Clinical Biochemistry 50 (2017) 73–79

Contents lists available at ScienceDirect

Clinical Biochemistry

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

Measurement of levofloxacin in human plasma samples for a reliable and accessible drug monitoring

Othoniel Hugo Aragon-Martinez [a], Mario Alberto Isiordia-Espinoza [b], Othir Galicia [a], Saray Aranda Romo [c], Alejandro Gómez Gómez [d], Silvia Romano-Moreno [e], Flavio Martinez-Morales [a,*]

[a] Department of Pharmacology, School of Medicine, University of San Luis Potosi, San Luis Potosi, S.L.P., Mexico
[b] Department of Research, School of Dentistry, Cuauhtémoc University, San Luis Potosi, S.L.P., Mexico
[c] Diagnostic Clinic Dentistry School, University of San Luis Potosí, San Luis Potosí, S.L.P., Mexico
[d] Center of Respiratory Diseases, San Luis Potosí, S.L.P., Mexico
[e] Department of Pharmacy and Drug Technology, School of Chemistry Sciences, University of San Luis Potosi, San Luis Potosi, S.L.P., Mexico

ARTICLE INFO

Article history:
Received 19 February 2016
Received in revised form 4 September 2016
Accepted 8 September 2016
Available online 13 September 2016

Keywords:
Levofloxacin
Validation
Liquid chromatography
Diode array technology
Drug monitoring

ABSTRACT

Objectives: Levofloxacin monitoring is recommended to obtain clinical cure and low incidence of antimicrobial resistance. During the monitoring procedure, levofloxacin should be measured in plasma samples and several assays are reported for this purpose. However, those methods do not have all of the characteristics for an accessible and reliable drug monitoring. For this reason, we develop a method that has all of the essential characteristics for levofloxacin monitoring.

Design and methods: The procedure of validation was done in terms of Food and Drug Administration guidelines. Subsequently, our assay was applied in plasma samples obtained from healthy volunteers with a single oral administration of levofloxacin as well as patients with respiratory diseases under levofloxacin therapy.

Results: Levofloxacin was extracted from samples using only two precipitation steps. Our assay had a rapid run time (5 min), adequate sensitivity (0.05 μg/ml of lower limit of quantification), and acceptable parameters of validation. Moreover, compound identities were supported using three dimensional spectra and purities were confirmed employing similarity factors (values > 900). Variable concentrations of levofloxacin in samples were observed during the application.

Conclusions: Levofloxacin is successfully quantified using our method that shows reliable results, appropriate range, rapid analyses, and cost-effective measurements under a simple and easy technique while all prior methods did not have it all together. Consequently, our method is a valuable tool for routine drug monitoring. Moreover, a complete evaluation of specificity was done for levofloxacin in plasma samples for the first time. Meanwhile, the application data supported the necessity of levofloxacin monitoring.

© 2016 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.

1. Introduction

Levofloxacin (LVX) is the active S-isomer isolated from the racemic ofloxacin and belongs to fluoroquinolone antibiotics that are employed extensively in anti-infective therapy [1,2]. LVX exerts its activity via inhibition of the bacterial synthesis of deoxyribonucleic acid showing a spectrum activity against Gram-positive bacteria, Gram-negative organisms, and intracellular pathogens [3]. For this reason, LVX is used widely for the treatment of several diseases such as respiratory, urinary, and skin infections [4].

