Diclofenac Sodium Determination in Pharmaceutical Preparations After Complexation With Calcium and Magnesium and Molecular Modelling Study.

Waheed Ali Soomro (waheedsoomro88@yahoo.com)
University of Sindh

Muhammad Yar Khuhawar
University of Sindh

Taj Muhammad Jahaangir
University of Sindh

Muhammad Farooque Lanjwani
University of Sindh

Rafi-u-Zaman Brohi
University of Sindh

Imran Khan Rind
University of Sindh

Research Article

Keywords: diclofenac sodium, Calcium, Magnesium, molecular modelling, indirect determination

DOI: https://doi.org/10.21203/rs.3.rs-224136/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

The study was carried out for indirect determination of diclofenac sodium in preparations after complexation with Ca or Mg. 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) or by complexometric titration with EDTA. The linear calibration range observed were from 40-200 µg /mL and 40-200ug/mL diclofenac sodium respectively by using both Ca and Mg. The limit of detection was 15 µg/mL diclofenac using Ca or Mg ions using both solvent extraction and precipitation methods with AAS. 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, Voltral, Voren, Qufen, Dicloplus, Dicloran with RSD% 1.3%-8.2%.

Introduction

Calcium is 5th and Magnesium is 9th most abundant elements found in the Earth's crust [1][2]. Both are the key element of a strong nutrition and a mineral essential for life. These are vital for all livings; calcium is essential for the bones and teeth. The chief component of bones is calcium phosphate [3]. Magnesium is an important element for animals as well as plants. In plants photosynthesis process is taking place by chlorophyll that contain magnesium. [4]. Calcium and Magnesium are also essential generally for all cells of human body, the number of cellular processes functioning by calcium ion [5]. Significant collaboration between magnesium ions and phosphate is essential for humans. In humans, several enzymes are functioning through magnesium ion which are related compounds like RNA, ATP, and DNA. Magnesium ions form complexes with these compounds [4]. The average human body has approximately 1 kg of calcium and 20g of magnesium in intra and extra cellular, especially in bones [6] [7]. Maintenance of the magnesium level in serum is by renal secretion and gastric absorption [8]. Calcium is also essential for pregnant females and children for their growth. Deficiency of calcium rises to poor blood clotting and rickets a condition in which the bone weakens and cracks. Calcium complements are used to inhibit calcium deficits. Vitamin D content in things is main source of calcium [9]. Hypomagnesaemia is the cause of magnesium deficiency. Due to increasing loss of magnesium by gastrointestinal and renal also cause of deficiency [10]. [11][12].

Diclofenac, (2,(2,6-dichlorophenylamino) phenylacetate) is a phenylacetic acid derivative[13], the most usually used as pain killer and is chemically used as sodium and potassium salts. It is a nonsteroidal anti-inflammatory drug (NSAID) that exposes anti-inflammatory and pain-relieving actions in both animals and human beings [14]. Diclofenac reacts with numerous 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
The complex formation of diclofenac with several metal ions have been reported such as copper, iron, nickel, cadmium, calcium, magnesium and others in the presence of excess of metal ion [15]. 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. Structurally calcium ion is comparatively big ion that inclines to accept a great coordination number in the complexes. Preparation and characterization of stable monomeric Calcium and magnesium complexes with anti-inflammatory drug diclofenac is reported with formula

\[
\text{[Ca (diclofenac)2(H2O)2]2H2O and } [\text{Mg(diclofenac)2(H2O)2]2H2O respectively. Complex formation is in 100 }\%
\]

water medium. Calcium carbonate solution and Magnesium chloride solution reacted with diclofenac sodium salt. The reaction conditions for both the complex formation was at room temperature in acidic condition pH3. The precipitates of calcium- diclofenac and magnesium-diclofenac complexes are in white colour [15].

Several procedures have been reported for the determination of diclofenac sodium in pharmaceutical preparations including indirect flow injection spectrophotometric method [16], indirect spectrophotometric method [17], square wave voltammatric method [18]. Spectrophotometry [19], Spectrofluorometry [20,21], Indirect fluorometric determination [22], Indirect digital image based (webcam) flame emission spectrometric [23] and indirect atomic spectrometric methods [24][25]. 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. Currently diclofenac sodium has been determined indirectly after complexation with copper or iron [25].

This study focuses on the formation of diclofenac-metal complexes with calcium or magnesium which are in the form of insoluble precipitates. These precipitates were extracted in chloroform or centrifuged from centrifuge machine. For the preparation of diclofenacmetal 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 with calcium or magnesium in aqueous solution, the content for metal ion was analysed instead of diclofenac-metal complex.

