Indirect Determination of Diclofenac Sodium in Formulation by Atomic Absorption Spectrophotometry after Complexation with Copper or Iron

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ABSTRACT
The study was carried out for indirect determination of diclofenac sodium in formulations by AAS after complexation with Cu or Fe. The diclofenac was treated with the metals individually and the metal complexes formed as precipitates were extracted in chloroform or separated by centrifugation. A decrease in concentration of metal ions was observed in aqueous phase which was correlated with concentration of diclofenac sodium. The complexation reactions were optimized in terms of pH, nature of metal ion, extraction in chloroform or separation by centrifugation. The decrease in concentration of metal ions in aqueous phase was monitored using flame Atomic Absorption Spectrophotometry (AAS). The linear calibration range observed was from 10–80 µg mL⁻¹ for both methods. The limit of detection was 4 µg mL⁻¹ diclofenac using Cu or Fe ions using solvent extraction 5 µg mL⁻¹ by precipitation. The method was repeatable with interday and intraday reproducibility with relative standard deviation within 5%. The method was applied for the determination of diclofenac from pharmaceutical preparations, Voltoral, Voren, Qufen, Dicloplus, Dicloran with RSD% 0.01-8.8 with relteve deviation within 0.6-6.5%.

Keywords: Diclofenac sodium, copper, iron, complexation, atomic absorption spectrometry

INTRODUCTION
Copper and Iron are the important metals because of their biological activities in living⁴. Cu is fundamental component of respiratory enzyme complex and also has synergetic functions with drugs⁵. Iron is also the metallic element present at the various sites of numerous significances including redox enzymes dealing with cellular breathing and reduction and oxidation in animals and plants cells⁶. Several complex compounds of iron are recognized. A characteristic six-coordinate anion is originated in the mixed salt⁴. Geometric isomers are example of iron complexes with multi bidentate ligands⁷. Coordination complexes are formed by copper with ligands. Complexes of copper are formed with oxy-anion contain copper (II) acetate, copper (II) carbonate and copper (II) nitrate. Cu (II) complexes are generally in octahedral geometry and most of the complexes solutions are in blue color⁸. Diclofenac, (2,2,6-dichlorophenylamino) phenylacetate) is a phenylacetic acid imitative⁹, the most commonly used as pain killer
and is chemically used as sodium salt. It is a nonsteroidal anti-inflammatory drug (NSAID) that reveals anti-inflammatory and pain-relieving actions in both animals and human beings. Diclofenac ligand reacts with various metals to form complexes. The diclofenac ligand has been found to act as bidentate chelating agent. The diclofenac coordinate through the oxygens of carboxyl group. The molar ratio chelation is 1:2 (M:Diclofenac) with general formula (M(diclofenac)₂(H₂O)₃) nH₂O. The complex formation of diclofenac with several metal ions have been reported such as copper, iron, nickel, cadmium, calcium, magnesium and also others in the presence of excess of metal ion. Formation of Diclofenac-metal complex depends on nature of metal, pH of the solution and speed for the precipitation. According to survey of literature some diclofenac-metal complexes are soluble and others are insoluble precipitates in aqueous medium. Preparation and characterization of stable monomeric copper and iron complexes with anti-inflammatory drug diclofenac are reported with formula [Cu (diclofenac)₂(H₂O)₃] H₂O and [Fe(diclofenac)₂(H₂O)₃]₂H₂O respectively. Complex formation occurs in water medium.

A number of procedures have been reported for the determination of diclofenac sodium in pharmaceutical preparations including Raman spectoscopy, Spectrophotometry, Spectrofluorometry, Indirect fluorometric determination, Indirect digital image based (webcam) flame emission spectrometric and indirect atomic spectrometric methods. The reported procedures are sensitive, but the determinations using atomic absorption spectrometry are considered as easy and equipment is also frequently available in most of analytical laboratories. Issa et al. determined diclofenac sodium by oxidation of the drug by iron (III). The excess of iron (III) was extracted in diethyl ether and iron (II) remaining in aqueous solution was determined by air-acetylene flame AAS.

This study focuses on the formation of diclofenac-metal complexes which are in the form of insoluble precipitates. These precipitates were extracted in chloroform or centrifuged from centrifuge machine. For the preparation of diclofenac-metal complexes, first pure diclofenac drug was used for the formation of complex with metal ion, then used pharmaceutical preparations of diclofenac sodium. The research is indirect determination of diclofenac, therefore after complexation aqueous solution content for metal ion was analyzed instead of diclofenac-metal complex.

