Title: A simple LC-MS/MS method using HILIC chromatography for the determination of fosfomycin in plasma and urine: application to a pilot pharmacokinetic study in humans

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Fosfomycin

HILIC and LC-MS/MS bioanalysis

pharmacokinetic profile

ICU patients plasma and urine

Graphical Abstract
A simple LC-MS/MS method using HILIC chromatography for the determination of fosfomycin in plasma and urine: application to a pilot pharmacokinetic study in humans.

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Keywords:

Fosfomycin; Antibiotic; LC-MS/MS; Pharmacokinetic; HILIC
Abstract

A high performance liquid chromatography - tandem mass spectrometry (LC-MS/MS) method, using hydrophilic interaction liquid chromatography (HILIC) chromatography for the analysis of fosfomycin in human plasma and urine, has been developed and validated. The plasma method uses a simple protein precipitation using a low volume sample (10 μL) and is suitable for the concentration range of 1 to 2000 μg/mL. The urine method involves a simple dilution of 10 μL of sample and is suitable for a concentration range of 0.1 to 10 mg/mL. The plasma and urine results, reported respectively, are for recovery (68, 72%), inter-assay precision (≤9.1%, ≤8.1%) and accuracy (range -7.2 to 3.3%, -1.9 to 1.6%), LLOQ precision (4.7%, 3.1%) and accuracy (1.7% and 1.2%), and includes investigations into the linearity, stability and matrix effects. The method was used in a pilot pharmacokinetic study of a critically ill patient receiving IV fosfomycin, which measured a maximum and minimum plasma concentration of 222 μg/mL and 172 μg/mL, respectively, after the initial dose, and a maximum and minimum plasma concentration of 868 μg/mL and 591 μg/mL, respectively, after the fifth dose. The urine concentration was 2.03 mg/mL after the initial dose and 0.29 mg/mL after the fifth dose.

HIGHLIGHTS

- A simple and robust LC-MS/MS method for the quantification of fosfomycin in human plasma and urine has been developed.
- The method uses HILIC chromatography that supports a simple treatment of fosfomycin from biological fluids.
- The developed LC–MS/MS method has been validated according to published US FDA guidelines and shows excellent performance.
- Results of a critically ill patient from a pilot pharmacokinetic study with receiving IV fosfomycin are included.
- This is the first method published that is suitable for the quantification of fosfomycin in both plasma and urine.
1. Introduction

Fosfomycin (FOM) is a broad-spectrum antibiotic that is generating substantial interest as an intravenous or enteral therapy for the treatment of multi-drug resistant (MDR) infections [1, 2]. Antibiotic resistance is a significant and immediate global health concern and an increasing prevalence of MDR bacteria is steadily decreasing the number of usable antibiotics available [3]. In this context, fosfomycin represents an important treatment option.

FOM was first discovered in Spain in 1969 and is used as treatment therapy for uncomplicated urinary tract infections [4] and often combined with beta-lactams or aminoglycosides for a synergistic effect against *Pseudomonas aeruginosa* [5, 6]. FOM is structurally unrelated to other antibiotics: it is a small (138 Da), highly hydrophilic phosphonic acid and, with negligible protein binding [7], exhibits excellent penetration into tissue [8]. FOM has a unique mechanism of action by way of its ability to inhibit the synthesis of peptidoglycan found in the inner cell wall of Gram-negative and Gram-positive bacteria [9]. These characteristics support the effectiveness of FOM for the treatment of MDR pathogens and it has been used extensively as a last-line antibiotic for treatment of critically ill patients [2].

Investigating the pharmacokinetic (PK)/pharmacodynamic (PD) characteristics of FOM may enable development of robust dosing strategies that maximize the PD response to treatment while minimizing the potential future development of antibiotic resistance. A reliable method of quantification of FOM in plasma is needed to define the pharmacokinetic profile of the drug and urine data can provide valuable information on elimination rates. The information obtained can be used to characterize the PK/PD relationship.

There are several analytical techniques available for the determination of FOM in biological fluids, using gas chromatography [10-14], liquid chromatography (LC) - spectrophotometric detection [15], LC - photometric detection [16], capillary zone electrophoresis [17, 18], and, more recently, with derivatization and LC -
atmospheric pressure chemical ionization mass spectrometry [19] and LC–tandem mass spectrometry (MS/MS) [20-22].

