A fast and sensitive LC-MS/MS method for the quantification of fosfomycin in human urine and plasma using one sample preparation method and HILIC chromatography

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ABSTRACT

Fosfomycin is an old antibiotic that is increasingly prescribed because of emergence of the antibiotic resistance and the growing incidence of multi-drug resistant infections. Surprisingly, little is known about its pharmacokinetics (PK) and the pharmacodynamics (PD). Quantification of fosfomycin in both urine and plasma provides insight into the PK/PD characteristics of fosfomycin, which is crucial for the optimization of the therapy and the prevention of the emergence of resistance. An analytical method is therefore needed for the quantification of fosfomycin in both urine and plasma. A fast and sensitive tandem mass spectrometry method in combination with HILIC chromatography for the quantification of fosfomycin with a universal sample preparation method for urine and plasma was developed and validated according to FDA guidelines. The universal sample preparation method only requires 100 µL of a sample, the addition of the internal standard fosfomycin-$13C_3$ benzylamine and an ultrafiltration step. The method is applicable for the concentration range of 0.75 to 375 mg/L (R^2 of 0.9998 in both matrices) encompassing the clinically relevant concentration range based on the susceptibility of possible (uro)pathogens in the clinical setting. The validation results for urine and plasma for all QC levels, were <2.1% and <3.2% for accuracy, <1.5% and <1.7% for within day precision and <5.0% and<3.8% for between day precision, respectively. No matrix effects were encountered and the total recovery in urine and plasma was high (102.5% and 99.4%). Prepared samples were stable at 4°C and 15°C for at least 72 hours and stored samples at -80°C were stable for at least 6 months. Selectivity and sensitivity were confirmed and no carry-over was observed. The method was successfully applied in two pharmacokinetic studies in healthy volunteers and patients respectively.

Keywords

Fosfomycin; Pharmacokinetics/Pharmacodynamics; UPLC-MS/MS; HILIC; Antibiotics

INTRODUCTION

In an era of emerging drug resistance and lack of new antibiotics, old off-patent antibiotics are increasingly being prescribed. Oral fosfomycin has gained more attention as an alternative, or even as first line treatment, for uncomplicated urinary tract infections (UTIs) caused by extended spectrum beta lactamase (ESBL)-producing bacteria (1–3). Oral fosfomycin is also used for the treatment of complicated urinary tract infection in some countries and has been used as prophylactic therapy in prostate resection procedures. Fosfomycin remains active against many multidrug-resistant (MDR) pathogens (4–6). For this reason, intravenous (IV) fosfomycin is now given a more prominent role in the treatment of (critically ill) patients due to the higher prevalence of MDR pathogens (7). The IV formulation of fosfomycin was recently approved in several countries worldwide, thereby it is expected that the use of this administration form will increase over the next few years. Fosfomycin, discovered in the late 1960s, is an old antibiotic agent, specifically suited to the treatment of UTIs (4). The chemical structure of fosfomycin (figure 1a) is unique and not related to any other antibiotic drug: it is small (138 Dalton) and highly hydrophilic.

Urinary or plasma concentrations (PK) directly influence the kill-rate of the (uro) pathogen in vitro and hereby the effectivity of the antibiotic treatment (8). Since resistance rates have dramatically increased over the last few years (9), it is important to investigate the pharmacokinetics of fosfomycin in order to optimize the treatment response (PD), minimize the duration of treatment and minimize the risk of the development of resistant pathogens (10).