**MATERIALS AND METHODS**

**Apparatus and chemicals:** The 10 mL of glass test tubes with stand and holder, pipet filler, volumetric flasks, funnel, whatman filter papers, paper tape, iron stand, etc. were used. Diclofenac sodium (Novartis Pharms Jamshoro), Chloroform (Merck Germany), Copper sulphate, ferrous sulphate, (Fisher Scientific USA) were also used. Granted reagents grade ammonium chloride, potassium chloride, boric acid, acetic acid, sodium tetraborate, ammonium acetate, sodium acetate, ammonia solution and hydrochloric acid (37%) were from Merck, Dramstadt, Germany. Stock solutions of diclofenac drug contain 1 mg mL⁻¹ was prepared in distilled water. Copper sulphate and ferrous sulphate solutions containing 1 mg mL⁻¹ of metal ion were prepared in distilled water. A few drops of acid were added before the adjusting the volume. Buffer solution (0.1 M) between pH 1 to 10 at unit interval were prepared from the following, potassium chloride adjusted with hydrochloric acid (pH 1-2), acetic acid with sodium acetate (pH 3-6), ammonium acetate (pH 7), boric acid-sodium tetraborate (pH 8-9) ammonium chloride-ammonia (pH 10).

**Procedure:** Formation of Copper and Iron-diclofenac Complexes: Stock solutions of Cu (II) and Fe (II) (1000 ppm) were diluted to 100 ppm. Five clean and dry well stopper test tubes were arranged in stand and were marked up numbers on each test tube in increasing concentration of drug solution. 1 mL of (100 μg mL⁻¹) solution of Cu (II) or Fe (II) was added to each test tube. Standard solution of diclofenac drug (100 μg mL⁻¹) was then added in increasing order 0.1, 0.2, 0.4, 0.6 and 0.8 mL (10-80 μg), followed by 0.5 mL of pH 8 buffer solution in Cu-diclofenac and pH 7 in Fe-diclofenac were added and the contents were mixed well. The reaction mixture was kept for 2 h at room temperature and green colored precipitates of both metal-diclofenac complexes appeared in aqueous solutions. A decrease in concentration of metal ions was observed through the increasing precipitates of complex yield. The precipitates of Cu (II) or Fe (II) complex formed were separated by two methods. Solvent Extraction Method; 1 mL of chloroform was added in each test tube contain precipitate of metal-diclofenac (Cu or Fe) and the contents were mixed well. The supernatant liquid containing excess of metal ion was separated. Centrifuge method: The test tubes containing Cu (II) or Fe (II) complex, were centrifuged at 4500 rpm for 20 min at 25°C. The precipitate settled down in the bottom of test tube and the supernatant liquid containing Cu (II) or Fe (II) ion was separated. The Supernatant liquid from the test tubes was transferred to volumetric flask and final volume was adjusted to 10 mL with distilled water and analyzed by AAS for metal contents. The concentration of copper and iron in the solutions was evaluated from linear regression equation of
external calibration curve prepared on AAS at 324 and 248.3 nm for copper and iron respectively in the range of 1-10 µg mL⁻¹.

**Determination of diclofenac sodium in pharmaceutical preparations:** The pharmaceutical preparations Voltral [Novartis Pharma Jamshoro], Voren [Asian Continental Pharma Karachi], Qufen [High-Q Pharma Karachi], Dicloran [Sami Pharma Karachi] and diclofenac were obtained from local market and were analyzed as follows.

The pharmaceutical tablets containing 100 mg of diclofenac sodium were weighed. The weight of the Voltral tablet was (292 mg), Voren (170 mg), Qufen (205 mg), Dicloran (384 mg) and Dicloplus (310 mg). Five tablets of each sample were ground to fine powder and the powder containing 25 mg of diclofenac sodium was weighed and dissolved in distilled water, filtered and volume adjusted to 25 mL. Each of solution was diluted 10 times. Solution of Cu (II) or Fe (II) containing 100 µg each was transferred to test tube followed by 0.4 mL (40 µg) of drug solution of each sample separately. The remaining procedure was followed as above. The concentration of diclofenac sodium drug in sample was calculated from the regression equation of calibration curve:

\[ Y = ax + b \]

**Instruments for samples analysis:** The pH of solutions was measured with an Orion 420A pH meter (Orion Research Inc., Boston, USA) combined with glass electrode and reference internal electrode, Air-acetylene flame Atomic Absorption Spectrophotometer (AAS) (Perkin-Elmer AA 800 model Singapore), with standard burner head was operated at the conditions recommended by the manufacturer. The equipment was controlled by the computer with winlab software. The analysis was carried out at least in triplicate (n=3), with integration time 3 sec and delay time 3 sec. Centrifuged machine (Allegra 64R centrifuge Backman, USA) was used throughout the study.

**RESULTS AND DISCUSSION**

The diclofenac sodium reacts with copper (II) and Iron (II) to form metal complexes. The complexes are slightly soluble in water and turbidity or precipitate formation generally takes place in aqueous phase. Therefore excess of Cu (II) or Fe (II) was added to diclofenac sodium solution and turbidity or precipitate formed was separated by solvent extraction or centrifugation. The decrease in the concentration of Cu (II) or Fe (II) in aqueous phase was proportional to the concentration of diclofenac sodium. The concentration of Cu (II) or Fe (II) in aqueous phase was monitored by flame atomic absorption spectrophotometer. The effect of pH on the complexation, solvent extraction or precipitation by centrifugation of Cu (II) or Fe (II) complexes with diclofenac sodium was examined within pH 2-10 at unit interval following analytical procedure. Both the metal ions indicated the formation of greenish precipitates at near to neutral pH. The pH for Cu (II) and Fe (II) were optimized at 8 and 7, respectively, based on the maximum decrease in the concentration of Cu (II) and Fe (II) in aqueous solution, after the addition of same amount of standard of diclofenac solution to Cu (II) or Fe (II) solution.

