ASSAY OF DICLOFENAC SODIUM TABLETS FROM DIFFERENT PHARMACEUTICAL MANUFACTURING SOURCES IN IRAQI PHARMACEUTICAL MARKETS

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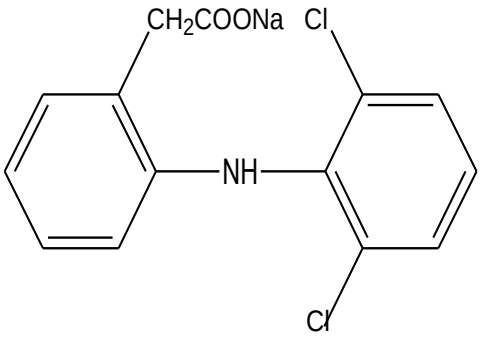
Abstract

Diclofenac sodium is a non-steroidal anti-inflammatory analgesic drug used in the relief of pain and inflammation witch associated with certain conditions. It can be assayed using HPLC to estimate the weight of the active constituent of the drug in various dosage forms. In this work we studied four commercial brands of the oral dosage form of the drug from different pharmaceutical companies in the Iraqi pharmaceutical market, to evaluate the content of the drug in these brands.

This has been achieved by making a calibration curve, using standard solutions of different concentration of the external standard diclofenac sodium u.s.p. different readings of the area under the peak were obtained following the straight line equation. Diclofenac was extracted from the tablet and injected in to HPLC system to measure the area under the peak from which we calculate the concentration then we evaluate the weight of the active constituent of the drug.

Introduction

Diclofenac sodium is a non-steroidal anti-inflammatory drug. It is referred as Voltaren.



$C_{14}H_{10}Cl_2NNaO_2$: Molecular Weight= 318.13
o- [(2,6 DICHLORO PHENYL) AMINO] phenyl] Acetate (1).

Mechanism of action

The action of single dose of the drug is much longer (6 to 8 hours) comparing to the short half-life that the drug indicates. This could be partly due to a particular high concentration achieved in synovial fluids. The exact mechanism of action is not entirely known, but it is thought that the primary possible mechanism which responsible for its anti-inflammatory/antipyretic/analgesic action is inhibition of prostaglandin synthesis by inhibiting of cyclooxygenase (COX). Inhibition of COX also decreases prostaglandins in the epithelium of the stomach, making it more sensitive to corrosion by gastric acid. This explane the side effect of diclofenac. Diclofenac has a low to moderate preference to block the COX2-isoenzyme (approximately 10-fold) so it show lower incidence of gastrointestinal complaints than noted with indomethacin and aspirin. Diclofenac may also be a unique member of the NSAIDs (1). There is some evidence that diclofenac inhibits the lipoxygenase pathways, thus reducing formation of the leukotrienes (also pro-inflammatory autacoids). There is also speculation that diclofenac may inhibit phospholipase A2 as part of its mechanism of action. These additional actions may explain the high potency of diclofenac - it is the most potent NSAID on a broad basis (2).

There are signifcant differences among NSAIDs in their selective inhibition of the two subtypes of cyclo-oxygenase, COX-1 and COX-2. Much pharmaceutical drug design has attempted to focus on selective COX-2 inhibition as a way to minimize the gastrointestinal side effects of NSAIDs like aspirin. In practice, use of some COX-2 inhibitors has led to massive numbers of patient family lawsuits alleging wrongful death by heart attack, yet other significantly COX-selective NSAIDs like diclofenac have been well-tolerated by most of the population (2; 3).

Uses of Diclofenac

Diclofenac is used for musculoskeletal complaints, especially arthritis (rheumatoid arthritis, osteoarthritis, spondylarthritis, ankylosing spondylitis), gout attacks, and pain management in case of kidney stones and gallstones. An additional indication is the treatment of acute migraines. Diclofenac is used commonly to treat mild to moderate post-operative or post-traumatic pain, particularly when inflammation is also present, and is effective against menstrual pain (3; 4).

