Research Article

Development and Validation of UV Method for the Estimation of Diclofenac Sodium in Human Plasma

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ABSTRACT

A simple, rapid method for the estimation of diclofenac sodium in human plasma by using ultraviolet spectroscopy UV) is described. The method was developed and validated according, to ICH guidelines, for quantitative analysis and therapeutic drug monitoring of diclofenac sodium (DS) in human plasma. Plasma samples (0.7mL) were acid hydrolysis by 100µL, (1M) hydrochloric acid. Analytes were concentrated from plasma by liquid–liquid extraction with 2mL ethyl acetate by repeated twice, which allows to obtain good extraction yields. The Diclofenac sodium were detected at 276nm respectively. Method showed linearity with very good determination coefficients r^2=0.998, over the concentration range of 5-20µg/mL.

Keywords: Diclofenac sodium, human plasma, UV Visible spectrophotometer.

INTRODUCTION

Diclofenac sodium (DS), 2-*[(2,6-dichlorophenyl)amino]phenylacetic acid,* (Figure 1) is non-steroidal anti-inflammatory analgesic (NSAID) with potent cyclooxygenase (COX) inhibition activity[1]. DS has a well-documented safety profile, which is comparable to those of other NSAIDs[2]. It inhibits prostaglandin synthesis by inhibition of enzymatic transformation of arachidonic acid into prostaglandins[3,4]. This drug is widely used in clinical medicine for the pain control and treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis also acute injury. In addition to, it is used to treat chronic pain associated with cancer[5]. DS has anti-uricosuric feature. The development of Alzheimer disease may be prevent if use of DS which low-dose in long term.

Common side effects include abdominal pain, astrointestinal bleeding, nausea, dizziness, headache, and swelling[6]. Serious side effects may include heart disease, stroke, kidney problems, and stomach ulceration. Use is not recommended in the third trimester of pregnancy[6]. It is likely safe during breastfeeding. It is believed to work by decreasing the production of prostaglandin[7]. It blocks both cycloxygenase-1 (COX-1) and cycloxygenase-2 (COX-2).

DS is well-absorbed after oral administration with extensive hepatic metabolism. It is extensively (>99.7%) protein bound (albumin) in plasma and serum at therapeutic concentrations. Terminal half-life is 1-2 hours. Food can cause a delay in the onset of absorption and a reduction in plasma levels of approximately 30%. After absorption, approximately half of the absorbed dose is metabolized immediately by the liver, due to first pass metabolism. 35% of absorbed DS enters entero hepatic circulation. The distribution volume (Vd) of DS is 1.4 L/Kg. Cmax is reached at approximately 4 hours. Tmax is approximately 0.5-1 h. Elimination is rapid with 90% of the drug clearance taking between 3 to 4 hours. The DS metabolism products, which are mainly 4'- hydroxy (OH) diclofenac and minor monohydroxy metabolites are 3'-OHdiclofenac and 5'-OH diclofenac, are excreted by the urine (65%) and biliary (35%)[8].

The use of DS has been associated with occasional hepatictoxicity[9]. Although the etiology of this toxicity is not known, clinical evidence suggests that it may be due to an immune[10,11] or a non-immune mechanism[9,12,13]. In both cases, covalent modification of liver proteins may play an important role in the etiology of DS hepatotoxicity [14]. The fact that, CYP2C9 was known to have a major role in the oxidative metabolism of DS [15,16]. It seemed possible that CYP2C9 might metabolically activate DS into a reactive metabolite(s), which may have a role in DS hepatitis in humans.

Bioavailability, bioequivalence and therapeutic drug monitoring studies have received major attention from the pharmaceutical industry, health authorities and clinic. These studies are performed to evaluate the safety and efficacy of a genetic structure. Which studies as well as drug product development studies require rapid, simple, sensitive and reliable bioanalytical methods to monitoring the target drug in human plasma sample. Also for clinical studies, it is essential to establish accurate, sensitive and selective analytical techniques that permit detection and quantitative measurement of drug entities in biological and pharmaceutical samples[17]. Several methods have been reported for determination of DS including spectrophotometric[18,19], spectrofluorimetric[20,21],

polarographic[22], conductometric[23], high-performance liquid chromatography (HPLC)[24,25], gas chromatography mass spectrometry (GC-MS)[26] and capillary electrophoresis [27] in human plasma and other biological fluids. Some of these methods are not suitable for routine analysis because they need sophisticated instruments, not yet available in many routine control laboratories. Since, UV method is reliable, inexpensive and widely utilized.

The biological sample volume (0.7mL plasma), with small volume samples include simple, efficient and inexpensive extraction procedure. In the present study, our objective is to develop and validate a reliable, simple, fast, and inexpensive using UV detection for determination of DS in human plasma with the lower volume sample preparation according to ICH guidelines. The developed method is validated by using linearity, accuracy, robustness and specificity parameters according to ICH guidelines. Figure1.Shows the chemical structure of diclofenac sodium.

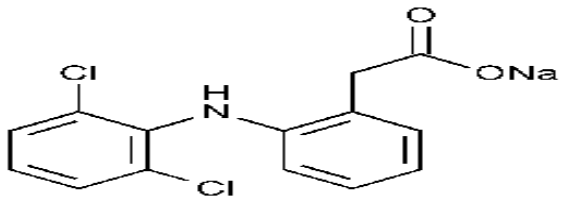

Figure 1: The chemical structure of Diclofenac sodium.