**Materials And Methods**

Apparatus and chemicals: The 10 mL of glass test tubes with stand and holder, pipit filler, volumetric flasks, funnel, Whatmanfilter papers, paper tape, iron stand, buret, conical flask etc. were used. Diclofenac sodium (Novartis Pharmas Jamshoro), Chloroform (Merck, Germany), Copper sulphate, ferrous sulphate, EDTA, merioxide, (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 was prepared in distilled water. Calcium (II) and magnesium (II) solutions containing 1 mg/mL metal ions were prepared in distilled water from calcium carbonate and magnesium chloride. 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 Calcium and Magnesium-diclofenac Complexes:
Stock solutions of Ca (II) and Mg (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) solution of Ca (II) or Mg (II) was added to each test tube. Standard solution of diclofenac drug (100 µg /mL) was then added in increasing order 0.0, 0.4, 1.0, 1.5 and 2.0 ml (40-200µg/ml), followed by 0.5 ml of (pH3) buffer solution in Ca-diclofenac and Mg-diclofenac and the contents were mixed well. The reaction mixture was kept for 2 h at room temperature and whitecoloured 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 Ca (II) or Mg (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 (Ca or Mg) and the contents were mixed well. The supernatant liquid containing excess of metal ion was separated. Centrifuge method: The test tubes containing Ca (II) or Mg (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 Ca (II) or Mg (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 analysed by AAS for metal contents. The concentration of Ca and Mg in the solutions was evaluated from linear regression equation of external calibration curve prepared on at 422.7 nm and 285.2 nm for calcium and Magnesium respectively in the range of 1-10 µg/ ml.

The Supernatant liquid containing Ca (II) or Mg (II) ions were also analysed by EDTA titration method for the analysis of Calcium and magnesium. The 5 ml of sample was taken, added few mash of “muri oxide“ indicator and also 1 ml of (0.1M NaOH) then titrated it against 0.1 M solution of EDTA, the wine red colour changed in to the blue at the end point and noted the burette reading and repeated similar procedure for three times. Obtained values were aggregate with the results obtained through AAS.

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 dicloplus (Abbot phama) 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 (1 mL) of Ca (II) or Mg (II) containing 100 µg each was transferred to test tube followed by 1.0
mL (100 µg) of drug solution of each sample separately. The remaining procedure was followed as above
and analysed through AAS as well as EDTA titration techniques. 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.

**Computational Study**

In this study the computational models showing interactions of calcium with diclofenac and magnesium
with diclofenac were also studied. Molecular modelling calculations were conducted using Gaussian 09W
software package. The optimizations of molecular models were performed using semi empirical
calculation procedure with PM6 level.

**Results And Discussion**

The diclofenac sodium reacts with Calcium (II) and Magnesium (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 Ca (II) or Mg (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 Ca (II) or Mg (II) in aqueous phase was proportional to the concentration of diclofenac
sodium. The concentration of Ca (II) or Mg (II) in aqueous phase was monitored by flame atomic
absorption spectrophotometer or EDTA titration. The effect of pH on the complexation, solvent
extraction or precipitation by centrifugation of Ca (II) or Mg (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 whitish precipitates at acidic pH. The pH for Ca (II) and Mg (II) were optimised at 3, , based
on the maximum decrease in the concentration of Ca (II) and Mg (II) in aqueous solution, after the
addition of same amount of standard of diclofenac solution to Ca (II) or Mg (II) solution. Chloroform,
carbon tetrachloride, benzene, n-butanol and amyl alcohol were examined for the extraction of Ca (II) or
Mg (II) complex of diclofenac sodium, but chloroform gave better results and was selected. The effect of
The concentration of diclofenac sodium on the decrease in the concentration of Ca (II) and Mg (II) was examined using both solvent extraction and centrifugation methods. A linear calibration curves were obtained with 40-200 µg/ml diclofenac sodium using either Ca (II) or Mg (II) as monitoring metal ion using either solvent extraction or centrifugation technique. The coefficient of determination (r²) for the calibration curves were obtained with 0.9945-0.9938 (Fig. 1, 2) and 0.9897-0.9853 (Fig. 3, 4).

Limit of detection (LOD) measured as 3 times the standard deviation of the slope was calculated 15µg/ml using solvent extraction procedure and centrifugation technique separately using both Ca (II) or Mg (II). In order to test the validity of the calibration curves 4 test solutions of diclofenac sodium within the calibration range for each determination were analyzed and relative errors were obtained within ±4.5%. The effect of drug additives glucose, fructose, gum acacia and methylparaben for their possible interfering effects on the determination of diclofenac sodium were examined using Ca (II) or Mg (II) as indicating ions. The concentration of additives added was at least twice the concentration of the analyte. The results obtained were compared with the analyte without addition of the additives.

