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Determination of fosmidomycin in human serum and urine by capillary electrophoresis

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Abstract

A capillary electrophoresis method with direct UV detection was developed for the determination of fosmidomycin, a promising new anti-malarial drug, in human serum and urine. Optimization of the separation parameters resulted in a buffer system adjusted to pH 10.8 containing a cationic reagent and an organic modifier. Under these conditions, the migration time of fosmidomycin was 5.2 min with serum and 7.4 min with urine samples. Validation of the method revealed good recoveries, precision and accuracy. The limit of quantification was 0.5 μg/ml in serum and 10 μg/ml in urine. The determination of fosmidomycin in serum was linear over a range of 0.1–150 μg/ml. Short and long-term stability tests resulted in no significant loss of fosmidomycin. The described technique will provide a fast and accurate analytical method for future pharmacokinetic studies.
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1. Introduction

Fosmidomycin, a phosphonic acid derivative, was originally isolated as natural antibiotic from *Streptomyces lavendulae* (Fig. 1) [1]. With respect to its activity against various Gram-negative bacteria, early clinical studies conducted during the 1980s focused on a potential role of fosmidomycin in the management of urinary tract infections. In 1998, it was demonstrated that fosmidomycin inhibits 1-deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase, an enzyme of the non-mevalonate pathway (DOXP pathway) of isoprenoid synthesis [2,3]. This pathway is utilized by most eubacteria and the plastids of algae and higher plants, but is absent in animals and humans which use the mevalonate pathway, instead. Recently it was demonstrated that fosmidomycin possesses potent antimalarial activity, since the isoprenoid biosynthesis of the malaria parasite depends on the DOXP pathway which is localized inside a plastid-like organelle, the so-called apicoplast [4,5]. The efficacy of fosmidomycin against acute uncomplicated *Plasmodium falciparum* infections was demonstrated in phase II trials using a regimen of 1.2 g fosmidomycin administered orally every 8 h [6]. Clinical studies with a combination of fosmidomycin and clindamycin, which was shown to act synergistically, are currently ongoing [7–9]. In previous pharmacokinetic studies, a microbiological assay with *Enterobacter cloacae* as test organism was used for the determination of fosmidomycin in biological fluids [10]. For the current studies, a more convenient and reliable assay is required in order to monitor the efficiency of the dose regimen and to optimize the therapy. Since only poor absorption of fosmidomycin was previously reported, such a method needs to be sensitive enough to detect the relatively low plasma levels expected to be achieved after oral or intravenous administration [11]. Here, we report on the development of a high-performance capillary electrophoresis (HPCE) technique with direct UV detection for the determination of fosmidomycin in serum and urine. First, the electrophoresis parameters were optimized, and a extraction procedure for the drug established. Then, the method

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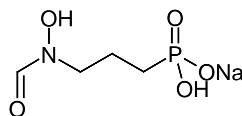

Fig. 1. Chemical structure of fosmidomycin.

was subjected to a validation procedure including intra- and inter-day precision, linearity, and recovery. Also, the stability of fosmidomycin under different storage conditions was determined.

2. Experimental

2.1. Reagents

Fosmidomycin [3-(formylhydroxy-amino)propylphosphonic acid mono-sodium salt, 3-(*N*-formyl-*N*-hydroxy-amino)propylphosphonic acid mono-sodium salt, FR-31564] was synthesized by a combinations of the protocols from work by Kamiya et al. and Öhler and Kanzler [12,13]. Dichloromethane and acetonitrile, chromatographic grade were purchased from Merck (Darmstadt, Germany); Hexadecyltrimethylammonium bromide (HTAB) was from Fluka (Buchs, Switzerland); and methanol was from Prolabo (VWR International S.A.S., Fontenay-sous-Bois, France).

2.2. Capillary electrophoresis system

Electrophoresis was carried out using a Beckman P-ACE MDQ HPCE system (Beckman-Coulter, Fullerton, CA, USA) with direct UV detection at 214 nm wavelength. The capillary thermostat was set to 25 °C. A fused-silica capillary of 75 cm total length (62.5 cm length to detection window) with 75 μm i.d. was used for the experiments. For the first use, the capillary was filled with buffer and equilibrated for at least 2 h applying a voltage of 15 kV. Then, it was rinsed with 0.1 M NaOH for 10 min, followed by 10 min ultra-pure water. The samples were injected by pressure (0.5 psi) for 15 or 25 s. Before the injection of the first sample and between two runs the capillary was rinsed successively with 0.1 M NaOH, water, and the working buffer for 1 min each. The electrophoresis of the samples was performed in reverse mode from the cathode towards the anode at 15 kV.