A method suitable for use in a pharmacokinetic study with patients receiving multiple intravenous doses, up to around 12-24 g/day (as are now being used clinically [2]) would require a lower limit of quantification (LLOQ) of around 1 μg/mL for plasma and 0.1 mg/mL for urine. However, patients with renal insufficiency or with altered pharmacokinetics – as is commonly seen in the critically ill – receiving multiple doses of antibiotics can exhibit very high concentrations in their plasma. This has, therefore, led to an unusually extended concentration range (from 1 to 2000 μg/mL for plasma) being described here, which is supported by the data from the pilot study. While there are many methods currently available for the analysis of FOM in biological fluids, and the method by Li offers a rapid and sensitive alternative for plasma [20], we are unaware of any methods suitable for the analysis of FOM in both plasma and urine that offer the concentration range to meet these clinical specifications at this time.

This paper describes an analytical technique using hydrophilic interaction chromatography (HILIC) – tandem mass spectrometry that offers a simple and reliable determination of FOM in plasma and urine, with a quick and reproducible sample preparation.

2. Experimental

2.1. Chemicals and materials

Fosfomycin (FOM), ethylphosphonic acid (EPA, internal standard) and acetonitrile (HPLC gradient-grade solvent) were purchased from Sigma-Aldrich and ammonium acetate was obtained from Ajax Univar. Ultra-pure water was obtained using a Permutit system. Drug-free human plasma was obtained from the Australian Red Cross Blood Service and drug-free urine was obtained from healthy volunteers.

2.2. Instrumentation and conditions

The LC-MS/MS used is a Shimadzu Nexera UHPLC equipped with a triple quadrupole 8030+ Shimadzu mass spectrometer (MS) detector. An electro-spray ionization (ESI)
The source interface operating in negative-ion mode was used for the multiple reaction monitoring (MRM) LC-MS/MS analysis. MS conditions for FOM and the internal standard (IS; EPA) are reported in Table 1. The interface settings consisted of the nebulizing gas flow of 3 L/min, a de-solvation line temperature of 250°C, heat block temperature of 400°C and a drying gas flow of 15 L/min.

The compounds were separated on a Merck SeQuant zic-HILIC, 2.1 x 50 mm, 5.0 μm analytical column (operated at 24°C) protected by a 20 mm SeQuant zic-HILIC guard cartridge using an isocratic mobile phase containing acetonitrile with 2 mM ammonium acetate, pH 4.8 (85/15 v/v) at a flow rate of 0.3 mL/min. The injection volumes used were 0.1 μL for the plasma assay and 0.5 μL for the urine assay. The retention time for both FOM and EPA was 2.5 min.

2.3. Stock and standard solution preparation

2.3.1. Standards for plasma analysis

Aqueous stock solutions for plasma standard preparation (at 1, 2 and 10 mg/mL) were stored at -80°C. On the day of assay these were diluted with drug free plasma to yield calibration standards from 1 to 2000 μg/mL that was processed alongside the clinical samples.

2.3.2. Standards for urine analysis

Aqueous stock solutions for urine standards of FOM (2, 5, 10, and 50 mg/mL) were stored at -20°C. On the day of assay these were diluted with drug free urine to prepare calibration standards from 0.1 to 10 mg/mL that were processed alongside the clinical samples.

2.3.3. Internal standard solution

Ethylphosphonic acid (EPA) in acetonitrile was used as internal standard for the plasma assay (10 μg/mL), and an aqueous EPA solution was used as internal standard for the urine assay (1 mg/mL). Solutions were stored at -20°C.
2.3.4. Quality Controls

Quality controls were prepared by spiking drug free plasma with FOM to concentrations of 3, 800 and 1600 µg/mL, and stored at -80°C until assay. On the day of assay an additional QC at 80 µg/mL was prepared by diluting with blank plasma the QC at 800 µg/mL. The four sets of QCs were assayed alongside clinical samples.

Quality controls for urine analysis were prepared at FOM concentrations of 0.3, 2 and 8 mg/mL. The urine QCS were stored at -20°C until assayed alongside clinical samples.

2.4. Analytical procedure

2.4.1. Clinical plasma sample preparation

Clinical samples were prepared by combining 10 µL of clinical sample, 10 µL of water, and 90 µL of drug-free blank plasma. Internal standard (300 µL, 10 µg/mL of EPA in acetonitrile) was then added and the sample vortexed and then centrifuged (for 5 min at 14,000 rpm) to remove precipitated proteins. A volume of 0.1 µL was injected onto the LC-MS/MS system.