The relative error was obtained within ±4.9%. The repeatability of the analytical procedures was examined inter (n=3) and intraday (n=3) by the same operator at the final concentration of diclofenac sodium at 50 µg/ml. The relative standard deviation obtained were within ±5% and ±4.5% using Ca (II) and Mg (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 1.4-5.3% (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 calcium (II) and magnesium (II), a better linearity of calibration curve was obtained using calcium (II). The method was compared with recent indirect spectrophotometric determination of diclofenac, based on oxidation with bromosuccimimide with linear calibration range 1-18 µg/ml [26] and HPLC analytical method for the determination of diclofenac sodium in tablets with linear calibration range 10-200 µg/ml and lower limit of detection 12.5 µg/ml[27]. 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.

**Computational Study Results**

During molecular modelling study, the models optimized were Ca interaction with diclofenac and Mg interaction with diclofenac. The Ca and Mg were interacted with carboxyl and hydroxyl oxygen atoms of diclofenac molecule. Carboxyl and hydroxyl oxygen interactions of diclofenac were selected because of negative charge which can easily interact with positive metals (Ca and Mg).

**Physical Characteristics of Optimized Molecular Models**
Physical properties computed during computational work included number of atoms, spin of structure, total energy, dipole moment and bond lengths. The results of all physical properties of optimized molecular models were shown in table 2.

Number of atoms measured were 67 atoms for both models. Both optimized molecular models exhibited singlet spin. Total energy measured for Ca-Diclo model was -15.83 eV and for Mg-Diclo was -11.44 eV. The total energy of Mg-Diclo model was higher than Ca-Diclo model which indicated least stability in Mg-Diclo structure than Ca-Diclo structure. Similarly, the dipole moment of Ca-Diclo was observed as 7.3956 debye which was higher than dipole moment of Mg-Diclo structure that was 5.8478 debye. Hence the optimized molecular model of Ca-Diclo is more polar than Mg-Diclo molecular model due to high dipole moment.

For bond length measurements, the optimized molecular models showing interactions of Ca and Mg with oxygen (O) atoms of carboxyl and hydroxyl groups were built. Shorter bond lengths indicate stronger bonding. So, the calculated bond length values after optimization of structures were shown in table 2. The calculated bond length values of Ca interaction with carboxyl oxygen (Ca-Diclo carboxyl oxygen) was 2.376 Å and for Ca-Diclo hydroxyl oxygen was 2.358, which indicated slight shorter and stronger bond than Ca-Diclo carboxyl oxygen. Hence the bond formation between Ca and hydroxyl oxygen is slightly stronger than Ca with carboxyl oxygen. On other hand, the bond lengths values of Mg-Diclo carboxyl and Mg-Diclo hydroxyl were 3.474 and 2.050, respectively. For both the metals (Ca and Mg), the hydroxyl interaction is more favourable than carboxyl interaction due to shorter bond lengths.

Consequently, from the computational molecular modelling studies it can be concluded that the complex formation between Ca diclofenac was likely to be more favourable than Mg diclofenac due to less energy and higher polarity.

**Conclusion**

The determination of diclofenac sodium was carried out indirectly via atomic absorption spectrophotometry from pharmaceutical preparations. Diclofenac sodium reacted with calcium (II) or magnesium (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 Ca (II) or Mg (II), as a probe ion was determined by Atomic Consequently, from the computational molecular modelling studies have been concluded that the complex formation between Ca diclofenac was more favourable than Mg diclofenac due to less energy and higher polarity.

**References**

1. A. G Dickson, & Goyet, C.. *Handbook of methods for the analysis of the various parameters of the carbon dioxide system in sea water. Version 2* (No. ORNL/CDIAC-74). Oak Ridge National Lab., TN
2. D. Predoi, S. L Iconaru, M. V Predoi, G. E Stan, & N. Buton,. Synthesis, characterization, and antimicrobial activity of magnesium-doped hydroxyapatite suspensions. *Nanomaterials*, 9(9), 1295 (2019).

3. World Health Organization (WHO). "WHO Model List of Essential Medicines-19th List. April 2015-Amended November 2015." *Acesso em* 23 (2016).