Diclofenac sodium has been demonstrated to be effective in preventing proliferation of lens epithelial cells both in vitro and in animal study. The effects of Diclofenac sodium given during the hydrodissection stage of phacoemulsification surgery on posterior capsule opacification (PCO) were investigated (4).

Also, Diclofenac sodium has been shown to be effective for post tonsillectomy pain management (5).

Diclofenac sodium is available as tablets, ampoules, gel, drops and suppositories. It is given after meals. The maximum total daily dose of Diclofenac sodium by any route is 150 mg. The usual dose, oral or rectal, is 75 mg to 150 mg (6).

Several high performance liquid chromatographic methods have been developed for the determination of Diclofenac in its dosage forms and body fluids. This study, describes a simple helpful technique for the determination of Diclofenac sodium following extraction from tablet dosage form, the sensitivity and easy sample processing make it useful for companies products. Also it is suitable for the monitoring of the drug in the biological samples such as plasma blood, serum and it is useful for the estimation of the bioavailability and bioequivalency of several drugs.

The Aim of the Study

The aim of this study was to assay Diclofenac sodium from different pharmaceutical brands in Iraq pharmaceutical market to:

1- Prove that the weight of each tablet is within the range of maximum difference allowed.

2- Assay the active constituent of different samples using HPLC-UV method and comparing the results obtained.

Experimental Work

Materials

1- Methanol of HPLC grade (Scharlau) M.E: 0306 U.N.: 1230.

2- Phosphoric acid for HPLC Man. Date: 3/02 Exp. Date: 4/07 GCC Analyt reagent. U.K.

3- Sodium dihydrogen orthophosphate of HPLC grade Cat No. 11570 GCC Laboratory Reagent U.K.

4- Diclofenac sodium (U.S.P. Reference Standard) was obtained from the National Center for drug research and quality control .Cat No. 1880 Lot. No. HOB 150.

Instruments

1- CeCIL HPLC System Liquid Delivery System Detector: 1200 CE Pump:CE1105 Integrator: CE 1320 150 mm , 4.6 mm, CI8 Stainless steel column.

2- PH meter microprocessor PH meter HANA type PH211.

3- Ultrasonic Mixer. FRAITSCH Laboratte type 17.202 number 1390.

4- Spectrophotometer Type Specord 40, Analytic Jenqaa, Germany.

5- METTLCfR TO LEDO Made in Switzerland AB204-S 50/60 Hz, 6 VA.

6- KARL KDLB. Scientific Technical Supplies D-6072 Dereieich, West Germany.

Samples

The brands of (Diclofenac tablet) were collected from the Iraqi pharmaceutical market. The table below explains the data obtained concerning the proprietary name, source, manufacture date (M.D.), expire date (E.D.), batch number and average tablet weight of each one**.**

Table (1): The criteria of Diclofenac sodium tablet in the Iraqi markets.

Type	Company	M.D.	E.D	Batch Number	AverageTablet Wt (g)
Voltadin, (25 mg)	S.D.I Iraq	12/2004	12/2007	3	0.1417
Optifenac (25 mg)	AJANTA Pharma India	09/2004	08/2008	3251	0.2232
Divon (25 mg)	EROS Pharma India	09/2004	08/2007	DkOlO	0.1094
Voltagin (25 mg)	MEDI Chemi India	05/2005	04/2008	VV-007	0.2153

Identification

1- Melting point, observed m.p. 284 °C Recorded m.p. 283 - 285.
2- Solubility test, DICLOFENAC is sparingly soluble in water, soluble in alcohol, and in methanol. It dissolves in dilute solutions, alkali hydroxides, slightly soluble in acetone. It is weak organic acid. It is belong to be from class A2.
3- Ignition test, Ignited with sooty flame.
4- Infrared absorption spectrophotometry comparing with the spectrum obtained with Diclofenac sodium CRS.

Methods

After preparing the standard curve by using the external standard of (Diclofenac sodium), we can test the Diclofenac sodium 25 mg tablets of the four brands of different pharmaceutical companies. In this research we use standard Diclofenac sodium U.S.P. to prepare stock solution and from this stock solution make different dilutions to be ready for the HPLC study.