2.4.2. Clinical urine sample preparation

All urine samples were filtered using a 0.22 µm filter prior to use. Clinical samples were prepared by combining 10 µL of sample with 10 µL water, followed by internal standard (20 µL, 1 mg/mL of EPA). The sample was then diluted with 200 µL of mobile phase and 0.5 µL was injected into the LC-MS/MS system.

2.4.3. Data analysis

For both plasma and urine the concentration of each clinical sample was obtained using the data from the calibration curve prepared (in either plasma or urine) within the batch using a quadratic regression with peak-area ratio (drug/internal standard area responses) against concentration (x), with a 1/x² weighting as the mathematical basis of the quantification.
2.5. Validation

The validation was performed in accordance with the guidelines provided by the US Food and Drug Administration (USFDA) and met the criteria required to demonstrate the method is suitable for intended purpose [23]. The validation for both plasma and urine was assessed for matrix effects, lower limit of quantification (LLOQ), linearity, inter-day precision and accuracy, freeze-thaw stability of quality control samples and the stability of standard solutions.

2.5.1. Limit of quantification

The lower limits of quantification for FOM were evaluated by analysis of replicate standards, for both plasma and for urine samples.

2.5.2. Linearity

To investigate linearity, calibration curves were prepared using the corresponding concentration ranges suitable for each matrix.

2.5.3. Inter-day precision and accuracy

Precision and accuracy for FOM throughout the calibration range of both plasma and urine was evaluated by the analysis of QC samples at four different concentrations for plasma and three different concentrations for urine with the QC concentrations determined against freshly prepared standard curves. In addition to precision and accuracy data obtained from QC samples, an incurred sample reanalysis was performed.

Acceptance criteria were applied according to the US Food and Drug Administration guidelines [23]; with acceptance criteria on an incurred sample reanalysis applied according to the European Medicines Agency guidelines [24].

2.5.4. Matrix effects

Plasma matrix effects were evaluated to identify any suppression or enhancement of signal from an interfering substance around the retention time of FOM and EPA by using the matrix factor test. Five blank plasma samples were assayed at spiked low and high concentration levels and with internal standard, and the area results
compared to those produced following the same extraction procedure using water
instead of plasma. The precision of the matrix factor (normalized against the internal
standard) was used to determine if any concentration level demonstrated a trend of
variation from the expected result.

Five urine blanks were assayed at a spiked low and high concentration level and the
precision and accuracy calculated, with respect to the nominal concentrations, to
determine if any concentration level demonstrated a trend of variation from the
expected result.

2.5.5. Recovery

The recovery of FOM and EPA was evaluated by comparing the peak area for plasma
or urine samples spiked prior to protein precipitation (for plasma) or dilution (for
urine) with samples spiked after protein precipitation or dilution. Care was taken to
ensure the injection matrix was identical in comparable samples.

2.5.6. Stability

Stability of FOM in plasma and urine was assessed across three freeze-thaw cycles
(from -80°C to ambient temperature) using three replicates of the QC samples at
low, medium and high concentrations. Stability of stock solutions was assessed
comparing aqueous solutions stored at both -20°C and -80°C to freshly prepared
solutions.

2.6. Pharmacokinetic application

The method was developed for the analysis of plasma and urine samples from a
pharmacokinetic study with critically ill patients receiving an intravenous dose of 6 g
of FOM every 6 hours with expected peak plasma concentrations of around 200
µg/mL, an expected plasma half-life of 2 h, and urinary concentrations of around 5
mg/mL [25].

One critically ill patient was administered an intravenous dose of 6 g fosfomycin
disodium. Blood samples (3 mL) were taken prior to dosing (0 h) and 0.5, 0.75, 1, 1.5,
2, 4, and 6 h post administration using heparinized vacuum tubes (Greiner Bio-One,
Vacuette® LiHep) on the first day of FOM administration and after receiving the fifth
Blood samples were centrifuged at 3000 rpm for 10 min to obtain plasma samples. Plasma samples were transferred into 2 mL polypropylene tubes, capped and stored at -80°C until analysis.

Similarly, a urine sample was collected 6 h post administration of the FOM dose. The urine was transferred into a urine specimen vial, capped and stored at -80°C until analysis.

