomycin in human plasma samples. The calibration range used in the method is at range of 0.02 ppm ~ 6 ppm, which correspond to its concentrations of 1 ppm ~ 300 ppm (dilution factor = 50) in plasma. The LLOQ of the method is determined to be 0.02 ppm in solution, which corresponds to the concentration of 1.0 ppm in plasma. Recovery and matrix effect were investigated with pre-spiked, post-spiked and neat standard solution.

### Reference

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2. Fekade Bruck Sime, Adam Johnson, Sarah Whalley, Anahi Santoyo-Castelazo, A. Bruce Montgomery, Kathie Ann Walters, Jeffrey Lipman, William W. Hope, Jason A. Roberts, Antimicrobial Agents and Chemotherapy, Jan. 2017 Vol 61, Issue 1, e01763-16

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