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Short communication

Development and validation of a high performance liquid chromatography assay for the determination of temocillin in serum of haemodialysis patients

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ABSTRACT

Therapeutic drug monitoring of β-lactams can be useful for the optimization of therapy, especially when little reference data exist on actual pharmacokinetic profiles such as in patients undergoing haemodialysis. There is no reported validated method for temocillin assay in serum samples, and preliminary studies evidenced potential for interferences by acidic metabolites and co-administered drugs in patients with advanced kidney failure. This paper describes a fully validated method for the determination of temocillin in human serum, and its applicability in haemodialysis patients. Temocillin was extracted from human serum by a solid phase extraction methodology, and then assayed by reversed-phase HPLC with UV-detection. The method was validated according to the accuracy profile methodology, using total error to verify the trueness, precision and overall accuracy. It showed high specificity and precision and was accurate in the concentration range of 5–400 mg/L. LOD and LOQ were 1.2 and 5 mg/L, respectively. No interference with 30 co-administered drugs was evidenced. The method was successfully applied to clinical samples from haemodialysis patients, showing a high degree of dialysability of temocillin.

1. Introduction

Temocillin is a β-lactam antibiotic active against Gram-negative bacteria. Its spectrum covers *Enterobacteriaceae* (including strains producing β-lactamases), *Haemophilus influenzae* and *Neisseria* spp. [1], but not *Pseudomonas*. It is licensed for use in septicemia, urinary tract and lower respiratory tract infections [2]. Its interest has been recently renewed due to the increased incidence of organisms producing extended-spectrum β-lactamases, making it to become a sparing-drug for carbapenems [3]. Temocillin is administered as an isomeric mixture (both isomers rapidly interconvert in aqueous solution to the equilibrium mixture) [4]. Although not performed in routine, β-lactam therapeutic monitoring is considered of potential interest in specific situations, in order to optimize the dosing regimens for reducing the risk of treatment failures and of emergence of resistance [5]. Little is known about dosing and elimination rate of temocillin in haemodialysis patients, who may also present a wide pharmacokinetic variability for antibiotics eliminated by renal route [6]. Determining temocillin serum concentrations in haemodialysis patients is challenged by the fact they often receive several other medications and accumulate several acidic metabolites (uric acid, hippuric acid, indoxylsulfate, p-cresylglucuronide, p-cresylsulfate, indole-3-acetic acid, or 3–carboxy-4-methyl-5-propyl-2-furanpropionic acid [7]) that can interfere in the assay [8]. There is no reported validated analytical method for temocillin assay in human serum. One study done in an intensive care setting [9] briefly describes the HPLC method used to analyze temocillin. However, when applied to samples from haemodialysis patients, it gave unsatisfactory results due to major interferences. The aim of the present study was therefore to establish and validate a reliable HPLC method to accurately quantify temocillin concentrations in the serum of patients with advanced kidney failure that could also be used while these patients were undergoing dialysis.

2. Materials and methods

2.1. Chemicals and reagents

Temocillin (NEGABAN®; ~68% R and ~32% S isomers) was provided by Eumedica s.a. (Brussels, Belgium). Ticarcillin disodium

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(~55% R and ~45% S isomers) used as internal standard (IS) was purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). HPLC-grade acetonitrile and other chemicals were obtained from VWR International (Radnor, PA, USA), Merck KGaA (Darmstadt, Germany) and Acros Organics (Subsidiary of Thermo-Fisher Scientific, Waltham MA, USA). Ultrapure water was obtained through a Milli-Q Academic apparatus (Millipore Corporation, Billerica, MA, USA).

2.2. Instrumentation and HPLC analysis

The HPLC system involved a Waters Alliance 2695 Separations Module equipped with a solvent degasser, a quaternary pump, and a temperature-controlled auto-sampler maintained at 4 °C, and was coupled to a Waters 2998 photodiode array (PDA) detector and operated with the Empower 2 software. Elution and resolution of the compounds were carried out with a RP-18 LiChrospher® column (250 × 4 mm, 100 Å, 5 μm) (Merck KGaA), with a isocratic gradient of sodium acetate (pH 7; 100 mM)–acetonitrile (95:5, v/v) at a flow rate of 1 mL/min, at 25 °C and in a 12 min run. The injection volume was 25 μL. PDA detection was monitored between 210 and 400 nm, with instrumental response expressed as the ratio between the areas of total temocillin (both isomers) and IS (both isomers) at 235 nm.

2.3. Calibrators and quality controls

Stock solutions of temocillin (10 mg/mL) were prepared in water, and used to prepare calibrators in human serum at 6 concentration levels in the 5–400 mg/L range. Four quality control (QC) samples were prepared from an independent stock solution. Both calibrators and QC samples were aliquoted, stored at −80 °C, and prepared with temocillin-free serum samples obtained from healthy donors (Centre de Transfusion de Woluwé-Saint-Lambert, Brussels, Belgium). The IS solution was prepared by reconstituting ticarcillin in ultrapure water to a final concentration of 1 mg/mL. Calibrators, QC samples, and IS solution were thawed on the day of use and discarded at the end of the day.