This procedure was conducted in accordance with the principles laid down by the ICH guidelines for Good Clinical Practice and approved by the University of Queensland Medical Research Review Committee (clearance # 2012000870) and the Epistimoniko Symvouleio (Scientific Committee) of Attikon University Hospital (approval MEΘ-84/13-3-12).

3. Results and discussion

3.1. Chromatography

This method has been established using HILIC technology which offers excellent selectivity for polar hydrophilic compounds like FOM, and the use of a high organic-solvent content in the mobile phase leads to a rapid evaporation of the solvent during electrospray ionization \[26\], endowing the method with a simple compatibility with mass spectrometry. Additionally, the use of HILIC technology in this method obviates the requirement for further modification of the sample, after a solvent-based protein precipitation, to closely correspond with the organic content of the mobile phase for improved peak shape. This simplicity of extraction leads to low detection levels when using low sample volumes.

Retention of the analyte by the stationary phase is caused by hydrophilic partitioning within an aqueous-enriched liquid layer and/or with the positive or negative charges on the HILIC stationary phase \[27\]; the balance of the partition is provided by the aqueous content and pH of the mobile phase. Therefore, the aqueous layer is critical to the efficiency of HILIC separation. Published HILIC methodology often recommends mobile phases consisting of high ionic strength (from 5 – 20 mM, with upper limits of 200-300 mM) \[28\] but this presented difficulties in development. The
use of mobile phases with high ionic strength of buffers, low and variable aqueous content, combined with the high pressure applied in an HPLC system led to blocking of the column and the capillary in the ionization source on the mass spectrometer. This isocratic method uses a low ionic strength of 2 mM ammonium acetate buffer in 85% acetonitrile which has been reliable and provided consistent results with minimal loss to chromatographic shape and reproducibility. Regenerating the column after every 300–400 injections of samples, particularly the urine samples, and keeping buffer concentration to as low an ionic strength as possible, was advantageous to long term use. Using a guard column extended the column life but as the separation is highly dependent on the salt concentration and its impact on the stationary phases aqueous-rich layer, the regeneration of the column was critical to maintaining the quality of chromatography.

Another aspect of the method development that was found to affect both the chromatographic retention and peak shape was the injection volume and the composition of the sample being injected. Despite the low injection volumes being used, 0.1 μL for plasma and 0.5 μL for urine, we conclude that the changes in the quality of the chromatography observed were due to the sensitivity of the interaction of FOM with the stationary phase from changes in ionic strength and pH of the buffer [26, 27]. For the development of a bio-analytical assay using HILIC chromatography, consideration of the organic to aqueous ratio, concentration of salts, and finally, the presence of acids or base, is required. A dilution with plasma was used in the plasma method as the ratio of acetonitrile to aqueous concentration had an impact on the quality of the chromatographic peak shape and retention; the concentrations found in clinical samples from the pilot study allowed this dilution. The urine method included a dilution of sample that improved the chromatographic separation by either reducing the presence of endogenous salts in the sample or controlling the pH.

Buszewski [26] and Alpert describe the mechanism of HILIC separation as being based on an interplay of a partitioning equilibrium in the aqueous layer (based on the hydrophilicity of the analyte), weak electrostatic mechanisms, and dipole-dipole interactions (including hydrogen-bonding) [29] the impact of each parameter on the
selectivity and reproducibility of chromatography requires a more sophisticated management than in general reversed-phase chromatography, but which once overcome can lead to a highly stable and robust method.

3.2. Validation

The LLOQ was determined as 1 μg/mL for plasma and 0.1 mg/mL urine with precision calculated as 4.7 and 3.1%, respectively, and accuracy calculated as 1.7 and 1.2%, respectively (Table 2). The signal to noise ratio of the lowest standard in the calibration curve was 23.2 for plasma and 178 for urine and this data, combined with the excellent precision and accuracy obtained at 1 μg/mL for plasma and 0.1 mg/mL for urine, suggests substantial scope for achieving a lower LLOQ for both matrices (see Figure 2 and 3 for a representative chromatogram of the LLOQ standard extracted from plasma and urine, respectively). The lower limit of detection (LLOD) is defined as being reliably distinguished from the background noise and calculated as ≥ three-times the noise of the blank plasma sample. From the validation the LLOD was determined as being approximately 0.01 μg/mL for plasma and <0.01 mg/mL for urine.