4. Romani and M. P. Andrea, "Chapter 3. Magnesium in Health and Disease". In Astrid Sigel; Helmut Sigel; Roland K. O. Sigel."Interrelations between Essential Metal Ions and Human Diseases”, Metal Ions in Life Sciences.13. Springer. pp 49–79 (2013).

5. Santulli, Gaetano; Marks and Andrew, "Essential roles of intracellular calcium release channels in muscle, brain, metabolism, and aging". Current Molecular Pharmacology, vol. 8(2) pp 206–222, (2015).

6. “National Institutes of Health. "Dietary supplement fact sheet: Calcium." *Retrieved February* 19 (2013): 2014.

7. Saidoka, Kamaran Mahmud, Salam Naser Zangana, and Rebaz Tahir Lak. "Estimation of Serum Magnesium level among Patients with Bronchial Asthma in Erbil-Iraq." *Diyala Journal of Medicine* 17, no. 1 (2019): 36-43.

8. Kaczmarczyk, Joseph M., Alice Chuang, Lorraine Dugoff, Jodi F. Abbott, Amie J. Cullimore, John Dalrymple, Katrina R. Davis et al. "e-Professionalism: a new frontier in medical education." Teaching and learning in medicine 25, no. 2 (2013): 165-170.

9. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine (1997).

10. J. Ayuk, N. J. Gittoes, "Contemporary view of the clinical relevance of magnesium homeostasis". Annals of Clinical Biochemistry, vol. 51(2), pp 179–88, (2014).

11. M. Firoz, M. Graber, "Bioavailability of US commercial magnesium preparations". Magnes Res, vol.14(4) pp 257–262, (2001).

12. J. S. Lindberg, M. M. Zobitz, J. R. Poindexter and C. Y. Pak, "Magnesium bioavailability from magnesium citrate and magnesium oxide". J Am Coll Nutr, vol9(1), pp 48–55, (1990).

13. N. M. Vieno. H. Härkki, T. Tuhkanen and L. Kronberg, Occurrence of pharmaceuticals in river water and their elimination in a pilot-scale drinking water treatment plant. Environ. Sci. Technol., 41: 5077-5084. (2007).

14. M. Arvand, M., T.M. Gholizadeh and M.A. Zanjanchi, MWCNTs/Cu(OH)2 nanoparticles/IL nanocomposite modified glassy carbon electrode as a voltammetric sensor for determination of the non-steroidal anti-inflammatory drug diclofenac. Mater. Sci. Eng., 32: 1682-1689, (2012).

15. R. M. El-Megharbel, R.Z. Hamza and M.S. Refat, Synthesis, spectroscopic and thermal studies of Mg (II), Ca (II), Sr (II) and Ba (II) diclofenac sodium complexes as anti-inflammatory drug and their protective effects on renal functions impairment and oxidative stress. Spectrochimica Acta Part A: Mol. Bimolec. Spectrosc., 135: 915-928, (2015).
16. Idrees F. Al-Momani, “Indirect Flow-Injection Spectrophotometric determination of Meloxicam, Tenoxicam and Piroxicam in pharmaceutical formulations”, Analytical Sciences, vol. 22, pp 1611-1614, (2006).

17. Nief Rahman Ahmed, “Indirect spectrophotometric determination of captopril in pharmaceutical tablets and spiked environmental samples”, Iraqi National Journal of Chemistry, vol. 49, pp 1-11, (2013).

18. Bilal Yilmaz, Uluvihan Ciltas, “determination of diclofenac in pharmaceutical preparations by voltmmetry and gas chromatography method”. Journal of Pharmaceutical analysis vol. 5(3) pp 153-160, (2015).

19. S. Agrawal, J. Temsamani and J.Y. Tang. Pharmacokinetics, biodistribution and stability of oligodeoxynucleotide phosphorothioates in mice. Proceedings of the National Academy of Sciences, 88: 7595-7599, (1991)

20. Carreira, L.A., M. Rizk, Y. El-Shabrawy, N.A.Zakhari and S.S. Toubar,. Europium (III) ion probe spectrofluorometric determination of diclofenac sodium. J. Pharm. Biomed. Anal., 13: 1331-1337 (1995).

21. Arancibia, J.A., M.A. Boldrini and G.M. Escandar,. Spectrofluorimetric determination of diclofenac in the presence of "-cyclodextrin. Talanta, 52: 261-268 (2000).

22. M.A Castillo, and L. Bruzzone, Indirect fluorometric determination of diclofenac sodium. Anal. Sci., 22: 431-433 (2006).