2.3. Electrophoresis buffer

Various instrumental setting for migration were tested (voltage, capillary temperature), and the buffer properties were optimized (pH values, component molarities). The effect of the ionic strength of the buffer was assessed by different concentrations of Na_2HPO_4 (10–200 mM) or KH_2PO_4/K_2HPO_4. The electroosmotic flow (EOF) was modified by cationic ion pairing reagents, such as hexadecyltrimethylammonium bromide (HTAB). Also, various organic modifiers were tested (methanol, acetonitrile). Finally, the working buffer was 14 mM KH_2PO_4/56 mM K_2HPO_4, 0.2 mM HTAB, 5% (serum samples) or 20% (urine samples) methanol as additives. The pH was adjusted to 10.8 with 1 M NaOH. The resulting current was in part modulated by methanol percentages, and it was 150 μA with serum samples and 110 μA with urine samples.

2.4. Sample preparation

2.4.1. Serum

The extraction procedure was based on the technique published by Rudrik and Bawdon followed by an ultra-filtration step [14]. Briefly, the serum samples (500 μl) were deproteinized with 500 μl acetonitrile. After mixing on a vortex-mixer, the tubes were gently shaken by rotation (20 rpm, 10 min). After centrifugation (2000 × *g*), the supernatants were mixed for 10 min with 3.5 ml dichloromethane to remove the excess of acetonitrile. The samples were centrifuged for 10 min, and 150 μl of the supernatants passed over Ultrafree-MC filter units (molecular mass cut-off 5000 Da; Millipore Corporation, Bedford, MA, USA) for 15 min. The samples were stored at +10 °C in the storage compartment of the P/ACE MDQ CE system before analysis. The validation procedure was performed with human type AB serum (off clot, sterile filtered, male donors) from ICN Biomedicals France S.A., Orsay (Lot: 4507F, 6433F).

2.4.2. Urine

A pool of five urine samples from healthy humans or patients with urinary tract infections was used for the validation procedure. Prior to the injection into the capillary, the urine samples were diluted 1 in 10 with ultra-pure water.

2.5. Validation procedure

2.5.1. Serum

2.5.1.1. Linearity and accuracy. Six linearity curves were determined with an injection time of 15 s (150, 75, 50, 25, 7.5, 5, 2.5, 0.75, 0.5 μg/ml, nine concentrations), and three concentrations were used as quality control (QC) samples (100, 10, 1 μg/ml). For the lower concentrations (10, 7.5, 2.5, 1, 0.75, 0.5, 0.1 μg/ml, seven concentrations) four calibration curves were constructed, and one concentration (5 μg/ml) was used as QC sample. Consequently, two mean calibration curves were determined.

The accuracy was determined as the deviation of the mean results obtained by our method from the spiked concentrations, and determined with the mean calibration curves.

2.5.1.2. Limits of detection and quantification. The limit of detection (LOD) in human serum was defined as the minimum detectable concentration for which the signal-to-noise ratio is three.

The limit of quantification (LOQ) was defined as the minimum fosmidomycin concentration resulting in an accuracy higher than 80% (determined with the standard mean curve), or a deviation of the mean concentration from the spiked value lower than 20%.

2.5.1.3. Precision.

Repeatability. In order to assess the repeatability of the injection, each of three samples containing 0.75, 10, or 100 μg/ml fosmidomycin were measured 10 times. Between two runs the capillary was rinsed as described in the Section 2.2.

Day-to-day and intra-day reproducibility. The day-to-day reproducibility was determined on serum samples spiked with three different concentrations (100, 10 and 1 μg/ml). For each concentration, 10 samples were extracted and analyzed one by one at different days and/or time.