A regression model with a weighted (1/x²) quadratic curve provided the lowest distribution of error across the substantial concentration range (1 to 2000 μg/mL for plasma and 0.1 to 10 mg/mL for urine). The results of the linearity study are described in Table 3.

Precision and accuracy of the QC samples are shown in Table 4 for both plasma and urine. All results were within the acceptance criteria of ± 15% of the nominal concentration, indeed the results of all plasma QCs samples were within 9.1% and urine within 4.2%. An incurred sample reanalysis was performed on a subset of clinical samples and the results meet the current guidelines [23, 24] with >67% of repeated results being within 30% of the mean. Indeed, 100% of the repeated results were within 11%.

No signal suppression/enhancement was evident for either FOM or the internal standard from the matrix study performed. The matrix effect evaluation is reported in Table 5.
Despite using a very simple protein-precipitation for the extraction of FOM from plasma the recovery was somewhat low at 68%. However, this extraction recovery is not atypical for a drug with a highly hydrophilic nature due to preferential aqueous partitioning. As was seen from the LLOQ testing, the variability was reliable (precision 6.1%) and sensitivity (LLOD ca. 0.01 μg/mL) easily achievable. The recovery of the internal standard EPA was good at 72% when tested at the undiluted concentration of 10 μg/mL. The urine preparation was a simple dilution with internal standard and as such provided recoveries of 98% when tested at 0.4 mg/mL. The recovery results are reported in Table 5.

Stock solution stability for FOM was tested for aqueous solutions stored for over 16 months at -80°C and for over 11 months at -20°C and it was found to be stable. FOM was also found to be stable in plasma and urine across three freeze-thaw cycles when stored at -80°C and thawed at ambient temperature in water (see Table 5).

Overall, the validation of this method was highly successful for both plasma and urine with the method showing an excellent degree of reproducibility and accuracy, and is suitable for use in the analysis of patient samples in a pharmacokinetic study. This technique offers a simple and robust method for the analysis of FOM in both plasma and urine in patient samples. Other quantitative methods have been described for the determination of FOM in serum or plasma [10, 12, 14, 15, 20, 22] and urine [11]. However, these methods often require a significant amount of time in sample preparation or technique, and none offer a chromatographic system suitable for a pharmacokinetic study of FOM in both plasma and urine.

3.3. Pharmacokinetic analysis

The plasma concentration-time profile obtained in the pilot pharmacokinetic study is shown in Figure 4. The peak plasma concentration in this patient after receiving the initial dose was 222 μg/mL, and the trough concentration was 141 μg/mL. Increased plasma concentrations were observed after receiving the fifth dose of FOM, with the peak plasma concentration recorded as 868 μg/mL, and the trough concentration was 592 μg/mL. The urinary concentration determined from a 6 h sample taken
post-dose and was 2.03 mg/mL after the initial dose and 0.29 mg/mL after the fifth
dose of FOM.

4. Conclusion

The developed analytical method is a sensitive, simple and robust tool to analyse
FOM in plasma and urine of patients. With the increasing prevalence of MDR
organisms and the reduced effectiveness of currently available antibiotics this
method allows the opportunity to study the disposition of FOM, particularly in at-risk
patient groups. This research may improve dosing strategies which could minimize
the risk of increasing resistance and bring an effective antibiotic back into the hands
of treating physicians.
Table 1. MS conditions for FOM and EPA

<table>
<thead>
<tr>
<th>MS</th>
<th>FOM</th>
<th>EPA</th>
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</thead>
<tbody>
<tr>
<td>Product Ion</td>
<td>137.1 (M.H-)</td>
<td>109.1 (M.H-)</td>
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<tr>
<td>Daughter Ion</td>
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<td>78.9</td>
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<tr>
<td>Dwell Time (ms)</td>
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<td>100</td>
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<tr>
<td>Q1 (V)</td>
<td>+14</td>
<td>+10</td>
</tr>
<tr>
<td>Q3 (V)</td>
<td>+28</td>
<td>+13</td>
</tr>
<tr>
<td>Collision Energy (V)</td>
<td>+25</td>
<td>+20</td>
</tr>
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</table>

