1. Overview

Fosfomycin (FOM) was analyzed in human plasma using a HILIC-LC/MS/MS method in this study. The fosfomycin (FOM) concentrations were calculated in the samples as the internal standard. The MRM transitions for quantitation are 137.05→79.0 for fosfomycin and 137.05→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 unavailable for treatment of several infections in the past. 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 and be helpful in clinical practice. 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 µg/mL fosfomycin was made by using 10% acetonitrile in water to prepare calibration standards in blank plasma samples. The stock solution was stored at -20°C before use. Racemic fosfomycin-1C, benzylamine as the internal standard, was used in this experiment. A stock solution of 100 µg/mL racemic fosfomycin-1C, 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 precipitation procedure, adding mixed organic solvent (ACN/Methanol, 1:1). The ratio of plasma and solvent mixture was 1:4 (v/v). The centrifuged plasma was filtrated for 10 min and then filtered using a 0.22 µm nylon filter. The filtrated solution was diluted with 5mL 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 LCQ-MS/MS triple-quadrupole system and a Shim-pack GDS column (150*2.0mm, 5µm) was employed in the study.

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 LCQ-MS/MS. The LCQ-MS/MS chromatogram of pre-spiked plasma samples and the internal standard are shown in Figure 1. Racemic fosfomycin-1C, benzylamine was used as internal standard added to the plasma samples. MRM transition of 137.05→79.0 was selected as the quantitation ion for fosfomycin, and transitions of 137.05→63.0 and 137.05→81.0 were used as reference ions. For the internal standard fosfomycin-1C, the MRM transition of 140.0→79.2 was used as the reference ion. A calibration of series eight concentration levels were prepared by pre-spiked fosfomycin standards in the blank human plasma. The concentrations of the calibration curve were 0.020, 0.10, 0.34, 1.2, 4.0, 6.0 ppm, which correspond to concentrations of fosfomycin of 1.0, 5.0, 20, 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 reference fosfomycin standards. QC samples were in prepared in the same way (pre-spiked) for evaluation of method performance.

4.2 Performance evaluation for quantitation method of fosfomycin

Linearity and LLOQ of MRM quantitation:

The linearity of the plotted calibration curve (FF) with 15% method was 0.098 for the range from 0.02 ppm to 6.0 ppm. The LLOQ was determined with 0.02 ppm pre-spiked, selecting S/N>10 and R=1%. The LLOQ was 0.22 ppm (Figure 3). The selected MRM transitions of the precursor preparation procedure without addition of fosfomycin showed no interference peak 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 was below 15% in three levels for each sample (Figure 4). In order to investigate the accuracy of the method, QC samples of varying concentrations (0.1 ppm and 3 ppm) were prepared in the same manner of the calibration standards for both sets of plasma samples. The accuracy, deviation and precision were calculated. The results are summarized in Table 3. All calculated values were within the acceptance criteria of ±15% of the mean concentrations.

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 corresponds to its concentrations of 1 ppm – 300 ppm (matrix 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


Table 1. Accuracy, precision and recovery results of 3 QC samples obtained from the human plasma.

Table 2. Summary of MRM quantitation method for analysis of fosfomycin in plasma on LCQ-MS/MS calibration standards.

Table 3. Summary of MRM quantitation method for analysis of fosfomycin in plasma on LCQ-MS/MS calibration standards.

Table 4. Accuracy, precision and recovery results of 3 QC samples obtained from the human plasma.

Table 5. Summary of MRM quantitation method for analysis of fosfomycin in plasma on LCQ-MS/MS calibration standards.

Table 6. Summary of MRM quantitation method for analysis of fosfomycin in plasma on LCQ-MS/MS calibration standards.

Table 7. Summary of MRM quantitation method for analysis of fosfomycin in plasma on LCQ-MS/MS calibration standards.

Table 8. Summary of MRM quantitation method for analysis of fosfomycin in plasma on LCQ-MS/MS calibration standards.