Several methods for the quantification of fosfomycin in urine and/or plasma have been developed during the last years. These vary from an older microbiological assay (11), gas chromatography methods (12, 13), a flow injection spectrophotometric method (14) and ion exchange chromatography (15) to the more sophisticated method as high performance liquid chromatography tandem mass spectrometry (HPLC-MS) (16), LC-MS combined with atmospheric pressure chemical ionization (17) and Hydrophilic Interaction Liquid Chromatography (HILIC) (18). They were all successfully validated, but lack the ability to quantitate fosfomycin in the lower range of the clinically relevant concentrations, relating to the wild-type distribution of (uro)pathogens, with a minimal inhibitory concentration (MIC) less than (or equal to) 8 mg/L (19). The MIC is a measure for the susceptibility of the pathogen to fosfomycin. Therefore, concentrations from 1 to 256 mg/L in both urine and plasma should be able to be quantified (19). Earlier published methods using LC-MS/MS are lacking this sensitivity (16, 18). Also, retention times of these methods are long which indicates a longer runtime (8-10 minutes). Only two of these methods are applicable for both urine and plasma samples (12, 18), but they use different sample preparation methods for both matrices. The aim of this study

was to develop a sensitive and rapid ultra performance LC-MS/MS method with HILIC chromatography for the quantification of fosfomycin in urine and plasma.

EXPERIMENTAL

Chemicals and reagents

Fosfomycin was purchased from Santa Cruz Biotechnology Inc. (Huissen, the Netherlands, purity >98%) and racemic fosfomycin-13C_3 benzylamine salt, which was used as the internal standard, was purchased from Toronto Research Chemicals (North York, Canada, purity 96%). Acetonitrile and methanol were both purchased from Biosolve BV (Valkenswaard, the Netherlands) and were of LC-MS quality. Ammonium formate was purchased from Sigma-Aldrich (Zwijndrecht, the Netherlands). The water was purified using a Milli-Q Ultrapure Water System (Merck Millipore, Darmstadt, Germany). Antibiotic free urine was donated just prior to analysis by five healthy volunteers without history of antibiotic use including fosfomycin over the past four weeks. Blank plasma was obtained from drug-free volunteers who donated blood in the blood donation center (Sanquin, Rotterdam, the Netherlands). After donation the blood was centrifuged and plasma was pooled and stored at -18°C prior to analysis.

Solutions

A stock solution of 10000 mg/L fosfomycin disodium salt in Milli-Q Ultrapure water was used to prepare calibration standards in blank urine or plasma at eight concentration levels between 3.75 and 375 mg/L. The stock solution was stored at -20°C. Quality control samples were prepared in the same manner as the calibration standards at concentrations of 7.5 mg/L (QC low (L)), 115 mg/L (QC medium (M)) and 335 mg/L (QC high (H)). Also, a lower limit of quantification (LLOQ) standard was prepared in a concentration of 0.75 mg/L. 100 µL of each standard or quality control sample was transferred to a 1.5 mL safe-lock Eppendorf tube and stored at -80°C until to analysis.

A stock solution of 100 mg/L racemic fosfomycin-13C_3 benzylamine salt was prepared in an ammonium formate/ultrapure water solution (pH 7; 4 mM).This ammonium formate solution was also used in the preparation of the mobile phase (see section 2.4). The stock solution of the internal standard was stored in a refrigerator at 2-8°C and was brought to room temperature before use.

Instruments

The equipment used was a Dionex Ultimate UPLC system which was connected to a triple Quadrupole mass spectrometer with a Heated Electrospray Ionization-probe operating in the negative mode (Thermo Scientific, Waltham, MA). A spray voltage of

4000 kV with a capillary temperature of 250°C and a vaporizer temperature of 400°C were used to produce the parent ions with a mass/charge (m/z) ratio of 137.040 for fosfomycin and 137.021 for the internal standard. With nitrogen used as sheet gas and auxiliary gas, and collision gas pressure of 1.5 mTorr, the product ions with m/z=79.170 for fosfomycin and m/z=79.171 for the internal standard were produced. The fragmentation energies were respectively 26 eV (S-lens of 10 V) and 41 eV (S-lens of 76 V). The UPLC system consisted of a UPLC-pump, an auto sampler with flow through needle injection and a column compartment (all RS 3000 Ultimate). The software programs Chromeleon 6.80 (Dionex, Thermo Scientific), LCquan 2.6.1.32 (Thermo Scientific) and Xcalibur 2.1 (Thermo Scientific) were used for data processing.