The intra-day reproducibility determines the closeness of individual measures when the procedure is applied to multiple aliquots of a single volume of serum. This parameters reflects in particular the reproducibility of the extraction procedure. To study the intra-day reproducibility, volumes of serum spiked with three concentrations (100, 10, and 0.75 μg/ml) were distributed in nine (500 μl each), and the aliquots of the same concentration were extracted simultaneously.

2.5.1.4. Recovery. Pretreatment of the serum samples was necessary to improve the sensitivity and to reduce the matrix components which potentially interfered with the migration of fosmidomycin. Cleaner electropherograms were obtained after ultra-filtration of the samples. The recovery of the extraction procedure was defined as the ratio of the corrected area determined after extraction to the corrected area determined in water at the same concentration ($n = 7$). The recovery was determined for three concentrations (100, 10, and 1 μg/ml).

2.5.1.5. Stability.

Short-term stability. Short-term stability (24 h) of three concentrations of fosmidomycin (1, 10, and 100 μg/ml) in human serum was assessed at room temperature and +4 °C.

Long-term stability. Long-term stability in serum was determined at three concentrations (1, 10, and 100 μg/ml). At day 0, samples were extracted in triplicate, and for each concentration, the resulting solutions were divided before freezing at −80 °C. For each time point, three aliquots of each concentration were thawed and extracted until 6 months of storage.

Freeze–thaw stability. Freeze–thaw stability was assessed with three concentrations (1, 10, and 100 μg/ml). At day 0, samples were extracted in triplicate immediately after the preparation of the solutions and before freezing at −80 °C. The solutions were thawed at room temperature, and aliquots extracted in triplicate at day 1, 2, 3 and 4. On each day, the solutions were refrozen.

2.5.1.6. Exogenous interferences. The effect of molecules potentially co-administered with fosmidomycin (clindamycin) or used in blood sample tubes as anti-clogging agents (heparin, EDTA), which may interfere with the assay, were analyzed directly or after pretreatment.

2.5.2. Urine

2.5.2.1. Linearity and accuracy. Linearity in urine samples was assessed over the concentration range 10–500 μg/ml before 1 in 10 dilution (500, 125, 100, 62.5, 25, 10 μg/ml), and two concentrations were used as QC samples, 250 and 31.25 μg/ml. The sample injection time was 15 s. Five linearity curves were recorded, and the accuracy of the concentration was determined with the mean curve.

2.5.2.2. Limits of detection and quantification. Both the LOD and LOQ were determined as described for the serum (Section 2.5.1.2).

2.5.2.3. Precision.

Repeatability. The precision of injection (repeatability) was determined at three concentrations (250, 100, and 10 μg/ml). The samples were diluted 1 in 10 before injection. Each sample was injected 10 times, and the capillary rinsed between two runs. The variation coefficients of the corrected areas between the single runs were determined to evaluate the injection reproducibility.

2.6. Analyzing and integrating data

Peak integration was done using the 32 Karat™ Software (Beckman-Coulter). To compensate for potential mobility changes between the single runs, results were expressed as corrected areas (A_c), which corresponds to the ratio of the peak area to the migration time (arbitrary units) [15].

3. Results

3.1. Migration time and specificity

A typical electropherogram of human serum spiked with 10 μg/ml fosmidomycin before extraction in comparison to blank serum is shown in Fig. 2. Fig. 3 shows the electropherograms of pooled human urine diluted 1 in 10 without

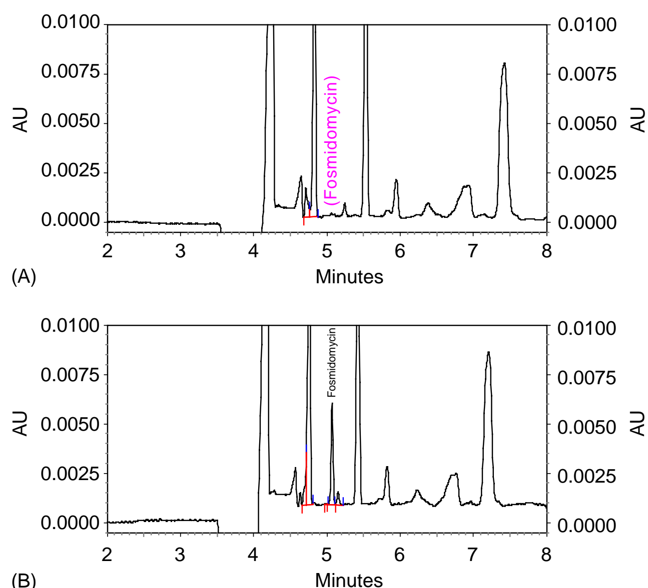

Fig. 2. Detection of fosmidomycin in human serum. Blank serum (A) and serum spiked with 10 μg/ml fosmidomycin (B) was extracted and analyzed as described in the experimental section.

