Journal of Chromatography A, 1332 (2014) 1–7

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

A novel derivatization method for the determination of Fosfomycin in human plasma by liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometric detection via phase transfer catalyzed derivatization☆

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ARTICLE INFO

Article history:
Received 31 August 2013
Received in revised form 13 January 2014
Accepted 14 January 2014
Available online 24 January 2014

Keywords:
Fosfomycin
Phase transfer catalysis (PTC)
Liquid chromatography
Atmospheric-pressure chemical ionization (APCI)

ABSTRACT

An analytical method employing novel sample preparation and liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometric detection (LC–APCI/MS) was developed for the determination of fosfomycin in human plasma. Sample preparation involves derivatization through phase transfer catalysis (PTC) which offers multiple advantages due to the simultaneous extraction, preconcentration and derivatization of the analyte. Using a PT catalyst, fosfomycin was extracted from plasma in an organic phase and, then converted to a pentafluorobenzyl ester with the use of pentafluorobenzyl bromide (PFBBr) derivatization reagent. The method was fully optimized by taking into account both PTC and derivatization parameters. Several catalysts, in a wide range of concentrations, with different counter ions and polarities were tested along with different extraction solvents and pH values. Thereafter, the derivatization procedure was optimized by altering the amount of the derivatization reagent, the temperature of the reaction and finally, the derivatization duration. As internal standard (I.S.) ethylphosphonic acid was chosen and underwent the same pretreatment. The derivatives were separated on a pentafluorophenyl (PFP)-C18 analytical column, which provides unique selectivity, using an isocratic elution with acetonitrile–water (70–30, v/v). The method was validated according to US Food and Drug Administration (FDA) guidelines and can be used for a bioequivalence study of fosfomycin in human plasma. The correlation coefficient (r^2) of the calibration curve of spiked plasma solutions in the range of 50–12000 ng/mL was found greater than 0.999 with a limit of quantitation (LOQ) equal to 50 ng/ml (for 500 μL plasma sample).

1. Introduction

Fosfomycin, [(2R,3*S*)-3-methyloxiran-2yl]phosphonic acid, is a highly hydrophilic compound with low molecular weight [Fig. 1]. Fosfomycin is considered as a broad-spectrum antibiotic, which is commonly used for the therapy of uncomplicated lower urinary tract infections [1]. Furthermore, fosfomycin could be used as an alternative treatment agent for infections caused by gram-positive and gram-negative bacteria and also, for gastrointestinal infections [2]. Fosfomycin is available in the form of sodium salt (used for injection), calcium salt (for oral use) and also, trometamol derivative. Fosfomycin trometamol is highly soluble in water and provides enhanced bioavailability compared to the other forms [3], and for this reason is the most common used form.

Due to the high polarity of the compound and the complexity of plasma matrices, extensive sample preparation is required in bioanalysis. Commonly, sample preparation in plasma includes (a) liquid–liquid extraction, (b) solid-phase extraction or (c) protein precipitation[4]. Phase transfer catalysis (PTC) could be considered as an alternative technique of sample preparation and presents advantages because it combines the simultaneous derivatization, reaction, extraction and preconcentration [5–7].

Fosfomycin lacks ultraviolet (UV) absorption. Despite its extend use as antibiotic, only few methods have been developed for the determination of fosfomycin in biological matrices. The developed analytical methods include gas chromatography [8–12], liquid chromatography [13], ion exchange chromatography through

☆ Presented at the 39th International Symposium on High-Performance Liquid-Phase Separations and Related Techniques, Amsterdam, Netherlands, 16–20 June 2013.

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http://dx.doi.org/10.1016/j.chroma.2014.01.041

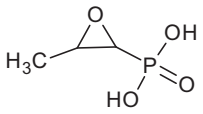

Fig. 1. Chemical structure of fosfomycin.

spectrometric detection [14], flow injection spectrophotometry [15], capillary electrophoresis [16–19] and microbiology [20]. However, the main drawback of the above methods is the low sensitivity/detectability [10–12,14,16,18].

The mechanism of PTC was, firstly, proposed by Starks in 1971 [21] and involves a quaternary ammonium salt (Q^+X^-) dissolved in the aqueous phase, which undergoes anion exchange at/or near the interface with the analyte anion (Y^-) in the aqueous phase. The ion-pair (Q^+Y^-) formed can cross the liquid–liquid interface due to its lipophilic nature and diffuses from the interface into the organic phase, this step being the "phase-transfer". In the organic phase, the species of (Q^+Y^-) being poorly solvated and quite nucleophilic (QY), undergoes a nucleophilic displacement reaction with the organic reagent (RX) to form the product (RY). The new ion pair (QX) returns to the aqueous phase and the cycle continues [5]. An overview of PTC reactions of fosfomycin is schematically described in Fig. 2.

The goal of the current work is to develop a sensitive, accurate and selective analytical method for the determination of fosfomycin in human plasma. Sample pretreatment includes the pentafluorobenzylation of fosfomycin through PTC, which provides multiple advantages compared to the common techniques of sample preparation. Also, to our knowledge is the first method employing PTC for the determination of fosfomycin in human plasma, achieving a LOQ (50 ng/ml), less than those reported in the bibliography. The method was optimized by univariate approach and validated, taking into account both PTC and derivatization parameters, and the results are theoretically interpreted.