LC-MS/MS conditions

A HILIC column (2.1 x 100 mm Acquity UPLC BEH Amide 1.7 µm, Waters, Etten-Leur, the Netherlands), operating at 40°C was used to perform the chromatographic separation. An isocratic mobile phase containing a mixture of the previous described ammonium formate solution in ultrapure water (pH 7; 4 mM) and acetonitrile (20:80, v/v) at a flow rate of 0.4 mL/min. The retention time for both components was 1.8 minutes. The column was intensively preconditioned with the mobile phase for stabilization prior to the analysis.

Sample preparation

The following sample preparation method was applicable to both urine and plasma samples: 100 µL of a sample and the same volume of the internal standard solution were added together and mixed on a shaker for 10 seconds. The mixture was transferred to an ultrafilter tube (Amicon Ultra 0.5 ml Ultracel 10k, Millipore) and then centrifuged at 16.100 g for 5 minutes. Ultrafiltration is a method to determine the free, protein unbound fraction of a drug. 50 µL of the filtrate was mixed with 200 µL of acetonitrile in an auto sampler insert vial (snap ring vial, 32 x 11.6 mm with integrated 0.2 ml glass micro-insert, VWR). 2 µL for urine or 4 µL for plasma was injected into the LC-MS/MS system.

Analytical validation

The following validation parameters were investigated, according to the US Food and Drug Administration guidelines for bio analytical method validations (20):

Linearity

To investigate the linearity of the method, eight calibration standards (table 1) were prepared together with two blank samples (n=2 per concentration). The responses, defined as the ratio between the response of fosfomycin and the internal standard,

were plotted against the theoretical sample concentrations. The determination coefficient (R^2) was calculated and had to be at least 0.995.

Limits of quantification and detection

To assess the ability of the method to quantitate the LLOQ precise and accurate, six replicates of the LLOQ standard (table 1) were prepared where after the mean and the standard deviation of the response ratios were calculated as well as the accuracy and precision. The LLOQ concentration should be below the lowest calibration standard (standard 1), the accuracy between 80%-120% and the precision ≤20%. The lower limit of detection (LOD) was determined by the measurement of five blank samples . The LOD was defined as the mean response plus three times the standard deviation (SD)..

Table 1: Concentrations of eight calibration standards (S1-S8), quality control samples (QC-L, QC-M and QC-H) and the LLOQ standard. The concentrations are the same in both matrices. Standard 3 and 6 are used during routine analysis to prepare the calibration line.

Calibrations standards (mg/L)								Internal quality control samples (mg/L)			
S1	S2	S3	S4	S5	S6	S7	S8	QC-L	QC-M	QC-H	LLOQ
5	15	35	75	150	225	300	375	7.50	110	335	0.75

Accuracy and Precision

Six QC samples of each level (table 1) were prepared in order to investigate the accuracy of the method. The deviation of the measured concentrations compared to the theoretical concentration was calculated. Two types of precision were investigated: the within-day precision by analyzing the 18 QC samples which were also used for the accuracy and the between-day precision by analyzing two QC samples of each level on six different days. For accuracy as well as the precision, the measured concentrations should be within the acceptance criteria of ±15% of the nominal concentration.

Matrix variability and Recovery

The method of Matuszewski et al. was used to investigate the presence of matrix effects (21). Human urine and plasma were collected from five different sources. QC samples of the highest and the lowest concentration (table 1) were prepared in the six different matrices together with blank samples. In total, three sets (A, B and C) of samples were prepared (QC-L,QC- H and blanks). In set B, fosfomycin was added after the ultrafiltration step during sample preparation. These samples were prepared in the five different sources of urine or plasma (2 samples per source; 30 samples in total). The samples for set C, also consisting of 30 samples, were prepared with the standard sample preparation method and the last set (A) was prepared without using urine of plasma. These samples were prepared in ultrapure water (6 samples in total).