Infectious diseases are the second cause of deaths worldwide [5]. During the treatment of infections, the drug monitoring is used to personalize the dosage for an antibiotic exposure related with a high probability of therapeutic success, low risk of toxicity, and low incidence of antimicrobial resistance [6]. Studies provide evidence that the recommended drug regimen for fluoroquinolones may be an inappropriate dosage and patients might be exposed to a high risk for appearance of quinolone resistance [7]. Actually, the crisis of antimicrobial resistance is one of the serious public health problems [8,9]. Consequently, the relationship between the maximum plasma concentration and the minimum inhibitory concentration (MIC) of the antibiotic alongside the relationship between the area under the concentration-time curve and MIC of the drug is the most relevant pharmacokinetic-pharmacodynamic ratios employed for the evaluation of the effective

Abbreviations: EDTA, ethylenediaminetetraacetic acid; FDA, Food and Drug Administration; LC, high-performance liquid chromatography; LLOQ, lower limit of quantification; LVX, levofloxacin; mAU, milli absorbance units; MIC, minimum inhibitory concentration; QC, quality control; rpm, revolutions per minute.

* Corresponding author at: Department of Pharmacology, School of Medicine, University of San Luis Potosi, 2405 Carranza Avenue, Los Filtros, 78210 San Luis Potosi, S.L.P., Mexico.
E-mail address: martinej@uaslp.mx (F. Martinez-Morales).

http://dx.doi.org/10.1016/j.clinbiochem.2016.09.011
0009-9120/© 2016 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.

clinical cure and prevention of antimicrobial resistance during fluoroquinolone therapy [10].

The therapeutic drug should be measured in plasma samples to obtain the pharmacokinetic parameters that are used for the calculation of fluoroquinolone ratios. In this way, several chromatographic methods are reported for the measurement of LVX in plasma or serum using chromatomass spectroscopy [11,12], fluorescence detection [1,13–15], or ultraviolet monitoring [2–4,16–21]. However, those techniques do not have all of the indispensable characteristics for the LVX monitoring because it is essential for the monitoring service to have rapid and cost-effective measurements, less laborious procedures, easy techniques, appropriate analytical ranges, and reliable results [22–25].

Consequently, the purpose of this work is to report a method that has all of the essential characteristics for an accessible and reliable LVX monitoring in plasma samples under a validation procedure according to international recommendations.

2. Materials and methods

2.1. Chemicals

LVX was acquired from Sigma-Aldrich (St. Louis, MO, USA). All reagents were of analytical grade except methanol and 2-propanol that were of LC grade (Mallinckrodt Baker Inc., Mexico City, Mexico). The ultra-pure water was obtained from a Millipore Milli-QUF Plus system (Millipore S.A.S., Molsheim, France).

2.2. Ethical aspects

This study was approved by the Health Research Ethics Committee from the University of San Luis Potosi (approval number 2015-001), and thus written informed consents were obtained from volunteers and patients. The present work was conducted in accordance with the Declaration of Helsinki and institutional standards.

2.3. Blank plasma sampling

Seven healthy volunteers above 30 years old were included in this sampling. From 32 to 40 ml of blood was collected in EDTA tubes via venipuncture of the antecubital vein of each volunteer in agreement with the Mexican normativity for disposal of human blood with therapeutic or research purpose [26]. The blood sample was immediately centrifuged at 4000 rpm for 10 min at 4 °C using a Sorvall RMC 14 Refrigerated Microcentrifuge (Thermo Fisher Scientific, Waltham, MA USA). Subsequently, the plasma was separated and frozen at −70 °C until use.

2.4. Stock solutions and quality control (QC) samples

Stock solutions of 500 μg LVX/ml were prepared in 7.0 mM potassium dihydrogen phosphate at a pH of 7.0 (phosphate buffer) each week. These solutions were immediately frozen at −70 °C until use. During the workday, blank plasma samples and stock solutions were thawed and handled at room temperature (15 ± 1 °C). The stock solutions were diluted at 100 μg LVX/ml using ice-cold phosphate buffer, and then these solutions were placed on ice. Blank plasma samples were added with different volumes of diluted stocks to obtain QC samples of 0.05, 0.10, 0.25, 0.50, 1.00, 2.50, and 5.00 μg LVX/ml. These QC samples were immediately processed as outlined in sample preparation. All of the described procedures in this section were kept out of direct sunlight.