MATERIALS AND METHODS

Chemicals & reagents: Diclofenac sodium, Ethyl acetate (EtOAc), hydrochloric acid (HCl) & distilled water. All chemicals were of analytical grade.

Instrument: A ELICO SL 210 UV and visible recording spectrophotometer with two 10-20 mm matched quartz cells were employed for all absorbance measurements.

METHODS

Preparation of stock and working standard solution

The Stock solution of diclofenac sodium was prepared in distilled water at the concentration of 1000µg/mL respectively. The working standard solution of diclofenac sodium were prepared from stock solution by accurately 0.02 ml was pipette out and transfer into 10ml volumetric flask and the volume was made upto the mark with distilled water to give 20µg/ml concentration and its absorbance is measured at 276nm.

Sample Preparation

An aliquot of spiked human plasma sample (0.7ml) was taken in clean glass tubes which is containing 0.1ml HCL (1M) after that it is mixed for 30 seconds. Then a volume of 2ml of ethyl acetate was added to the sample tube and mixed for 2min and then centrifuged for 5 minutes. This extraction procedure was repeated twice. Supernatant was collected to another sample tube and dried under nitrogen at 40^0C. The residue which is obtained is measured at 276nm.

Calibration Studies

Calibration curve of diclofenac sodium was linear over the concentration range. Diclofenac was extracted from plasma by liquid extraction procedure. These methods are also the most comprehensive method which can extract diclofenac in a single extraction procedure. The standard solutions for the drug having concentration 2, 4, 6, 8, 10, 12, 14, 16, 18, 20µg/ml was prepared from the stock solution. The absorbance of solutions of pure Diclofenac sodium drug were measured at 276 λmax and a calibration curve was plotted between concentration of drug (µg/ml) on x-axis v/s absorbance on y-axis to get the linearity and regression equation which has shown in fig. 2.

Validation of Method

The bioanalytical method was validated to demonstrate the Linearity, Accuracy and Robustness.

Linearity

Linearity was determined by plotting the standard curve in the concentration range of diclofenac sodium 5, 10, 15, 20µg/ml and the volume was made upto 10ml with distilled water. The absorbance was measured at 276nm, using distilled water as blank and calibration curve of concentration vs absorbance is plotted.

Accuracy

Accuracy is defines as the measure of how close the experimental value to the true value. Accuracy expressed as relative error (RE %) of the estimated concentration. The instrument was accurate as assay. 6ppm of standard solution is spiked to 6ppm of sample solution. 3ppm of standard solution is spiked to 6ppm of sample solution. 9ppm of standard solution is spiked to 6ppm of sample solution

Robustness

Six aliquots of 10pm test solution were prepared and was scanned at wavelength of +1nm of λmax. The absorbance values are noted down.

RESULTS AND DISCUSSIONS

The development and validation of diclofenac sodium in human plasma was estimated by UV method at the wavelength of 276nm and the absorbance was found to be 1.2298. The graph shown in figure 1.

Linearity

The linearity of diclofenac sodium was found to be in the range 5-20µg/ml at 276nm, the regression equation was found to be Y=0.030x + 0.005 and correlation coefficient (r^2=0.998) shown in fig 2. The calibration data is shown in table 1.

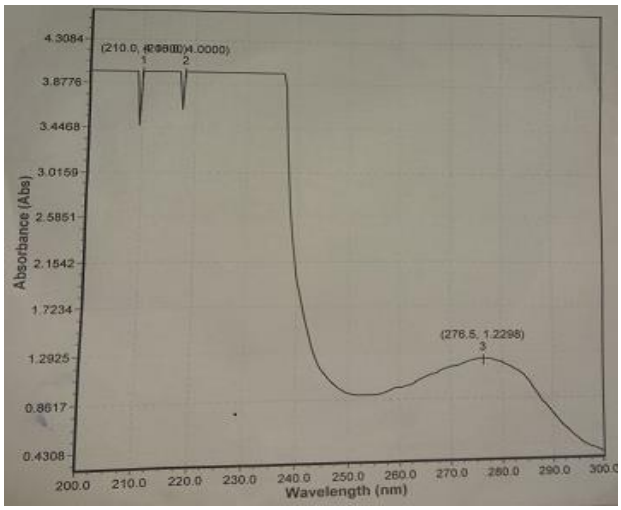

Figure 1: Spectrum of diclofenac sodium

Table 1: Calibration data of diclofenac sodium

Concentration	Absorbance at 276.5nm
5	0.164
10	0.318
15	0.446
20	0.617

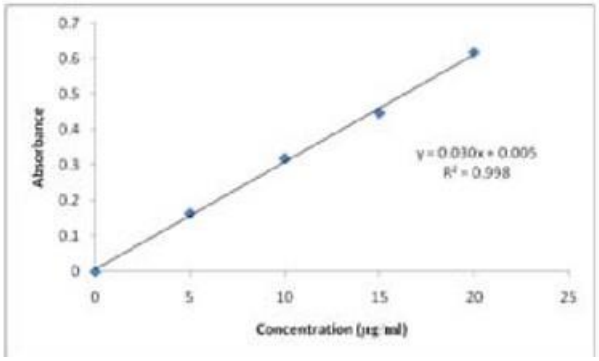

Figure 2: calibration curve of diclofenac sodium. Concentration on x-axis and absorbance on y-axis.

DICUSSIONS

Liquid extraction with UV detection is described for determination of diclofenac sodium in human plasma by UV detection of 276nm has been reported. The shorter time of analysis, simplicity of method is useful for pharmacokinetic and bioequivalence study of diclofenac sodium.

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