The chromophotographic procedure was carried out using:
1- A stainless steel column (4.6 mm x 25 cm) containing L7 (end-capped), the flow rate 1 ml/ minute.
2- Mobile phase is composed from a mixture of methanol and phosphate buffer, Ph =2.5 (700:300) filtered and degassed.

3- Diluent: prepare a mixture of methanol and water (70:30)
4- UV Detector with 254 nm wavelength.
5- We inject 20 micro liter of each solution.

Preparation of phosphate buffer PH#2.5

Equal volumes of 0.01 M phosphoric acid and 0.01 M monobasic sodium phosphate were mixed.

* Standard solutions and calibration curve.

1- Accurate 25 mg of Diclofenac, reference standard u.s.p. was weighed.

2- Dissolve the weighed powder in 25 ml volumetric flask containing solvent as diluents with the aid of ultrasound device for 10 minutes, complete the volume to 25 ml using the same diluents to form a stock solution with a concentration of 1 mg/ml (0.0785 m mole/L) (7). Take different volumes from this stock solution to prepare different dilutions by adding the precise volume and complete to the final volume with the diluents. Each one of these dilutions was injected three times in the HPLC device and then the average of the results was calculated according to area under the peak method as shown in table (2).

Table (2): Dilutions of Diclofenac and their peak areas**.**

The volume taken from the stock solution (ml)	Concentration (x-axis)	The AUP mm^2 (y-axis)
10	0.4 mg/l	40.59
8	0.32 mg/l	30.52
6	0.2 1 mg/1	26.05
4	0.16 mg/l	24.75
3	0.12 mg/l	21.01
1	0.04 mg/l	14. 26

Then we draw calibration curve by plotting the concentration of Diclofenac versus its peak area as in figure (1) knowing that The Calibration curve follow straight line equation. (Y = a + bx). By substituting - the statistical application - we obtained the following data (6).

a = 13.602 the intercept obtained to be applied in the Equation.
b = 65.838 the slope or regression coefficient.
R =0.9578 the coefficient of Determination.
R= 0.9786 the _correlation coefficient.
Then the straight line equation used in the calculation is rearranged to: (Y= 13.602+ 65.838X)

The highly significant linear correlation of the area on the concentration is indicated by the high value of r and r^2 which have the highest

value. This will ensure the accuracy of the work and the qualification of the HPLC device.

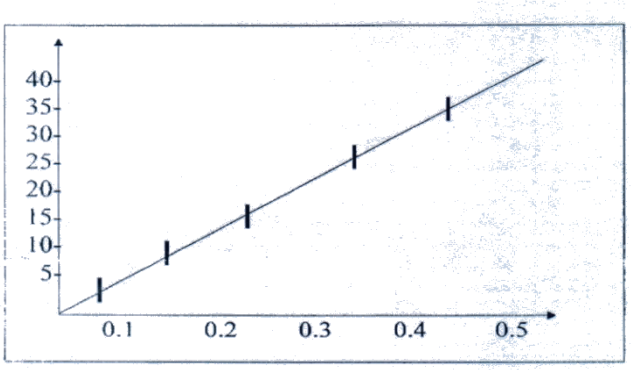


Figure (1): Calibration curve for Diclofenac sodium.

The chromatogram of 0.2 mg/l of standard solution of Diclofenac sodium is shown below in figure (2).

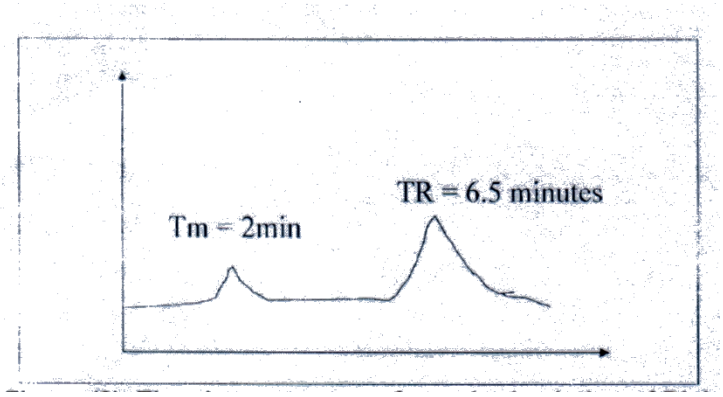


Figure (2): The chromatogram of standard solution of Diclofenac sodium/ retention time of Diclofenac (TR) is 6.5 minutes, and dead time (Tm) = 2 minutes.