2.4. Sample preparation

All serum samples (calibrators, QC samples, or patient clinical samples) underwent a preanalytical procedure prior to HPLC analysis. It consisted in a newly developed solid-phase extraction (SPE) method, using Oasis® MAX cartridges (sorbent mass 1 cm^3/30 mg) placed in a vacuum manifold. Five hundred μL of samples were mixed to 500 μL of 4% orthophosphoric acid and 50 μL of IS, centrifuged (8600 g) for 20 min at 4 °C and the corresponding supernatant collected. Cartridges preconditioned successively with 1 mL of methanol and 1 mL of water were loaded with the supernatant, washed with 1 mL of sodium acetate (pH 7; 100 mM), 1 mL of methanol, and 1 mL of methanol:water:formic acid (39.2:58.8:2, v/v/v) solution. Temocillin and the IS were eluted with 1 mL of methanol:water:formic acid (78.4:19.6:2, v/v/v) solution. The eluates were transferred into an autosampler vial for HPLC analysis.

2.5. Method validation

Validation was designed according to the procedure of the *Société Française des Sciences et Techniques Pharmaceutiques* (SFSTP) [10,11]. The calibration experimental design was 6 × 2 × 3 (calibrators of 6 concentration levels, each one replicated twice over 3 days). The QC samples (4 concentration levels) were replicated 5 times over 3 days (4 × 5 × 3 experimental design). Data from calibrators were used to build the calibration curves (peak area ratios of temocillin/IS versus concentration), and the equation of the linear regression used to predict the actual concentrations of the QC samples. The accuracy profile methodology for method validation was applied [10,11]. Results were evaluated according to the FDA acceptance criteria for bioanalytical method validation [12]. Regarding specificity, 6 different blank serum samples were processed with and without temocillin and IS to ensure the absence of interfering peaks. In addition, serum from haemodialyzed patients not treated by temocillin but receiving a variety of other drugs (see details in Section 3) were examined for the presence of interfering substances by comparing chromatograms before and after spiking with temocillin. Trueness was expressed in absolute and relative bias (%) at each concentration of the QC samples [10,13,14]. Precision was evaluated as relative standard deviation (RSD%) values for repeatability and intermediate precision [14]. Accuracy was evaluated based on the two-sided 80% β-expectation tolerance intervals for the total measurement error, i.e. the sum of systematic and random errors of the analytical procedure [10,13,15]. Based on the trueness, precision and β-expectation tolerance intervals for each concentration level, an accuracy profile was constructed [10,13]. The LOQ was determined with the accuracy profile as the smallest concentration level where 80% β-expectation tolerance limits remained inside the ±20% acceptance limits [14,15]. The LOD was estimated using the mean intercept of the calibration model and the residual variance [16].

2.6. Clinical evaluation in haemodialysis patients

Applicability was tested in 4 haemodialysis patients who received a 2 g intravenous dose of temocillin immediately after a haemodialysis session preceding a 48 h interdialytic interval (protocol approved by the ethical committee of the AZ Sint-Jan Brugge-Oostende AV hospital in which patients were hospitalized [unique Belgian number: B049201215528]; all patients provided informed consent). Blood samples were processed locally (*AZ St-Jan*) for preparation of serum, followed by rapid freezing at −80 °C and transfer under dry-ice to the analytical laboratory (*Université catholique de Louvain*) where they were kept at −80 °C until analysis. To estimate temocillin dialysability, extraction ratio (ER) and haemodialysis clearance (CL_{HD}) were determined. ER was calculated as $(C_{s,IN}-C_{s,OUT})/C_{s,IN}$ where $C_{s,IN}$ and $C_{s,OUT}$ are the serum concentrations of temocillin flowing into and out of the dialyser, respectively. CL_{HD} was calculated as $CL_{HD} = Q_{dial}$ x ER, where Q_{dial} is the blood flow into and out of the dialyzer in mL/min. A low flux dialyzer Fresenius Helixone® FX8 with an A_{eff} 1.4 m^2 (Fresenius Medical Care AG & Co., KGaA, Gad Homberg, Germany) was used.

3. Results and discussion

3.1. Specificity and method optimization

Fig. 1 shows the chromatogram of a representative serum sample from a haemodialysis patient receiving temocillin (patient [a]) using this new method. Temocillin and IS peaks were well resolved, with retention times of approximately 6.8 min and 7.6 min (*R-S* isomers) for temocillin, and 10.1 min and 11.3 min (*R-S* isomers) for ticarcillin. Fig. 2 shows the chromatograms for serum samples of 3 random, non-infected, haemodialysis patients spiked with temocillin. Interfering peaks were systematically contaminating chromatograms obtained using the previously published method [9] but were no more observed with the current methodology. We attribute this improvement to the optimization of the solid phase extraction procedure (replacing the Oasis® HLB with