All samples were measured with the standard analysis and the responses were normalized for response of the internal standard. Matrix effects were defined as the ratio of the response from the samples in set B and the samples from set A. Recovery was defined as the ratio of the response from the samples in set C and the samples from set A. Process efficiency is the product of the matrix effects and the recovery. Matrix effects, recovery and process efficiency are expressed as a percentage which should be between 80% and 120%. Deviation of the measured concentrations and the theoretical concentrations of the QC samples was required to be ≤15% in all cases.

Stability

To investigate the auto sampler stability and the in-process stability of prepared samples, QC samples of three levels were prepared, analyzed and then stored during 24, 48, 72 and 168 hours in the auto sampler at15°C or at 4°C. The recovery was calculated after each storage time and was compared to the recovery of the same sample after initial testing. Stability of stored samples over 168 hours was tested for three different conditions: room temperature (18°C), refrigerator (4°C) and freezer (-20°C). Six QC samples (n=2 for per level) were freshly prepared at T=0 hours and fosfomycin concentrations were quantified. After 168 hours of storage at the three different conditions, a second set of six QC samples was prepared and analyzed. The recovery was calculated at T=168 hours and was compared to the recovery at T=0 hours. Long term stability of stored samples in the freezer (-80°C) was investigated by the same process after a period of six-months storage had transpired. The recoveries should not deviate more than 10% from each other.

Clinical Validation

As part of two research projects into the PK of fosfomycin in urine, four healthy, female volunteers collected urine samples during 48 hours after they received a single, oral dose of 3 grams fosfomycin. Only females were included since this is the population who are most likely to have a UTI in daily practice.. In a second research project, three plasma samples of six male patients were collected after receiving an oral dose of 3 grams fosfomycin two hours before surgery as part of a prophylactic treatment. Plasma samples were immediately centrifuged and all samples were stored at -80°C prior to analysis. From all samples (urine and plasma), 100 µL was prepared as described in section 2.5.

Both studies were approved by the local ethical committee (MEC-2012-121 and MEC-15-047) and registered with EudraCT (2015-005700-28 and 2015-000626-11). Participation in both studies was voluntary and enrollment occurred after informed written consent had been obtained.

RESULTS AND DISCUSSION

Optimization of the method

Infusion experiments were conducted to determine the m/z values of the parent- and product ions together with the optimal MS settings. Therefore, two separate 1 mg/L solutions of fosfomycin and the internal standard in methanol were directly injected in the MS without chromatographic separation. The optimal MS setting of fosfomycin could be replicated for the internal standard.

This method is unique in the application of the $13C_3$-labeled internal standard. The use of a stable isotope, (C13) labeled internal standard is superior compared to an unlabeled standard, since the $13C_3$-labeled fosfomycin salt is a structure analogue of fosfomycin (figure 1a, 1b). This internal standard is perfectly able to adjust for variations other than those related to those associated with different fosfomycin concentrations, and thereby to improve quantitative detection (22).

This HILIC chromatographic method makes use of a hydrophilic stationary phase which binds hydrophilic compounds so that they are separated from the less hydrophilic matrix. The fact that fosfomycin has a highly hydrophilic character makes this chromatographic method very suitable for the quantification of this compound (23). The sensitivity of a method can be enhanced by increasing the organic content of the mobile phase whereby also the flow rate can be increased due to the reduced viscosity of the mobile phase (24). As the retention time of the analytes can be directly influenced by the pH of the mobile phase, it is of great importance to investigate the optimal contents of the mobile phase regarding the ratio between the organic solvent (acetonitrile), water and the buffer (ammonium formate in ultrapure water, pH 7; 4 mM). The buffer is added to regulate the pH of the mobile phase during the analysis to ensure a reproducible retention time (25). Different buffer concentrations, as well as different pH levels of the buffer were tested in order to achieve an optimal separation of fosfomycin and the internal standard from the matrix components as recommended by Kahsay et al. (23).