Table 2: Lower limit of quantification

<table>
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<th>Matrix</th>
<th>Mean</th>
<th>Precision (%)</th>
<th>Accuracy (%)</th>
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</thead>
<tbody>
<tr>
<td>Plasma (n = 13)</td>
<td>1.02 µg/mL</td>
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<td>+1.7</td>
</tr>
<tr>
<td>Urine (n = 7)</td>
<td>0.10 mg/mL</td>
<td>±3.1</td>
<td>+1.2</td>
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</table>

Table 3: Linearity analysis

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Calibration Range</th>
<th>Correlation Coefficient (Mean)</th>
<th>Maximum deviation* (%)</th>
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<tbody>
<tr>
<td>Plasma (n=12)</td>
<td>1 to 2000 µg/mL</td>
<td>0.9963</td>
<td>-14.0</td>
</tr>
<tr>
<td>Urine (n=5)</td>
<td>0.1 to 10 mg/mL</td>
<td>0.9959</td>
<td>+10.5</td>
</tr>
</tbody>
</table>

* Reported maximum deviation from nominal (%) across all standard curves and all concentration levels.
Table 4: Inter-assay Precision and Accuracy

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Concentration</th>
<th>Replicates</th>
<th>Mean</th>
<th>Accuracy (%)</th>
<th>Precision (%)</th>
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<tbody>
<tr>
<td>Plasma</td>
<td>3 μg/mL</td>
<td>22</td>
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<tr>
<td></td>
<td>80 μg/mL</td>
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<td>78.0</td>
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<tr>
<td></td>
<td>800 μg/mL</td>
<td>22</td>
<td>826</td>
<td>3.3</td>
<td>4.3</td>
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<tr>
<td></td>
<td>1600 μg/mL</td>
<td>23</td>
<td>1486</td>
<td>-7.2</td>
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<tr>
<td>Urine</td>
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<td>9</td>
<td>0.30</td>
<td>0.0</td>
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<tr>
<td></td>
<td>2 mg/mL</td>
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<tr>
<td></td>
<td>8 mg/mL</td>
<td>9</td>
<td>7.9</td>
<td>-1.9</td>
<td>2.3</td>
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Table 5: Matrix, recovery and freeze-thaw stability studies

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<tr>
<th>Study</th>
<th>Matrix</th>
<th>Concentration</th>
<th>Mean</th>
<th>Accuracy (%)</th>
<th>Precision (%)</th>
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<tr>
<td>Matrix</td>
<td>Plasma</td>
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<tr>
<td></td>
<td>Urine</td>
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<td>0.189</td>
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<td>7.2</td>
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<tr>
<td></td>
<td></td>
<td>5 mg/mL</td>
<td>5.36</td>
<td>-5.4</td>
<td>7.1</td>
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<tr>
<td>Recovery</td>
<td>Plasma</td>
<td>80 μg/mL</td>
<td>68</td>
<td>7.7</td>
<td></td>
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<tr>
<td></td>
<td>Urine</td>
<td>10 μg/mL</td>
<td>98</td>
<td>4.2</td>
<td></td>
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<tr>
<td>Stability (freeze-thaw)</td>
<td>Plasma</td>
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<td>4.0</td>
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<tr>
<td></td>
<td></td>
<td>5 μg/mL</td>
<td>4.8</td>
<td>-3.1</td>
<td>5.4</td>
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<tr>
<td></td>
<td></td>
<td>80 μg/mL</td>
<td>86</td>
<td>7.2</td>
<td>5.8</td>
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<tr>
<td></td>
<td>Urine</td>
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<td>0.9</td>
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<td>2.1 mg/mL</td>
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<td></td>
<td></td>
<td>7.8 mg/mL</td>
<td>8.2</td>
<td>4.4</td>
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</tbody>
</table>

*a matrix factor: calculated as a ratio of peak area of FOM in the presence of matrix to the peak area in the absence of matrix (normalized using the internal standard).
References


Figure 1: Structure of fosfomycin (FOM, left) and the internal standard, ethylphosphonic acid (EPA, right), [29,30].
Figure 2: Chromatograms of a blank sample (top) and the LLOQ (1 µg/mL) plasma standard (FOM, centre; EPA, bottom).
Figure 3: Chromatograms of blank sample (top) and the LLOQ (0.1 mg/mL) urine standard (FOM, centre; EPA, bottom)
Figure 4: Plasma concentration – time profiles of FOM in a critically ill patient receiving a 6 g FOM IV dose every 6 hours, for the first and fifth doses.