Chloroform, carbon tetrachloride, benzene, n-butanol and amyl alcohol were examined for the extraction of Cu (II) or Fe (II) complex of diclofenac sodium, but chloroform gave better results and was selected. The effect of concentration of diclofenac sodium on the decrease in the concentration of Cu (II) and Fe (II) was examined using both solvent extraction and centrifugation methods. A linear calibration curves were obtained with 10-80 µg mL⁻¹ diclofenac sodium using either Cu (II) or Fe (II) as monitoring metal ion using either solvent extraction or centrifugation technique. The coefficient of determination \( r^2 \) for the calibration curves were obtained with 0.9881-0.9937 (Fig. 1, 2) and 0.9745-0.9916 (Fig. 3, 4).

![Fig. 1: Linear calibration curve between Cu (II) and diclofenac sodium by solvent extraction](image1)

![Fig. 2: Linear calibration curve between Fe (II) and diclofenac sodium by solvent extraction](image2)
Table 1: AAS determination of diclofenac sodium in different pharmaceutical preparations by solvent extraction and centrifuge method

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Pharmaceutical preparations containing 100 mg/tablet diclofenac sodium</th>
<th>By solvent extraction method</th>
<th>By centrifuged method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diclofenac found by FAAS method using Cu (II)</td>
<td>Diclofenac found by FAAS method using Fe (II)</td>
<td>Diclofenac found by FAAS method using Cu (II)</td>
</tr>
<tr>
<td></td>
<td>RSD% n=3</td>
<td>RSD% n=3</td>
<td>RSD% n=3</td>
</tr>
<tr>
<td>1</td>
<td>Voltral</td>
<td>102.41 mg (6.0%)</td>
<td>102.15 mg (1.1%)</td>
</tr>
<tr>
<td>2</td>
<td>Voren</td>
<td>96.508 mg (2.5%)</td>
<td>100.94 mg (1.7%)</td>
</tr>
<tr>
<td>3</td>
<td>Qufen</td>
<td>102.55 mg (2.5%)</td>
<td>100.60 mg (2.2%)</td>
</tr>
<tr>
<td>4</td>
<td>Dicloplus</td>
<td>98.680 mg (3.7%)</td>
<td>95.448 mg (2.5%)</td>
</tr>
<tr>
<td>5</td>
<td>Dicloran</td>
<td>103.43 mg (6.8%)</td>
<td>99.725 mg (1.4%)</td>
</tr>
</tbody>
</table>

The relative error was obtained within ±4.9%. The repeatability of the analytical procedures was examined inter (n=5) and intraday (n=5) by the same operator at the final concentration of diclofenac sodium at 50 µg mL⁻¹. The relative standard deviation obtained were within ±5% and ±4.5% using Cu (II) and Fe (II) as monitoring ion respectively. The analytical methods developed were applied for the analysis of pharmaceutical preparations voltral, voren, Qufen, Dicloplus and Dicloran tablets, each containing 100 mg/tablet diclofenac sodium. The results of analyses are recorded in tablets and agreed with labeled values with RSDs within 0.01-6.8% (Table 1) for all the procedures. Now comparing the all four procedures examined, all indicated acceptable limits of quantitation, but the procedure with solvent extraction indicated better linear calibration curves than centrifugation methods. Again, comparing the results of copper (II) and iron (II), a better linearity of calibration curve was obtained using iron (II).

The method was compared with recent indirect spectrophotometric determination of diclofenac, based on oxidation with bromosuccinimide with linear calibration range 1-18 µg mL⁻¹ and HPLC analytical method for the determination of diclofenac sodium in tablets with linear calibration range 10-200 µg mL⁻¹ and lower limit of detection 12.5 µg mL⁻¹. The results indicate comparable similarity, but the use of simple chemicals, together with commonly used flame atomic absorption spectrophotometer are the added advantage of the present methods.

**CONCLUSION**

The determination of diclofenac sodium was carried out indirectly via atomic absorption spectrophotometry from pharmaceutical preparations. Diclofenac sodium reacted with copper (II) or ferrous (II) and the complexes were formed as precipitates. A decrease in the concentration of metal ion was correlated with the concentration of diclofenac sodium. The complex precipitates were separate out either by solvent extraction or by the centrifugation methods. The excess of Cu (II) or Fe (II), as a probe ion was determined by Atomic
Absorption Spectrophotometric technique for both the determination of diclofenac sodium in the pure form and in pharmaceutical preparations.

REFERENCES