Based on recommendations from a previous study with HILIC, buffer concentrations ranging from 2, 4, 5, 10 and 20 mM were tested with a pH of 4, 6 and 9 (26). The optimal appearance of the peak was observed with a buffer concentration of 4 mM and a pH of 6. Unfortunately, the retention time of fosfomycin under these circumstances was greatly reduced compared to a buffer concentration of 20 mM: 1.40 min to 0.98 min, which was slightly more than the column dead time. To increase the retention time, increased concentrations of acetonitrile were tested (26). 75%, 78% and 80% acetonitrile have been tested where after it could be concluded that the optimal peak appearance and retention time were obtained at a percentage of 80% acetonitrile. The retention time was eventually increased to 1.8 minutes so the combination of UPLC

in combination with HILIC chromatography resulted in a shorter run time (4 minutes) compared to other methods (16, 17). This is an advantage regarding the applicability of the method in daily lab routine.

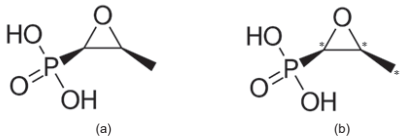


Figure 1: (a) Chemical structure of fosfomycin and **(b)** the internal standard: fosfomycin-13C_3. Only one of the enantiomers from the racemic mixture is depicted. The three 13C atoms are marked as C*.

The sample preparation method was initially developed for urine samples and consisted of the addition of the internal standard, an ultrafiltration step and a dilution step with acetonitrile to meet the starting conditions of the UPLC mobile phase. This sample preparation method was found to be applicable to the plasma samples. This is seen as a major advantage over previously developed methods because of its applicability to determine concentrations in both urine and plasma, thereby describing the PK process of drug distribution and elimination, contributing to the optimization of therapy and minimize the risk of emergence of resistance.

Analytical validation

Linearity

The method was successfully validated over a range of 3.75 to 375 mg/L in urine (R^2=0.9998, with a maximum deviation of 6%) and plasma (R^2=0.9998, with a maximum deviation of 5%) with a weighting factor of 1/x. Since the highest reported plasma concentration following oral administration of fosfomycin is 32 mg/L based on an earlier publication, it was expected that this calibration range was sufficient for the clinical plasma samples and no dilution step would be needed in the preparation of these samples (27). Standards 3 and 6 were used as calibration standards when the method was used in routine practice.

Urinary concentrations measuring up to 4000 mg/L were expected from clinical samples based on reports from earlier publications (28–30). Accuracy problems with such a wide range of concentrations were observed during the method development. Therefore, it was determined to validate the method for a smaller range, such that some urine samples would require dilution before analysis. This dilution step is not needed for plasma samples. With this method, all samples could be quantified with one calibration range. Another possible solution for the problem was used by Martens-Lobenhoffer et al. These authors used two calibration lines to cover the whole thera-

peutic range (16). As a consequence, one should estimate the expected concentration of a sample prior to analysis in order to decide which calibration range should be used. As this is usually difficult to predict, unknown samples always have to be prepared in both calibration ranges and thus have to be analyzed twice. The method presented here was developed to quantify fosfomycin concentrations in a specific, and clinically relevant concentration range. Therefore, the decision to validate the method over a smaller concentration range is not a limitation for the applicability of the method because it still covers the relevant concentration range (see also section 3.2.2). Preference was given here to develop the method over a smaller concentration range.

Limits of quantification and detection

The LLOQ, based on five LLOQ standards, was determined as 0.75 mg/L for both urine and plasma. The LOD for plasma was 0.65 mg/L and 0.70 mg/L for urine. The concentration range, together with the low LLOQ and LOD, appears adequate for studying fosfomycin in clinically relevant concentrations, based on the range of MICs of possible (uro)pathogens. This is based on the fact that oral fosfomycin is primary used for the treatment of uncomplicated urinary tract infections caused by gram-negative organisms of which *E. coli* is the most important (31). The concentrations in urine required to exceed the fosfomycin MICs of *E.coli* (uro)pathogens fall within the quantification range of the method, based on data from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (19). Plasma levels of fosfomycin that are expected in order to treat the infectionare also within the calibration range of this method (32–34).

