Journal of Pharmaceutical Analysis 2012;2(3):214–219

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

www.elsevier.com/locate/jpa
www.sciencedirect.com

ORIGINAL ARTICLE

Simple and sensitive determination of sparfloxacin in pharmaceuticals and biological samples by immunoassay

Hua-Jin Zeng[a], Ran Yang[b], Bing Liu[b], Li-Fang Lei[b], Jian-Jun Li[b], Ling-Bo Qu[b,c,*]

[a]*School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou 450001, China*
[b]*Department of Chemistry, Zhengzhou University, Zhengzhou 450001, China*
[c]*School of Chemistry & Chemical Engineering, Henan University of Technology, Zhengzhou 450001, China*

Received 20 November 2011; accepted 13 February 2012
Available online 21 February 2012

KEYWORDS

Sparfloxacin;
Enzyme-linked immunosorbent assay (ELISA);
Biological samples;
Pharmacokinetics;
Tissue distribution

Abstract Plasma quinolone concentrations are not routinely measured in clinical practice. However, in order to optimize quinolone treatment, monitoring of plasma concentrations could sometimes be useful particularly in critically ill patients. In this study, anti-sparfloxacin antibody was obtained by immunizing rabbits with sparfloxacin conjugated with bovine serum albumin using isobutyl chloroformate method. After the assay procedure was optimized, the standard curve of sparfloxacin was established. The practical measuring range of the competitive ELISA extended from 5 ng/mL to 2 μg/mL. The recovery rates and coefficients of variation for rat plasma, urine and tissues were 87.7–106.2% and 4.8–15.3%, respectively. To demonstrate the potential of the ELISA, a preliminary pharmacokinetics and tissue distribution study of sparfloxacin in rats and quantitative analysis of sparfloxacin in several pharmaceuticals were performed and compared with high-performance liquid chromatography (HPLC). The experimental data indicated that the proposed method would be a valuable tool in therapeutic drug monitoring (TDM) for sparfloxacin.

*Corresponding author at: School of Chemistry and Chemical Engineering, Henan University of Technology, Zhengzhou 450001, China. Tel.: 86 371 67756886.
E-mail address: qulingbo@zzu.edu.cn (L.-B. Qu)

Peer review under responsibility of Xi'an Jiaotong University.
http://dx.doi.org/10.1016/j.jpha.2012.02.001

Production and hosting by Elsevier

1. Introduction

Sparfloxacin (SPAR), a third-generation quinolone antimicrobial drug, is widely used in the treatment of urinary tract infections due to its excellent activity against various bacteria and good absorption on oral administration [1]. However, based on published results [2] and data on file with the manufacture of sparfloxacin, some adverse effects, including decreased appetite, vomiting, special sense adverse events and so on, would occur in some patients. Plasma quinolone concentrations are not routinely measured in clinical practice. However, in order to optimize quinolone treatment, monitoring of plasma concentrations could sometimes be useful particularly in critically ill patients.

In the past years, several methods, including bioassay [3], high-performance thin-layer chromatography [4], spectrophotometric method [5], high-performance liquid chromatography with ultraviolet [6,7] or fluorescent detection [8], and liquid chromatography-tandem mass spectrometry [9], have been applied in such a field; however, because of the complexity and diversity of biological samples, these methods have shown some disadvantages for the analysis of sparfloxacin, such as time-consuming, high background and requirement of sample pre-treatment. Moreover, owing to individual characteristics of the method, it is impossible to simultaneously determine multiple samples under the same conditions. So, it is necessary to establish different methods for different test samples, which made high throughput, real-time sparfloxacin detection difficult.

In recent years, because of its lower detection limit, high specificity, low background and no requirement of sample pre-treatment [10], enzyme-linked immunosorbent assay (ELISA) has been applied for the determination of fluoroquinolone antibiotics, such as ofloxacin [11], pefloxacin [12], lomefloxacin, norfloxacin, enrofloxacin [13] and ciprofloxacin [14,15]. However, few reports on the determination of sparfloxacin by ELISA were reported. Therefore, the aim of this article was to obtain the antibody of sparfloxacin and establish the ELISA method for determination of sparfloxacin by a comparative simple procedure. On such purpose, anti-sparfloxacin antibody was obtained by immunizing rabbits with sparfloxacin conjugated directly with bovine serum albumin (BSA) using isobutyl chloroformate method. A highly simple and sensitive ELISA for the determination of sparfloxacin in biological samples was developed.