2.5. Sample preparation

Two hundred fifty microliters of plasma sample was mixed with an equal volume of ice-cold 8.0% of perchloric acid solution. Subsequently, the mixture was centrifuged at 12,000 rpm for 10 min at 4 °C using a Sorvall RMC 14 Refrigerated Microcentrifuge (Thermo Fisher Scientific, Waltham, MA USA). The supernatant (308 μl) was added into a tube containing 17 μl of ice-cold 7.0 M of potassium hydroxide solution. This mixture was centrifuged at 12,000 rpm for 10 min at 4 °C. Finally, 180 μl of supernatant was separated, and then 100 μl of this plasma extract was placed in a vial insert inside an amber glass tube for chromatographic analysis. The sample preparation was performed at room temperature (15 ± 1 °C) and avoiding the sunlight exposure.

2.6. Equipment and separation-washing system

For the chromatographic analysis was employed a 1100 series Agilent LC system consisting of a quaternary pump with degasser, standard autosampler, thermostated column compartment, and diode array detector (Agilent Technologies, Palo Alto, CA, USA). The Agilent ChemStation software for LC 3D systems was used for the collection, data integration, and evaluation of specificity.

For the LC separation was used a Zorbax SB-C18 column with 5 μm of particle size, 4.6 mm of internal diameter, and 250 mm in length (Agilent Technologies, Palo Alto, CA, USA). The column compartment was maintained at 30 °C during the separation and the isocratic mobile phase was methanol-potassium dihydrogen phosphate (pH 3.5; 11.5 mM) (40:60, v/v). The analysis was performed with an injection volume of 25 μl of plasma extract, rate of 1.2 ml/min, monitoring at 294 nm, 5-min of run time, room temperature (15 ± 1 °C), out of direct sunlight, and drawing the spectra from 265 to 320 nm.

At the end of the workday, the column was eluted with mobile phase for 10 min followed by methanol-water (40:60, v/v) for 20 min at 40 °C. Subsequently, the washing protocol was applied starting in methanol-water (3:97, v/v) [27]. At the end of this protocol, the column was eluted with methanol-water (40:60, v/v) before starting the mobile phase for the LC separation.

2.7. Assay validation

The bioanalytical method was validated according to Food and Drug Administration (FDA) guidelines [28]. Consequently, the validation was done in terms of calibration curve, precision, accuracy, recovery, stability, and selectivity. In this manner, the linearity information was analyzed by both linear and second order polynomial regressions using the GraphPad Prism 5 software (San Diego, CA, USA) while between-day precision was done during three consecutive days. For the establishment of the lower limit of quantification (LLOQ), six independent blank plasma samples were added with different stocks of LVX, and then these samples were immediately processed as outlined in sample preparation. For the evaluation of stability, each QC sample was processed to obtain 360 μl of plasma extract, and then 180 μl of the extract was immediately placed in an amber LC vial for the measurement of LVX (zero value). This same sample was kept out of sunlight at room temperature (15 ± 1 °C) for their injection again after 2.5 and 5.0 h. Meanwhile, the remainder of plasma extract (180 μl) was immediately separated in two samples, and both rapidly frozen at −70 °C. Each sample was analyzed on day 3 or 7 after thawing at 15 ± 1 °C. Finally, every stability percentage was obtained to compare the experimental value with respect their zero value.

In addition, the diode array technology was employed to obtain spectra and purity values of LVX in samples. Each purity factor, so-called similarity factor, is a numerical value that characterizes the degree of dissimilarity of spectra records obtained from the chromatographic peak matching each spectrum to one another. For an adequate evaluation of purity, the spectra recording should have an appropriate wavelength range, corrected background, and normalized data. Values of <900 indicate dissimilarity; consequently, the presence of co-eluted or co-migrated impurities that contributed to the response of the chromatographic peak. More details about this topic are described in the report of Stahl [29].

Download English Version:

https://daneshyari.com/en/article/5510107

Download Persian Version:

https://daneshyari.com/article/5510107

Daneshyari.com