There were no interfering peaks found in both matrices at the retention times of fosfomycin and the internal standard. The areas in the blank samples were negligible compared to the fosfomycin area in the LLOQ standard. Therefore, the method was found to be selective and sensitive. Importantly, no carry over was observed when analyzing a sample with the highest concentration (calibration sample 8) after a blank sample. A typical chromatogram of fosfomycin (35 mg/L) and the internal standard in urine and in plasma is presented in figure 2.

Accuracy and Precision

The accuracy and precision data are shown in table 2. All calculated values were within the acceptance criteria of ±15% of the mean concentrations. Therefore, the within-day and the between-day variability of the method is low.

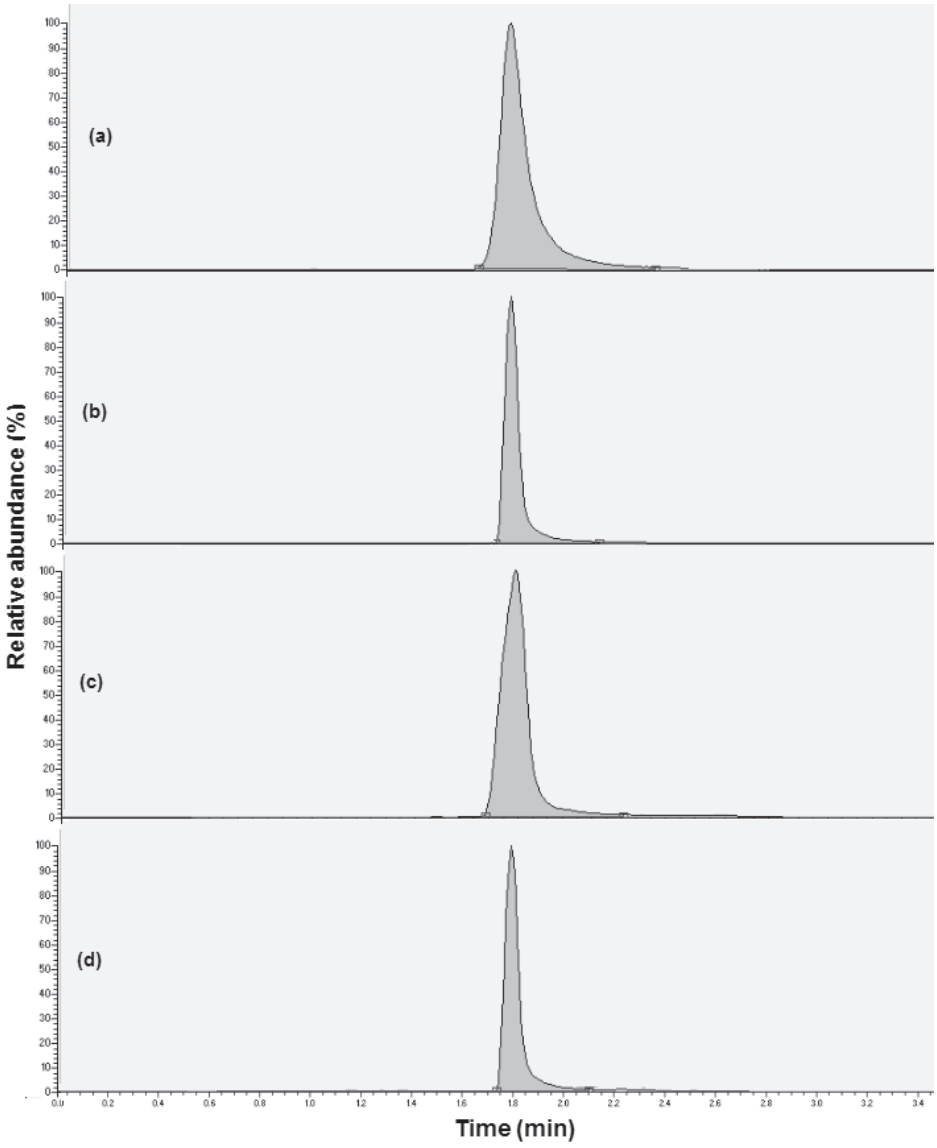


Figure 2: Chromatograms of fosfomycin in urine (a) and plasma (b) and the internal standard in urine (c) and in plasma (d) after injection of calibration standard 3.