2. Materials and methods

2.1. Materials and reagents

Sparfloxacin, enrofloxacin, lomefloxacin, ciprofloxacin, ofloxacin, norfloxacin, spiramycin and amoxicillin were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (China). Ovalbumin (OVA), bovine serum albumin (BSA), peroxidase-labeled anti-rabbit IgG, freund's complete and incomplete adjuvants were obtained from Huamei Bioengineering Co. (China). Tetramethybenzidine (TMB) substrate solution was obtained from Amresco Chemical Co. (USA). Polypropylene plate was purchased from Corning incorporated Co. (USA). All other chemicals used were of analytical grade.

Phosphate-buffered saline (PBS, 0.02 M phosphate buffer, pH 7.2, containing 0.15 M NaCl), coating buffer (0.05 M carbonate-hydrogen carbonate, pH 9.6), blocking buffer (0.02 M PBS containing 1% OVA), washing buffer (PBS-T, 0.02 M PBS containing 0.05% Tween 20) were used.

2.2. Preparation of an immunogen for sparfloxacin

Firstly, 10 μL of tribytylamine was added to a solution of sparfloxacin (35.7 mg, 0.91 mM) in 1.5 mL of 1,4-dioxane and the resulting solution was allowed to stand with vigorous stirring in the condition of no light and at room temperature for 40 min. 15 μL of isobutyl chloroformate was added slowly to the above solution and kept the solution with vigorous stirring for another 40 min at the same conditions. Subsequently, the reaction mixture was added dropwise to 7 mL BSA (123.9 mg, 1.81 μM) in water (pH 8.0), and under stirring incubated at 4 °C for 24 h. Last, the reaction mixture was dialyzed against deionized water for 3 days at 4 °C. The purified conjugate was lyophilized and used as the sparfloxacin immunogen.

The preparation of the sparfloxacin–OVA conjugate as a coating immunogen was similar to that of the sparfloxacin–BSA conjugate.

2.3. Preparation of sparfloxacin antibody

An aliquot containing 2 mg sparfloxacin–BSA complex was emulsified with an equal volume of Freund's complete adjuvant. Two Zealand white rabbits were each given multiple subcutaneous injections over sites along both sides of their backs. Booster injections were then given four times at bi-weekly intervals, using one-half the amount of the dose of the first immunization emulsified with incomplete Freund's adjuvant in the same ratio. After validation of antibody production by indirect ELISA, blood was collected from carotid artery. The sera were separated by centrifugation at 3000 rpm for 10 min and stored at −70 °C until use.

2.4. Standard solution preparation

The standard stock solution of sparfloxacin (0.5 mg/mL) was prepared in methanol and stored at 4 °C. Working solutions were prepared by appropriate dilution of the stock solution with 0.02 M phosphate buffer (pH 7.2) and eluent for ELISA and HPLC, respectively.

2.5. ELISA procedure

100 μL of the appropriate dilutions of sparfloxacin–OVA in coating buffer was added to each well of a polystyrene microtiter plate and incubated overnight at 4 °C. After incubation, the plates were washed three times with washing buffer. To reduce nonspecific binding, 150 μL of blocking buffer was added to each well and incubated at 37 °C for 2 h to block the unbound sites on the plastic surface. After washing the plate three times with washing buffer, 50 μL of the appropriate dilutions of anti-sparfloxacin antibody and 50 μL of the appropriate dilutions of sample solution were added to each well and incubated for 30 min at 37 °C, the plates were washed three times with washing buffer once more. Finally, 100 μL of TMB peroxidase substrate solution was added to each well. After incubation for 20 min in dark at room temperate, the reaction was terminated by addition of 50 μL of 2 M H_2SO_4. The activity of enzyme bound to the plate was measured spectrophotometrically at 450 nm using a microplate reader.

2.6. HPLC method

HPCL analysis was performed using an Agilent 1200 system, equipped with an Agilent UV–vis detector setting 290 nm and a Chromeleon software (Agilent) for calculation of peak area. The following conditions were maintained–column: Eclipse XDB-C_{18} (150 mm × 4.6 mm, 5.0 μm); eluent: acetonitrile-0.05 M phosphate buffer (pH 2.4) (15:85, v/v); flow rate: