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Determination of fosfomycin in pus by capillary zone electrophoresis

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Abstract

A method is described for the determination of fosfomycin in pus by capillary zone electrophoresis with reversed electroosmotic flow, and indirect UV absorbance detection. Sample pre-treatment is limited to removal of proteins and cell debris by adding the double volume of methanol, followed by vortexing for few seconds, and centrifugation at 15,000 × *g* for 2 min. The supernatant is directly injected into the instrument. Fosfomycin is separated from sample constituents with a background electrolyte at pH 7.25 (25 mM benzoate buffer with 0.5 mM hexadecyltrimethylammonium bromide added, adjusted to pH with tris(hydroxymethyl)-aminomethane (TRIS)). Separation is carried out in a capillary with 50 μm I.D., 64.5 cm total length, 56.0 cm to the detector, at 25 °C with −25 kV voltage applied. Due to the low absorbance of the analyte, indirect UV detection was performed at 254 nm using a bubble cell capillary. Sample was injected by pressure (450 mbar s). Repeatability for fosfomycin in spiked pus (from 8 or 10 consecutive injections of three different series at concentrations of 100 μg/mL of the antibiotic) was between 2.4 and 8.2% relative standard deviation (RSD). Accuracy (expressed as recovery of fosfomycin determined by three independent analysis at 10, 100 and 300 μg/mL fosfomycin added to plain pus) was between 75 and 102%. Intermediate reproducibility ($n = 9$ at three different days) was between 2 and 12% RSD. Limit of detection and limit of quantitation were 4.5 and 15 μg/mL, respectively. The concentration of fosfomycin in pus of patients treated with the antibiotic ranged up to 240 μg/mL. The concentration of other anionic pus constituents identified beside chloride (acetate, succinate, lactate, phosphate) ranged between 20 and 7800 μg/mL.

Keywords: Fosfomycin; Antibiotics; Clinical analysis; Body fluid; Pus; Capillary electrophoresis

1. Introduction

The antibiotic treatment of purulent bacterial infections is a complex matter. Generally, the requirements of an antibiotic are an adequate antibacterial spectrum and sufficient concentrations at the site of infection [1–4]. Particularly in infections of deep or encapsulated compartments, the penetration of antimicrobial agents to the target site is considered to be significantly impaired. In addition to a low pH [2,5,6], lack of oxygen [3,6] and the presence of enzymes that can deactivate the antimicrobial agent [3,6]. High protein concentrations in the range of 54 mg/mL [5–7] and the presence of cations and short-chain organic acids additionally hamper the antimicrobial activity of antibiotics. A clinical study was performed in order to gain information about the capability of an antibiotic to penetrate into the abscess cavity in human patients.

Different methods have been described so far for the analysis of different antibiotics in pus, e.g. for the determination of cephalosporins, fluoroquinolones, and penicillins. The most common method is high-performance liquid chromatography (HPLC) [1,8–12], but microbial culture experiments like agar diffusion assay [4], paper-disk methods [13] or bioassays [6] were used for the analysis of antibiotics as well.

Fosfomycin, (−)-(1*R*,2*S*)-(1,2-epoxypropyl) phosphonic acid (Fig. 1) is an antibiotic commonly used for the treatment of urinary tract infections and other infections that are caused by Gram-positive and Gram-negative bacterial species. Fosfomycin inhibits the synthesis of the bacterial cell walls and due to its different structure no cross-resistance with other

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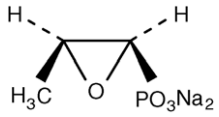

Fig. 1. Structural formula of fosfomycin (as disodium salt).

antibiotics is observed [14]. Fosfomycin concentrations in plasma have been determined by different analytical methods like gas chromatography [15–17], ion exchange chromatography [18] and capillary electrophoresis (CE) [19–21]. One paper reported the analysis of fosfomycin from pus obtained from cattle and sheep by standard bacteriological methods [22] and another showed the eligibility of fosfomycin for the treatment of abscesses in patients [23], also by bacteriological methods.