Table 2: Accuracy and within-day and between-day precision was tested. The values represent the deviation of the measured concentrations compared to the theoretical concentrations, expressed as a percentage of the theoretical concentrations. This value should be within the acceptance criteria of ±15%.

Matrix	Sample [a]	Accuracy (%) [b]	Within-day precision (%) [b]	Between-day precision (%) [c]
Urine	QC-L	-0.5	1.5	4.2
	QC-M	-2.1	0.5	5.0
	QC-H	0.0	1.1	1.0
Plasma	QC-L	3.2	1.7	3.8
	QC-M	1.9	0.8	1.2
	QC-H	0.7	1.2	1.1

[a] The sample concentrations are: QC-L = 7.50 mg/L, QC-M = 115 mg/L and QC-H=335 mg/L.
[b] Values are means and based on six QC samples for each level measured on one day.
[c] Values are means and based on two QC samples for each level measured on six different days.

Matrix Variability and Recovery

Matrix effect, recovery and process efficiency of fosfomycin in urine and in plasma are presented in table 3. No ion suppression and/or enhancement effects due to compound of the matrices was observed. Recoveries were high in both urine and plasma for both QC levels. This is remarkable for plasma samples since low recoveries were reported before (68% compared to 99.4% here) (18). This also resulted in higher LLOQ limits in urine (100 mg/L) and in plasma (1 mg/L) compared to this method (0.75 mg/L) (18). Ultrafiltration appears to result in a higher recovery compared to protein precipitation during the sample preparation. This can be explained by the fact that protein binding of fosfomycin is negligible (35) so the concentrations were not significantly influenced by the ultrafiltration step.

Table 3: Matrix effect, recovery and process efficiency of fosfomycin in urine and in plasma. The presented values, all expressed as a percentage, are corrected for the internal standard and obtained when using five different sources of urine and plasma. 'Diff' represents the deviation of the measured concentration compared to the theoretical concentration of the QC samples and should not exceed 15%.

	Matrix effect (%)				Recovery (%)				Process Efficiency (%)			
	QC-L	QC-H	mean	Diff	QC-L	QC-H	mean	Diff	QC-L	QC-H	mean	Diff
Urine	85.0	104.0	94.6	14.0	103.0	102.0	102.5	0.5	88.0	106.0	96.9	13.5
Plasma	93.0	102.0	97.4	6.1	100.0	99.0	99.4	0.2	93.0	101.0	96.8	5.9

Stability

Stability data of prepared samples and stored samples are presented in table 4. All QC levels for the prepared urine and plasma samples were stable for at least 72 hours with the exception of the QC-L sample in urine at 4°C. This sample was stable for at least 48 hours. No further time points were tested for the samples, but it is expected that the samples at both temperatures will have a longer expiration time than 72 hours. This is based on additional tests at 4°C were the QC-M and QC-H samples showed stability up to 168 hours in both matrices (data not shown). However, stability of 72 hours was found to be sufficient in the lab and did not provide practical problems. All stored urine samples are stable at each condition for one week and even for six months at -80°C. Plasma samples showed to be a little less stable since recoveries slightly decreased

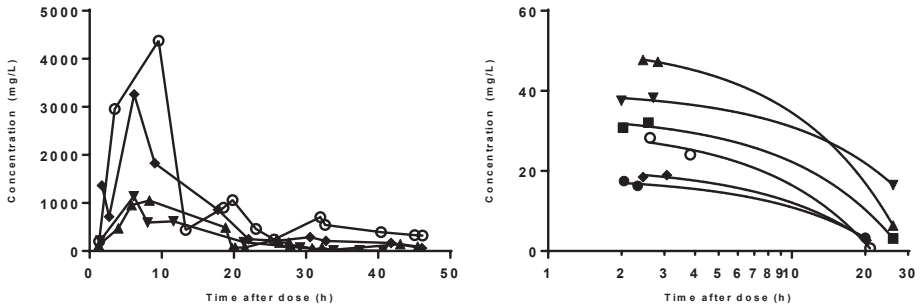
Table 4: Stability data of the assay where auto sampler (15°C) stability and in-process (4°C) stability was tested for prepared samples. Stability of stored samples was tested at 18°C, 4°C and -20°C during one week and at -80°C during six months. The values represent the mean recovery (%) of two QC samples of each level.

Matrix	Condition	Time	QC-L	QC-M	QC-H
Urine	15°C	24h	98.4	98.3	99.5
		48h	101.6	98.4	99.7
		72h	100.9	98.1	100.7
	4°C	24h	103.9	100.3	99.1
		48h	107.5	98.8	100.6
		72h	236.6	95.8	98.2
	18°C	1 week	102.3	99.3	98.2
	4°C	1 week	103.0	100.4	100.8
	-20°C	1 week	99.1	98.1	98.3
	-80°C	6 months	101.1	92.4	93.8
Plasma	15°C	24h	105.1	101.1	100.2
		48h	100.5	99.1	100.6
		72h	100.2	100.0	109.7
	4°C	24h	102.7	100.0	101.3
		48h	97.9	89.5	99.3
		72h	101.8	98.3	100.2
	18°C	1 week	89.1	87.4	87.8
	4°C	1 week	102.3	88.3	89.0
	-20°C	1 week	101.4	90.7	101.8
	-80°C	6 months	94.0	99.6	98.8

to <90 % at -18°C for all QC levels and at 4°C for QC-M and QC-H. Stability for six months at -80°C was considered to be the most important finding since QC samples and calibration standards, which are used during every analysis, are stored at -80°C.

Clinical validation

Concentrations in urine of four volunteers and in plasma of six patients are presented in figure 3a and 3b. No interfering peaks were found from the matrices. Also, distortion of the chromatograms by co-medication was not seen. The maximum urinary concentrations ranged from 1050.3 mg/L to 4378.9 mg/L and maximum plasma concentrations ranged from 17.5 mg/L to 47.7 mg/L. All concentrations were comparable with those found in previous studies (28–30, 36–38).



(a) (b)

Figure 3: (a) Urinary concentration – time curves of four healthy, female volunteers after receiving 3 grams of oral fosfomycin at T=0 h. A sample was collected from every urination in the following. **(b)** Plasma concentration – time curves of six male patients after receiving 3 grams of oral fosfomycin. The horizontal axis is presented on a logarithmic scale. Each symbol represents one subject.

The presented method was developed to quantify fosfomycin concentrations in the range achieved clinically and could also serve as a method for therapy optimization (19). Since this method is more sensitive compared to methods described earlier, it appears to be more suitable for monitoring the extent to which the (time above) MIC is achieved. Therefore, distinctions can be made in particular for strains with relatively low MIC values, such as *E. coli*. This is an important advantage since regrowth of bacteria is suspected to be correlated to low urinary concentrations. This highlights the importance of monitoring of these concentrations in order to optimize patient outcomes and to minimize the risk of emergence of resistance. The method may therefore serve as the basis of more individualized therapy with fosfomycin rather than the 'one size fits all' strategy which is currently used (39).

CONCLUSIONS

To the best of our knowledge, this is the first method suitable for the quantification of fosfomycin in urine and in plasma using one sample preparation method that includes an isotope labeled internal standard. The method is highly sensitive allowing quantification of fosfomycin concentrations in the clinically relevant concentration range. This makes the method applicable for optimization of both oral and IV therapy with fosfomycin. The use of ultra performance chromatography instead of high-pressure chromatography, offers the possibility to use a short retention time and therefore a shorter total runtime. The method has proven its applicability in daily clinical practice, and has been used in two clinical studies in which fosfomycin concentrations were quantified in urine and plasma samples.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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