Research Article

A new approach of ofloxacin analysis method in human blood plasma using solid-phase extraction - high-performance liquid chromatography - ultra violet

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ABSTRACT

Objective: The aim of this research was to develop a method for analyzing ofloxacin (OFX) assay in human plasma using solid-phase extraction (SPE)- high-performance liquid chromatography (HPLC)/ultra violet (UV) detector. In this work, SPE was employed in preparing for the analysis of OFX using HPLC-UV detector. **Materials and Methods:** Hydrophilic and lipophilic balance cartridge (100 mg, particle size 10 µm) of SPE was used in preparing a sample to determine further method of analysis using HPLC with phosphate buffer 0.025 M (pH 2.5) and acetonitrile (85.5:14.5) as mobile phase and a flow rate of 1.2 m l/min. UV detector was adjusted at 295 nm using internal standard ciprofloxacin. **Results:** Calibration curve was linear over the range of 0.1-6 µg/ml with correlation coefficient (r) = 0.9998-0.9999. The resolution was (Rs) > 1.5, and repeatability (% CV) <10%. Based on peak area and the peak height ratio of chromatogram, limit of detection and limit of quantification were 0.023 µg/ml and 0.076 µg/ml, respectively, and recovery of spiked OFX in human plasma was 94.32-100.45%. **Conclusion:** Based on the results of analysis, the analysis method was concluded as sensitive and valid for analysis of OFX in human plasma.

KEY WORDS: High-performance liquid chromatography, Human plasma, Ofloxacin, Solid phase extraction

INTRODUCTION

Ofloxacin (OFX) is a second generation formula of quinolone broad spectrum antibiotics (Figure 1). OFX is widely used for infections of the eye, urinary tract, digestive tract, respiratory tract, skin and soft tissue, joints and bones, infections by pneumococcus resistant to beta-lactam antibiotics, and macrolides, as well as for treating diseases transmitted through intercourse. The working mechanism of OFX inhibits bacterial protein synthesis, which in turn arrests topoisomerase II enzyme with the enzyme DNA gyrase and IV.[1]

Previous studies have investigated OFX in the biological fluid matrix, such as by the method of protein deposition in high-performance liquid chromatography (HPLC) with ultra violet (UV) detector, fluorescence detector, and photodiode array detector.[2-4] The methods of solid-phase extraction (SPE) - HPLC with fluorescence detector,[5] photodiode array detector,[6] and liquid chromatography-mass spectrometry[7] have been investigated. However, research analysis of OFX in human blood plasma using SPE for HPLC with UV detectors has not been reported. SPE extraction method is more effective compared to other extraction methods, one of the advantages being that it can isolate samples despite its small concentration in the matrix.[7,8]

Sample pretreatment is the main part that determines the efficacy in the analysis of drug or metabolites in a biological matrix. SPE is one solution since it can establish recovery and reproducibility of the matrix interference.[9-11] The parent metabolite compound (such as carbohydrate, protein, and lipid) should be eliminated as the presence of interference can cause misleading results in assay methods.[12] Therefore, it is necessary that the method can identify both the parent drug and metabolites accurately. The use of SPE in sample preparation can reduce time and solvent volume.[13,14] In the previous research, application of SPE in the determination of lead compounds of

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