Journal of Pharmaceutical Analysis 2015;5(1):18–26

HOSTED BY

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

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

ORIGINAL ARTICLE

Development and validation of microbial bioassay for quantification of Levofloxacin in pharmaceutical preparations

Nishant A. Dafale*, Uttam P. Semwal, Piyush K. Agarwal, Pradeep Sharma, G.N. Singh

Microbiology Division, Indian Pharmacopoeia Commission, Ghaziabad 201 001, India

Received 9 April 2014; revised 6 June 2014; accepted 7 July 2014
Available online 21 July 2014

KEYWORDS

Levofloxacin;
Antibiotic resistance;
Microbiological bioassay;
HPLC;
Pharmacopoeia

Abstract The aim of this study was to develop and validate a simple, sensitive, precise and cost-effective one-level agar diffusion (5+1) bioassay for estimation of potency and bioactivity of Levofloxacin in pharmaceutical preparation which has not yet been reported in any pharmacopoeia. Among 16 microbial strains, *Bacillus pumilus* ATCC-14884 was selected as the most significant strain against Levofloxacin. Bioassay was optimized by investigating several factors such as buffer pH, inoculums concentration and reference standard concentration. Identification of Levofloxacin in commercial sample Levoflox tablet was done by FTIR spectroscopy. Mean potency recovery value for Levofloxacin in Levoflox tablet was estimated as 100.90%. A validated bioassay method showed linearity (r^2=0.988), precision (Interday RSD=1.05%, between analyst RSD=1.02%) and accuracy (101.23%, RSD=0.72%). Bioassay was correlated with HPLC using same sample and estimated potencies were 100.90% and 99.37%, respectively. Results show that bioassay is a suitable method for estimation of potency and bioactivity of Levofloxacin pharmaceutical preparations.

1. Introduction

Levofloxacin is a synthetic broad-spectrum antibiotic of fluoroquinolone group and is used to treat severe bacterial infections which failed to respond to other antibiotic classes [1,2]. Levofloxacin is chemically(S)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7 H-pyrido [1,2,3–de]-1, 4 benzoxazine-6-carboxylic acid hemihydrate (Fig. 1) with molecular formula $C_{18}H_{20}FN_3O_4$, $\frac{1}{2}H_2O$ and a molecular weight of 370.4 [3]. It is a yellowish white to yellow powder [3,4].

Levofloxacin is active against both Gram-positive and Gram-negative bacteria [5]. It is used in the treatment of bronchitis, urinary tract infections, pneumonia, skin and soft tissues infections [6]. This antibiotic can also be used to prevent infection after exposure to inhaled anthrax. Levofloxacin inhibits bacterial

*Corresponding author. *Current address*: CSIR-National Environmental Engineering Research Institute (NEERI), Nagpur, India.
Tel.: +91 7768984888.
E-mail addresses: nishant_s_m@rediffmail.com, nishant.dafale@gmail.com (N.A. Dafale).

Peer review under responsibility of Xi'an Jiaotong University.

http://dx.doi.org/10.1016/j.jpha.2014.07.007

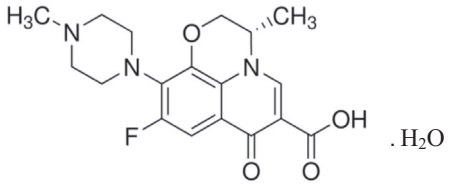

Fig. 1 Chemical structure of Levofloxacin.

topoisomerases II, topoisomerases IV and DNA gyrase, which are important enzymes required for DNA replication, transcription, repair and recombination, thereby inhibiting cell division [6,7].

Among all pharmaceutical products, the most commonly faked and adulterated ones are antibiotics probably because the frequency of their use is very high [8]. The misuse of antibiotics fosters the increase and spread of antibiotic resistance and may lead to superinfections [9]. An important factor in the development of drug-resistant strains of microorganisms is that many antibiotics are bacteriostatic rather than bactericidal [10]. In order to overcome the resistance problem and for the safe use of antibiotics, the correct measurement of potency and bioactivity of antibiotics is essential. Due to the increased resistance problem, the quantification of the actual concentration of active ingredients in antibiotic preparation is critical. A mild difference in the concentration of active ingredient in antibiotic preparations may have impact on actual efficacy. Therefore, quantification of active pharmaceutical ingredient (API) in antibiotic preparation is very necessary because most of the time these drugs are the lines that separate life from death [11]. These substances in very low concentrations are known to totally destroy or partially inhibit microorganisms [12].