fosmidomycin and spiked with 100 μg/ml of the drug. The migration time was 5.2 min with serum and 7.4 min with urine samples. There was no interference of endogenous signals with the fosmidomycin peak.

3.2. Stability of the stock solution

The long-term stability of the fosmidomycin stock solution was assessed. A solution of 10 mg/ml of fosmidomycin in water was divided at day 0 and the aliquots were stored at −80 °C pending analysis. Before analysis, the solution was diluted 1 in a 100 (100 μg/ml) in triplicate. After 3 months of storage, the difference of the mean corrected area to the corresponding value from day 0 was +7.5%.

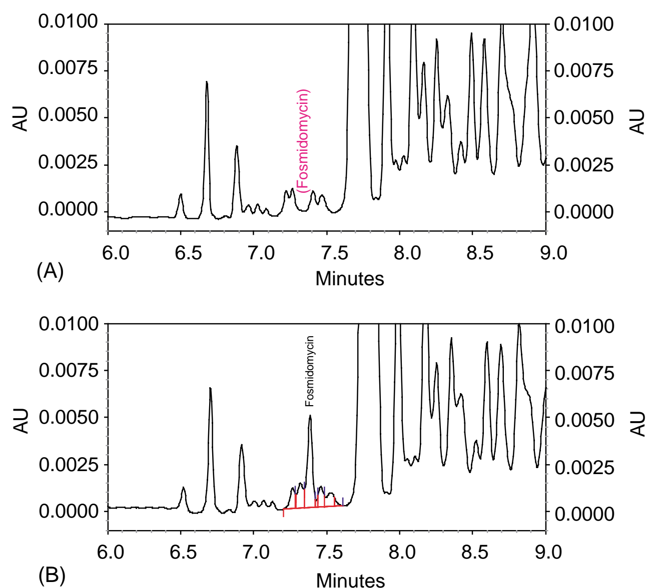

Fig. 3. Detection of fosmidomycin in human urine. Blank urine (A) and urine spiked with 100 μg/ml fosmidomycin (B) was diluted 1 in 10 and analyzed as described in the experimental section.

3.3. Serum

3.3.1. Linearity and accuracy

The assay was linear over the concentration ranges tested. The coefficient of correlation of the mean calibration curve was $r^2 = 0.99993$, and the equation was $y = 0.2828 + 0.0063x$ (15 s injection), where x corresponded to the corrected area measured by CE for each assay. The accuracy for the QC samples was good for high and medium concentrations (15 s injection), with a deviation of 2.3 and 0.6% for 100 and 10 μg/ml, respectively (Table 1).

For the low concentrations (0.1–10 μg/ml, 25 s injection time), the slope of the mean curve was 0.0036, and the intercept −0.0187 ($y = -0.0187 + 0.0036x$). The R^2 was 0.99972. The deviation of the QC sample value determined with the mean calibration curve from the spiked value (5 μg/ml) was 4.3% (Table 2).

3.3.2. Limits of detection and quantification

The limit of detection (LOD) of our method in human serum was 0.1 μg/ml. The LOQ value was 0.5 μg/ml, with an accuracy of 95.0% (Table 2).

3.3.3. Precision

For these experiments, an injection time of 15 s was used for the 10 and 100 μg/ml samples, and 25 s for the 0.75 and 1 μg/ml samples.

Table 1
Deviation of the concentration values determined with the mean calibration curve in human serum from the spiked values (15 s injection)

	Mean concentration determined with the mean standard curve (μg/ml)	Deviation (%)
Spiked concentrations (μg/ml)		
150	151.24	0.8
75	74.96	0.05
50	49.77	0.5
25	24.60	1.6
7.5	7.64	1.9
5	5.01	0.2
2.5	2.79	11.4
0.75	1.07	43.0
0.5	0.84	64.5
Quality control samples		
100	102.32	2.3
10	9.94	0.6
1	1.23	23.4

Three concentrations (100, 10, 1 μg/ml) were used as quality control samples.