23. da Silva Lyra, W., F.A.C. Sanches, F.A. da Silva Cunha, P.H.G.D. Diniz, S.G. Lemos, E.C. da Silva and M.C.U. de Araujo. Indirect determination of sodium diclofenac, sodium dipyrene and calcium gluconate in injection drugs using digital image-based (webcam) flame emission spectrometric method. Anal. Methods, 3: 1975-1980 (2011).

24. Issa, M.M., M. Nejem, M. Al-Kholy, S.N. El-Abadla, S.R. Helles and A.A. Saleh. An indirect atomic absorption spectrometric determination of ciprofloxacin, amoxycillin and diclofenac sodium in pharmaceutical formulations. J. Serbian Chem. Soc., 73: 569-576 (2008).

25. W. A. Soomro, M.Y. Khuhawar, T.M.J Khuhawar, M.F Lanjwani and I.K. Rind. Indirect Determination of Diclofenac Sodium in Formulation by Atomic Absorption Spectrophotometry after Complexation with Copper or Iron. Int.Res. J. Med. Sci., 2(1): 10-14,(2020)

26. Al-Sharook, M.M., and L.A. Al-Salim, Indirect Spectrophotometric Determination of Diclofenac and Isoxsuprine Drugs Using N-bromosuccinimide and Malachite Green Dye. J. Edu. Sci., 28: 18-32. 19 (2019).

27. B.T. Alquadeib, Development and validation of a new HPLC analytical method for the determination of diclofenac in tablets. Saudi Pharm. J., 27: 66-70 (2019).

Tables
Table 1: AAS determination of diclofenac sodium in various pharmaceutical formulations by solvent extraction and centrifuge method

| S.No: | Pharmaceutical Preparations containing 100mg/tablet diclofenac sodium | Solvent Extraction Method | Centrifuge Method |
|-------|-------------------------------------------------|--------------------------|------------------|
|       | Diclofenac found by FAAS method using Ca (II) | Diclofenac found by FAAS method using Mg (II) | Diclofenac found by FAAS method using Ca (II) | Diclofenac found by FAAS method using Mg (II) |
|       | RSD% n=3 | RSD% n=3 | RSD% n=3 | RSD% n=3 |
| 1     | Voltral | 99.510 mg (4.8) | 98.600 mg (2.1%) | 103.00 mg (4.2%) | 98.080 mg (1.8%) |
| 2     | Voren  | 107.00 mg (3.8%) | 96.000 mg (3.3%) | 98.00 mg (2.6%) | 96.600 mg (1.8%) |
| 3     | Qufen  | 104.00 mg (5.3%) | 103.00 mg (1.3%) | 101.07 mg (3.6%) | 100.20 mg (1.9%) |
| 4     | Dicloplus | 105.00 mg (2.9%) | 101.00 mg (2.2%) | 97.00 mg (5.3%) | 99.480 mg (1.9%) |
| 5     | Dicloran | 103.30 mg (1.4%) | 101.30 mg (1.8%) | 100.30 mg (1.8%) | 103.00 mg (1.9%) |

Table 2: Physical quantities of Optimized molecular models.

| Name of Molecular Model | No of atoms | Spin | Total Energy (eV) | Dipole Moment (Debye) | Bond Lengths (Angstrom) |
|------------------------|-------------|------|------------------|-----------------------|-------------------------|
| Ca-Diclo Interaction   | 67          | Singlet | -15.83          | 7.3956                |                         |
| Mg-Diclo Interaction   | 67          | Singlet | -11.44          | 5.8478                |                         |
| Ca-Diclo Carboxyl Oxygen |          |       |                  |                       | 2.376                   |
| Ca-Diclo Hydroxyl Oxygen |          |       |                  |                       | 2.358                   |
| Mg-Diclo Carboxyl Oxygen |          |       |                  |                       | 3.474                   |
| Mg-Diclo Hydroxyl Oxygen |          |       |                  |                       | 2.050                   |
Figures

Figure 1
Linear calibration curve between Ca (II) and diclofenac sodium by solvent extraction

Figure 2
Linear calibration curve between Mg (II) and diclofenac sodium by solvent

\[ y = -0.009x + 3.14 \]
\[ R^2 = 0.989 \]

Figure 3

Linear calibration curve between Ca (II) and diclofenac sodium by centrifuge method

\[ y = -0.004x + 1.26 \]
\[ R^2 = 0.985 \]

Figure 4

Linear calibration curve between Mg (II) and diclofenac sodium by centrifuge method
Figure 5

PM6 Level optimized molecular model of Ca Complex with two Diclofenac molecules

Figure 6

PM6 level optimized molecular model of Mg complex with two Diclofenac molecules