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A Fast and Sensitive LC/MS/MS Method for Quantitation of Fosfomycin in Human Plasma with HILIC Chromatography

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1. Overview

Fosfomycin (FOM) was analysed in human plasma using a HILIC-LC/MS/MS method in MRM mode on LCMS-8060. Racemic fosfomycin-$^{13}C_3$ benzylamine salt was added in the samples as the internal standard. The MRM transitions for quantitation are 137.05>79.0 for fosfomycin and 140.0>79.0 for the internal standard. The developed method is fast and sensitive for quantitation of fosfomycin in biological samples.

2. Introduction

Fosfomycin 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. 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.

3. Experimental

The standard of fosfomycin (in powder form) 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. The stock solution was stored at -20ºC before use. Racemic fosfomycin-$^{13}C_3$ benzylamine as the internal standard, was used in this experiment. A stock solution of 100 mg/L racemic fosfomycin-$^{13}C_3$ benzylamine was prepared in ammonium acetate solution (5 mM). Quality control samples (QC) were prepared in the same manner as the calibration standards. Sample pre-treatment was carried out by a protein crashing procedure, 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 5mM ammonium acetate to obtain standards of various concentrations. This procedure was applied in both pre-spiked and post-spiked samples. Calibration standards were prepared in plasma matrix. A LCMS-8060 triple-quadrupole system and a Shim-pack GIS HILIC column (150x 3.0mm, 3µm) were employed in the study.

Table 1. Analytical conditions of fosfomycin analysis on LCMS-8060

Column	Shimadzu Shim-pack GIS HILIC Column (150 x 3.0 mm, 3µm)	Interface & temp.	Heated ESI, 300ºC
Flow rate	0.3 mL/min	MS mode	MRM (-)
Mobile phase	A: 5 mM ammonium acetate in water B: Acetonitrile	Block temp.	350ºC
		DL temp.	250ºC
		CID gas	Ar (230 kPa)
Elution mode	Isocratic, 10% B	Nebulizing gas flow	N_2, 3 L/min
Oven temp.	40ºC	Drying gas flow	N_2, 10 L/min
Injection vol.	5.0 µL	Heating gas flow	Zero air, 10 L/min

4. Results and Discussion

4.1 Development of MRM method for fosfomycin in human plasma

A MRM method in negative mode was developed for quantitative analysis of fosfomycin in human plasma samples on a triple quadrupole LCMS-8060. The MRM chromatogram of pre-spiked plasma standard and the internal standard are shown in Figure 1. Racemic fosfomycin-13C_3 benzylamine was used as internal standard added to the plasma sample. 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-$^{13}C_3$, the MRM transition of 140.0>79.0 was used as the quantifier. A calibrant series of eight concentration levels were prepared by pre-spiked fosfomycin standards in the blank human plasma. The concentrations of the calibration curve 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 pre-spiked in the plasma (Table 2). The established calibration curve (Figure 2) was applied for determination of fosfomycin in post-spiked human plasma and the neat fosfomycin standards. QC samples were prepared in the same way (pre-spiked) for evaluation of method performance.

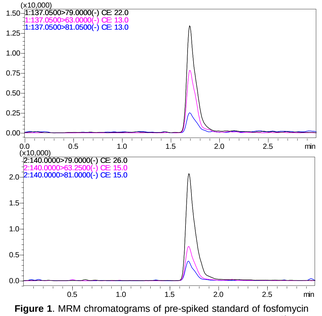

Figure 1. MRM chromatograms of pre-spiked standard of fosfomycin in human plasma, fosfomycin of 0.2 ppm (top) and fosfomycin-$^{13}C_3$ of 0.2 ppm (bottom).

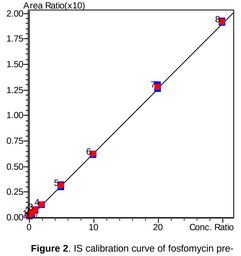

Figure 2. IS calibration curve of fosfomycin pre-spiked in blank human plasma, eight concentration levels ranging from 0.02 ppm to 6ppm on LCMS-8060 (IS: 0.2 ppm).

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^2	Accuracy (%)	%RSD (n=6)		
						Low conc. (0.1ppm)	Medium conc. (0.4 ppm)	High conc. (2 ppm)
FOM (Plasma 1)	1.70	137.05>79.0	0.02 ~ 6	0.999	93.4	4.2	4.7	2.1
FOM (Plasma 2)	1.72	137.05>79.0	0.02 ~ 6	0.999	97.0	4.7	2.0	0.9

4.2 Performance evaluation for quantitation method of fosfomycin

Linearity and LLOQ of MRM quantitation: The linearity of the plotted calibration curve (R^2) 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 3). 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 of the calibration standards for both sets of plasma samples. The accuracy, deviation and also precision were calculated. The results are summarized in Table 3. All calculated values were within the acceptance criteria of ±15% of the mean concentrations.

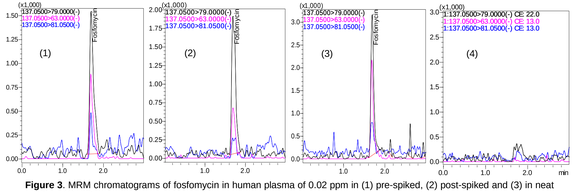

Figure 3. MRM chromatograms of fosfomycin in human plasma of 0.02 ppm in (1) pre-spiked, (2) post-spiked and (3) in neat solution. (4) MRM chromatogram of blank plasma sample.

Table 3. Accuracy, deviation and precision results of QC samples obtained from the two human plasma

Sample	Prepared QC conc. (ppm)	Measured QC conc. (ppm)	Accuracy (%)	Deviation (%)	%RSD (n=6) (peak area)
QC plasma 1	0.5	0.49	98.4	-1.6	1.5
	3.0	2.98	99.3	-0.7	2.5
QC plasma 2	0.5	0.50	100.8	0.8	2.4
	3.0	3.07	102.3	2.3	3.3

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 prepared 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 4. 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. Evaluation of recovery (%) and matrix effect (%) using various fosfomycin standards spiked in the plasma and neat solution

Sample	Conc. of FOM 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

5. Conclusion

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 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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