Table 2
Deviation of the concentration values determined with the mean calibration curve in human serum from the spiked values (25 s injection)

	Mean concentration determined with the mean standard curve (μg/ml)	Deviation %
Spiked concentrations (μg/ml)		
10	10.04	0.4
7.5	7.37	1.7
2.5	2.47	1.2
1	0.99	1.0
0.75	0.77	2.9
0.5	0.53	5.0
0.1	0.22	123.6
Quality control sample		
5	4.78	4.3

One concentration was used as quality control sample.

- *Repeatability*: the coefficients of variation between multiple injections were 2.3, 3.3, and 3.9% for 0.75, 10, and 100 μg/ml, respectively.
- *Day-to-day reproducibility*: the day-to-day reproducibility resulted in coefficients of variation (CV%) of 5.9, 4.7, and 3.1% for 1, 10, and 100 μg/ml, respectively ($n = 10$).
- *Intra-day reproducibility*: the intra-day reproducibility (CV%), was 6.4, 4.2, and 5.2% for 0.75, 10, and 100 μg/ml, respectively ($n = 9$).

3.3.4. Recovery

The recovery of the acetonitrile/dichloromethane pretreatment from serum was 122.0, 121.2 and 110.2% for 1, 10, and 100 μg/ml, respectively ($n = 7$).

3.3.5. Stability

High long-term stability of fosmidomycin in human serum was recorded at −80 °C (Table 3). Fosmidomycin was also stable for 24 h at room temperature or at +4 °C (Table 3). Moreover, fosmidomycin was resistant to numerous cycles of freezing and thawing. After four freeze–thaw cycles (4 days), 98.2, 102.7, and 99.7% of fosmidomycin were recovered from the 100, 10, and 1 μg/ml samples, respectively (Table 3).

3.3.6. Exogenous interferences

No interference with clindamycin, which is co-administered with fosmidomycin in the current clinical studies, occurred. Also, no interferences with heparin, which is frequently used as anti-clogging agent, did occur. However, strong interference with EDTA was observed. This compound is present in some blood sampling tubes as anti-clogging (K3E, Becton-Dickinson), and consequently, should not be used.

Table 3
Stability of fosmidomycin in human serum

		Difference to day 0 (%)		
		1 (μg/ml)	10 (μg/ml)	100 (μg/ml)
Long-term (3 months)	−80 °C	−5.5	−8.8	−14.6
Short-term (24 h)	+4 °C	−2.0	−2.7	−7.5
	Rt[a]	−0.3	+12.5	−15.3
Freeze–thaw (4 days)		−0.3	+2.7	−1.8

[a] Room temperature.

3.4. Urine

3.4.1. Linearity and accuracy

The assay was linear over the range of concentrations used (10–500 μg/ml) with an injection time of 15 s ($R^2 = 0.99991$). The slope of the mean curve was 0.0079 and the intercept −0.1792 ($y = -0.1792 + 0.0079x$). For the QC samples, the accuracy determined with the standard curves was 99.98 and 96.5% for 25 and 31.25 μg/ml, respectively (Table 4).

3.4.2. Limits of detection and quantification

The limit of detection was 0.5 μg/ml, corresponding to 5 μg/ml in the urine samples before dilution. The limit of quantification was determined using the linearity mean curve as reported for serum. The LOQ was 1 μg/ml (10 μg/ml in the urine samples), with a deviation of the mean value determined by the assay from the spiked value of 11.4%.