We have previously developed a CE method based on indirect UV and conductivity detection for this low-UV absorbing analyte in plasma and microdialysates [21] for clinical use. It is the aim of the present paper to adapt this method for the determination of the antibiotic in pus obtained from patients. The method should be feasible, including a simple procedure for sample pre-treatment. As it was found that the pus samples obtained from patients differ strongly in terms of their quantitative composition of matrix anions, these constituents were identified in order to select the appropriate separation conditions for fosfomycin.

2. Experimental

2.1. Chemicals

Fosfomycin disodium salt ("Sandoz 8 g Trockenstechampulle") was kindly provided by Sandoz GmbH (Kundl, Austria). Tris(hydroxymethyl)-aminomethane (TRIS) and benzoic acid (used for the background electrolyte (BGE)), phosphoric acid, acetic acid, lactic acid, methanol (HPLC grade) were purchased from E. Merck (Darmstadt, Germany); succinic acid was from Sigma (Milwaukee, WI, USA). All these chemicals were analytical grade. Hexadecyltrimethylammonium bromide (CTAB, >99.0% purity) was from Fluka Chemie (Buchs, Switzerland). Ringer's solution ÖAB (154 mM Na, 2.7 mM Ca, 4 mM K, 163.4 mM Cl) was from Mayrhofer Pharmazeutika (Linz, Austria). As solvent of the BGE, ultrapure water was used with resistance >18 MΩ cm, prepared by a Millipore Milli-Q apparatus (Bedford, MA, USA).

2.2. Instrumentation

Capillary zone electrophoresis was carried out with a ^{3D}CE apparatus (Agilent Technologies, Palo Alto, CA, USA), equipped with an uncoated fused silica capillary (50 μm I.D., total length 64.5 cm, effective length 56.0 cm; Agilent Technologies) and with a bubble cell (optical path length 150 μm) for indirect detection with a diode array detector at 254 nm. Determination of fosfomycin was performed using a 25 mM benzoate buffer solution with 0.5 mM CTAB added, adjusted to pH of 7.25 with 1 M TRIS for pus analysis. The short-chain organic acids were quantified in a BGE consisting of 25 mM benzoate solution with 0.5 mM CTAB added, but adjusted to pH 4.75 with TRIS. The buffer solutions were degassed by ultrasonication prior to use. Samples were hydrodynamically injected (450 mbar s pressure, applied at the cathodic end of the capillary). Voltage was −25 kV. The capillary cassette was thermostated to 25 °C.

New capillaries were conditioned before use with 1 and 0.1 M sodium hydroxide at 45 °C for 15 min each, followed by rinsing with water and buffer solution for 15 min each at 25 °C. Before each run, the capillary was flushed with 0.1 M sodium hydroxide (4.5 min), water (2 min) and buffer solution (6 min). To diminish buffer depletion during a sequence, the inlet and outlet buffer vial and the conditioning buffer vial were refilled before each run by the built-in replenishment system. For storage overnight, the capillary was flushed with water, 0.1 M sodium hydroxide followed again with water (flushing 3 or 2.5 min, respectively) as described for CTAB-treated capillaries in [24].

2.3. Procedures

A stock solution of fosfomycin (concentration 10 mg/mL) was prepared in Ringer's solution. This solution was stable for 4 months when kept at −80 °C. Pus samples from patients were stored at −80 °C directly after collection. Concentrations in patients' pus were determined by external calibration. Drug-free pus was spiked with concentrations of fosfomycin in the range of 10–1000 μg/mL and incubated at 37 °C for 20 min. Forty microlitres of pus from patients were mixed with 80 μL methanol, vortexed for few seconds and centrifuged at 15,000 × *g* for 2 min at room temperature to remove proteins and cell debris. Twenty-five microlitres of the supernatant were filled into an autosampler vial and injected into the CE system.

Evaluation of accuracy and precision of the method was performed at three different concentrations of fosfomycin spiked to drug-free pus, injected in triplicate on three different days.

3. Results and discussion

3.1. Separation of fosfomycin from matrix components

The selection of the conditions for separation of the anionic analyte from the anionic matrix components is less arbitrary when the kind of these components is known. In this case, one can derive information about a possible free migration window into which the analyte could be placed by varying the pH of the BGE. The typical electropherograms of blank pus obtained at two different pH values are shown in Fig. 2. pH 7.25 is chosen, because at this relatively high pH