2. Experimental

2.1. Reagents and chemicals

Fosfomycin trometamol (European pharmacopoeia grade) was purchased by LGC (UK). Ethylphosphonic acid (I.S., purity 98%), 2,3,4,5,6-pentafluorobenzyl bromide (PFBBr, purity 99%), tetrabutylammonium perchlorate (TBAPr, purity ≥ 98.0%) and tetrabutylammonium hydrogen sulfate (TBAHS, purity ≥ 99.0%) were purchased from Sigma–Aldrich (Steinheim, Germany). Tetrabutylammonium hydroxide (TBAOH, puriss p.a. for ion pair chromatography), tetrabutylammonium bromide (TBABr, puriss p.a for ion pair chromatography ≥ 99.0%), tetrabutylammonium phosphate monobasic (TBAP, purity ≥ 99.0%) and potassium dihydrogen phosphate (KH_2PO_4, purity ≥ 99.5%) were purchased from Fluka (Steinheim, Germany). All solvents used were of HPLC grade and obtained from Fisher Scientific (Loughbovough, UK). Water of HPLC grade (>18 MΩ cm) was used. Plasma, containing EDTA as anticoagulant, was kindly donated by plasma banks of domestic hospitals.

2.2. Solutions

Stock solutions of fosfomycin (1000 μg/mL) and I.S. (1000 μg/mL) were prepared in water and stored at 4 °C for 1 week (as has been proved by stability study). Working standard solutions of fosfomycin in the concentration range of 1.5–360 μg/mL used for the preparation of matrix (plasma) matched standards were prepared daily, by appropriate dilutions of stock solutions in water. The concentration of I.S. in the working solution was 200 μg/mL.

Solutions of TBAHS (0.20 M) and phosphate buffer (KH_2PO_4 0.5 M adjusted to pH 10.0 with NaOH) were prepared by dissolving the appropriate amounts of reagents in water. All the above solutions were stored at 4 °C up to 3 days.

2.3. Sample preparation

500 μL of plasma samples were transferred to a 10 mL-tube and 20 μL of the working standard solution of I.S. (200 μg/mL) was added. Thereafter, 500 μL of phosphate buffer pH 10.0 and 1000 μL of TBAHS 0.20 M were added. After vortex mixing for about 1 min, 1 mL of CH_2Cl_2 containing 50 μL of derivatization reagent (PFBBr) was added. The mixture was heated at 80 °C for 30 min, under vigorous agitation with magnetic stirring. The mixture was centrifuged at 40,000 rpm for 5 min and the organic layer was quantitatively transferred to a 10 mL-tube and evaporated, at ambient temperature, to dryness under nitrogen stream. The dry residue was redissolved in 100 μL of acetonitrile.

2.4. Preparation of spiked plasma standards

For the preparation of spiked plasma standards 2.9 mL of plasma were spiked with 100 μL of working standard solution of fosfomycin of the appropriate concentration. More specifically, for plasma standards of the concentrations of 0.050, 0.10, 0.50, 1.0, 4.0, 8.0, 12.0 μg/mL, 100 μL of working standard solutions of the following concentrations were added, respectively: 1.5, 3.0, 15, 30, 120, 240, 360 mg/mL. The plasma standards were treated as described in

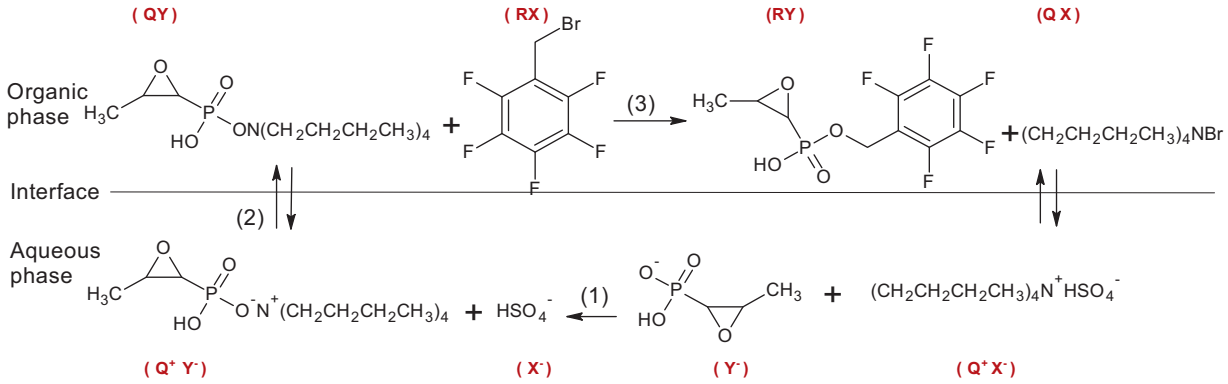

Fig. 2. Phase transfer catalytic reactions of fosfomycin with pentafluorobenzyl bromide (PFBBr): (1) Ion-exchange reaction between quaternary ammonium salt and the analyte anion. (2) The ion-pair crosses the liquid–liquid interface and is poorly solvated. (3) Nucleophilic displacement reaction with the derivatization reagent (PFBBr) takes place.