3.4.3. Repeatability and day-to-day reproducibility

The repeatability of the injection of urine samples was determined for three concentrations (250, 100, and 10 μg/ml). Before injection, the samples were diluted 1 in 10 with ultra-pure water. The repeatability of the injection (CV%) was 1.7, 2.0, and 2.2% for 250, 100, and 10 μg/ml, respectively ($n = 10$). Concomitantly, the variations of the migration times between the runs were determined. The variations were 1.3, 1.0, and 1.1% for 250, 100, and 10 μg/ml, respectively. The day-to-day reproducibilities were determined

Table 4
Deviation of the concentration values determined with the mean calibration curve in human urine from the spiked values (15 s injection, diluted 1 in 10)

	Mean concentration determined with the mean standard curve (μg/ml)	Deviation (%)
Spiked concentrations (μg/ml)		
50	49.70	0.6
12.5	12.63	1.0
10	10.14	1.4
6.25	6.25	0.01
2.5	2.28	8.9
1.0	0.89	11.4
Quality control sample		
25	25.01	0.02
3.125	3.23	3.5

Two concentrations (25 and 3.125 μg/ml) were used as quality control sample.

using the linearity assays, and by the variation between each assay spiked with the same concentration ($n = 5$). The day-to-day reproducibility was 4.7, 3.4, and 5.1%, for 250, 100, and 25 μg/ml, respectively.

4. Conclusion and discussion

Since it provides short analysis times and high separation efficiency capillary electrophoresis (CE) was applied for the determination of fosmidomycin in serum and urine. Direct UV absorption was chosen for detection taking advantage of the high absorption of fosmidomycin at 214 nm. This method is easier to handle in CE than indirect UV detection, which was applied, for example, for the determination of fosfomycin in biological samples [16].

However, the quantitative determination of the target molecule was complicated by numerous endogenous compounds with high UV absorption at 214 nm present in biological fluids. In addition, these compounds potentially interfere with the migration behavior of fosmidomycin, and direct injection of serum may reduce the lifetimes of the capillary due to clogging [15].

Therefore, an extraction procedure was necessary which, however, potentially reduces the sensitivity of the assay. For this purpose, a technique previously published by Rudrik and Bawdon was used resulting in good recovery and intra- and inter-days reproducibility and excellent linearity of the responses [14], indicating that significant variations of the injected volumes are unlikely to occur. Thus, an internal standard was not need. The peaks measured with the extracted serum samples were higher than those obtained with the aqueous samples, resulting in recovery above 100%. This behavior was described by Rudrik and Bawdon, who observed a relative concentration of cloxacillin in the aqueous layer during extraction [14]. The extraction procedure was followed by an ultra-filtration step leading to cleaner electropherograms and increased capillary lifetimes. The mean lifetime of the capillary was 220 runs. Capillaries were changed when the migration time became unstable or when the capillaries were clogged. The capillary specifications were chosen to offer the best compromise between migration time, resolution and heat production. As expected, an improved resolution was achieved with a capillary of 50 μm i.d. but the limit of quantification was shifted to unacceptably high values.

Various buffer compositions (Na_2HPO_4, KH_2PO_4/K_2HPO_4) were tested. An increase of the molarity and, consequently, of the ionic strength led to an improvement of the peak shape and an increase of peak height, but also to longer migration times. The fosmidomycin molecule is negatively charged at pH 10.8, and its migration from the cathode towards the anode is due to its electrophoretic mobility slowed down by the opposite electroosmotic flow. The addition of HTAB resulted in a strong effect on the analytical conditions, but had only minor effects on the current. These effects were concentration dependent and affected the migration time and the peak shape. The migration time decreased when the HTAB concentration was increased until a threshold concentration of about 0.2 mM was reached. This was probably the consequence of the reversion of the cathodic EOF to an anodic flow, which enhances the fosmidomycin mobility towards the anode.

The migration temperature was controlled by a thermostat device since the temperature is one of the most critical parameter in CE for robustness and reproducibility. Variations of the temperature potentially modify both pH and viscosity of the electrolyte and, consequently, the mobility of the test compound [17]. Adjusting the pH of the electrophoresis buffer to 10.8 by the addition of sodium hydroxide resulted in an increased current.

Methanol, which was added as organic modifier, had a strong influence on the migration, presumably by modifying the viscosity [17]. The increased methanol content of the buffer used for the urine samples resulted in an increased migration time of fosmidomycin and improved selectivity. Also, the current was reduced to 100 μA with 20% methanol, compared to 150 μA with 5% methanol.

The UV response of fosmidomycin in serum was linear over a large range of concentrations from 0.1 to 150 μg/ml. This is in good accordance with the commonly reported limit of quantification achieved by CE with UV detection ranging from 0.1 to 1 μg/ml [15]. Unfortunately, no well described microbiological procedure for the determination of fosmidomycin had been published, so it was not possible to compare the newly developed HPCE method with any other technique.