The potency of antibiotics can be determined by chemical and biological methods. Chemical methods such as capillary electrophoresis, ultraviolet (UV) spectrophotometry, high performance liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC) have been used for the quantitative determination of Levofloxacin in formulations as well as in human urine, and serum [6,13]. However, the microbiological assay for determination of potency of Levofloxacin has not yet been reported in any pharmacopoeia. Biological method is the most convenient way to determine the potency of antibiotics [14].

Determination of antimicrobial potency is extremely important for the quality control and quality assurance concerning pharmaceutical preparations, being thus necessary to develop practical and economical methods which can be applied in the validation and dosage of drugs [15,16]. The application of microbiological assay has been recently developed for intravenously administered antibiotics. This method is highly acceptable by regulating authorities to control antibiotic potency [17,18]. Microbiological bioassay plays an essential role in the manufacturing and quality control of antibiotic medicines and demands considerable skill and expertise to assure success [18,19]. Microbiological assay helps in estimating active constituents, biological activity and in monitoring the stability of antibiotics. Any small change in the antibiotic molecule, which may not be detected by chemical methods, will be revealed by a change in antimicrobial activity [4]. Hence, microbiological assay is very useful for resolving doubts regarding possible change in potency of antibiotics and their preparations. A microbial bioassay requires effective and fully characterized microbial strains. The identification and characterization of microbial strain are performed by culturable and non-culturable techniques [20,21].

The potency of antibiotics can be measured by microbial bioassay, in which their inhibitory effect on the growth of test microorganisms is evaluated [3,4,14,22]. Bioassays do not require specialized equipment or toxic solvents [23]. The agar diffusion method widely used in antibiotic assay relates the size of the zone of inhibition to the dose of the antibiotic assayed. The relation of the diameter of inhibitory zones to concentration of antibiotic in a solution applied in cups has been considered theoretically [24,25]. The ability of an antibiotic is to inhibit or to kill the growth of living microorganisms. The inhibition of microbial growth in standardized conditions may be utilized for demonstrating the therapeutic efficacy of antibiotics. The antimicrobial activity of Levofloxacin in ophthalmic solution was measured using *Bacillus subtilis*, ATCC-6633 [26]. The in vitro activity of Levofloxacin was evaluated against 234 strains of *Mycobacterium tuberculosis* and MIC_{50} and MIC_{90} were obtained as 0.25 mg/L and 0.5 mg/L, respectively [27].

The proposed article focuses on the development and validation of a simple, sensitive, accurate, precise and cost-effective one-level agar diffusion (5+1) bioassay for the quantification of potency and bioactivity of Levofloxacin in pharmaceutical preparations.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals and reagents used were of analytical grade (Merck Ltd., Mumbai). Milli-Q water (Millipore) was used to prepare solutions. United States Pharmacopoeia (USP) reference standard of Levofloxacin was used for standard solution preparation. Commercial sample Levoflox tablet containing Levofloxacin 500 mg was obtained from the local market.

2.2. Equipment

All equipments used for the bioassay study were calibrated and validated. Sterilized glassware (Class B) such as Petri plates, test tubes, volumetric flasks, pipettes and sterile borer were used in the experiment. Steam Sterilizer/Autoclave (Make-Nat steel) was used to sterilize the media at 121 °C and 15 psi for 15 min. Glycerol stocks of microbial cultures stored at –80 °C Deep freezers (Make – Haier) were used as test strains. Identification of Levofloxacin was performed by an FTIR spectroscope (Perkin Elmer) and HPLC (Make-Agilent Technologies) was used for comparative study. Bioassay plates were incubated at 37 °C inside the incubator (Make-Thermolab) for bacterial growth. Zones of inhibition were measured by an antibiotic zone reader (Make-Aarachal Corporation).

2.3. Test microbial strains

Microbial cultures were procured from American Type Culture Collection (ATCC), USA, and National Collection of Type Cultures (NCTC), UK. The different Gram-positive bacteria *Bacillus cereus* (ATCC-11778), *B. pumilus* (ATCC-14884), *B. subtilis* (ATCC-6633), *Staphylococcus aureus* (ATCC-6538, 29737, 9144), *Staphylococcus epidermidis* (ATCC-12228), *Kocuria rhizophila* (ATCC- 9341), *Micrococcus luteus* (ATCC-10240) and Gram-negative bacteria *Escherichia coli* (ATCC-10536, 8739), *Salmonella abony* (NCTC-6017), *Pseudomonas*