The limit of quantification of our method with urine samples was relatively high (10 μg/ml), but appears to be sufficient with respect to the high recovery of fosmidomycin in urine determined in previous human pharmacokinetic studies using a bioassay [10]. Indeed, according to references [10,11] the concentrations of fosmidomycin in human biological fluids, either after oral or intravenous administration, are in the range of 0.5–120 μg/ml in blood and 20 to about 300 μg/ml in urine, respectively. Thus, the concentrations used in the present study (0.1–150 μg/ml) to develop the HPCE assay are relevant.

In conclusion, we have developed a convenient CE technique for the determination of fosmidomycin in serum and urine which offers advantages of speed, sensitivity, and accuracy. This technique will be applied for pharmacokinetic studies in humans or animal models, and for therapeutic drug monitoring in order to improve the dose regimen for the treatment of malaria.

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References

[1] M. Okuhara, Y. Kuroda, T. Goto, M. Okamoto, H. Terano, M. Kohsaka, H. Aoki, H. Imanaka, J. Antibiot. (Tokyo) 33 (1980) 24.

[2] T. Kuzuyama, T. Shizimu, S. Takahashi, H. Seto, Tetrahedron Lett. 39 (1998) 7913.

[3] J. Zeidler, J. Schwender, C. Müller, J. Wiesner, C. Weidemeyer, E. Beck, H. Jomaa, H.K. Lichtenthaler, Z Naturforsch C 53 (1998) 980.

[4] H. Jomaa, J. Wiesner, S. Sanderbrand, B. Altincicek, C. Weidemeyer, M. Hintz, I. Türbachova, M. Eberl, J. Zeidler, H.K. Lichtenthaler, D. Soldati, E. Beck, Science 285 (1999) 1573.

[5] J. Wiesner, M. Hintz, B. Altincicek, S. Sanderbrand, C. Weidemeyer, E. Beck, H. Jomaa, Exp. Parasitol. 96 (2000) 182.

[6] M.A. Missinou, S. Borrmann, A. Schindler, S. Issifou, A.A. Adegnika, P.B. Matsiegui, R. Binder, B. Lell, J. Wiesner, T. Baranek, H. Jomaa, P.G. Kremsner, Lancet 360 (2002) 1941.

[7] J. Wiesner, D. Henschker, D.B. Hutchinson, E. Beck, H. Jomaa, Antimicrob. Agents Chemother. 46 (2002) 2889.

[8] S. Borrmann, A.A. Adegnika, P.-B. Matsiegui, S. Issifou, A. Schindler, D.P. Mawili-Mboumba, T. Baranek, J. Wiesner, H. Jomaa, P.G. Kremsner, J. Infect. Dis. 189 (2004) 901.

[9] S. Borrmann, S. Issifou, G. Esser, A. Adegnika, M. Ramharter, I. Lundgren, P.B. Matsiegui, S. Oyakhirome, P. Mawili-Mboumba, H. Jomaa, P.G. Kremsner, submitted for publication.

[10] T. Murakawa, H. Sakamoto, S. Fukada, T. Konishi, M. Nishida, Antimicrob. Agents Chemother. 21 (1982) 224.

[11] H.P. Kuemmerle, T. Murakawa, F. De Santis, Chemother. VI (1987) 113.

[12] T. Kamiya, M. Hashimoto, K. Hemmi, H. Takeno (Fujisawa Pharmaceutical Company), US Patent 4, 206 (1980) 156.

[13] E. Öhler, S. Kanzler. Synthesis (1995) 539.

[14] J.T. Rudrik, R.E. Bawdon, J. Liq. Chromatogr. 4 (1981) 1525.

[15] H. Wätzig, M. Degenhardt, A. Kunkel, Electrophoresis 19 (1998) 2695.

[16] D. Levêque, C. Gallion, E. Tarral, H. Monteil, F. Jehl, J. Chromatogr. B 655 (1994) 320.

[17] G.M. Mc Laughlin, J.A. Nolan, J.L. Lindahl, R.H. Palmieri, K.W. Anderson, S.C. Morris, J.A. Morrison, T.J. Bronzert, J. Liq. Chromatogr. 15 (1992) 961.