Sample testing:

Before starting assay, the weight variation tolerance for the enteric coated tablet should
be carried. It depends usually on tablet weight as shown in table (3).

Table (3): The weight variation of Diclofenac sodium enteric coated tablet.

Average weight of tablet in mg	Maximum % difference allowed
80 or less	70
80- 250	75
more than 250	5

The source of Diclofenac tablets obtained from Iraqi market and the tablet weights are shown in table (4) and all of the tested tablets were within the Range of Maximum % difference allowed.

Table (4): Diclofenac tablet weights from Iraqi markets are within the range of maximum percent difference allowed.

Type	OPTIFENAC Tab (wt) mg Average= 220mg	Voltadin Tab (wt) (SDI/IRAQ) (mg) Average=744.4mg	Divon Tab (wt) mg Average= 108.88mg	Voltagin Tab (wt) mg(Chemi/ INDIA) Average= 215.925mg
1	0.227	0.1370	0.1099	0.2199
2	0.2150	0.1440	0.1103	0.2155
3	0.2199	0.1407	0.1098	0.2162
4	0.2250	0.1513	0.1070	0.2214
5	0.2233	0.1443	0.1087	0.2112
6	0.2195	0.1355	0.1092	0.2099
7	0.2268	0.1517	0.1080	0.2089
8	0.2279	0.1493	0.1099	0.2137
9	0.2225	0.1438	0.1082	0.2189
10	0.2259	0.1417	0.1136	0.2174
11	0.202	0.1450	0.1090	0.2263
12	0.2122	0.1385	0.1030	0.2145
13	0.2235	0.1520	0.1120	0.2230
14	0.2289	0.1550	0.1150	0.2195
15	0.2216	0.1390	0.1015	0.2210
16	0.2197	0.1444	0.1060	0.2075
17	0.2202	0.1470	0.1160	0.2077
18	0.2216	0.1415	0.1025	0.2135
19	0.2213	0.1466	0.1075	0.2125
20	0.2170	0.1410	0.1105	0.2200

Assay

Procedure

The weight and powder of twenty tablets of each type of the drug was mixed, with aid of ultrasound, a quantity of the powdered tablets containing 25 mg (0.078 mmole) of Diclofenac in the diluents fully dispersed to form a homogenous solution, filter through a filter paper then complete to the final volume (25 ml) by the same diluents and centrifuge to complete purification of the solution, (10 ml) of this solution is poured into volumetric flask (50 ml capacity) and complete to the required volume with the aid of diluents solution. This final solution will be ready to be injected into HPLC system. Each one of the four samples will have its Aup (area under the peak) which reflect its concentration and the active ingredients contained in the tablet.

Standard Curve testing of the sample

Each one of the four samples is tested using the same conditions that has been used in the external standard in the HPLC system to get the Aup. Then the equation of straight line is applied to calculate diclofenac concentration and its weight. The maximum % difference allowed average of Aup and concentrations of the samples are shown in table (5).

Table (5): The maximum percent difference allowed, average of Aup, and concentration of the samples tested.

Type of Diclofenac	Max % difference allowed	Aup mm^2	Cone mg/mL
Voltadin	(133.57-155.23)	28.2	0.2217
Divon	(100.72-117.04)	27.5	0.211
OPTIFENAC	(203.98-238.58)	27.2	0.2065
Voltagin	(199.73-0.2321)	27.05	0.2042

RESULTS

From the data obtained in table (5) in which the cone of each Aup was determined. It is possible to calculate the weight, percentage of errors and recovery percent of each sample compared to the standard weight (25 mg) and the relative standard deviation percent (RSD %) or sample coefficient of variation (CV) of the three reading of area
(AUP) also calculated by the following equation:

$$\text{RSD \% or CV} = \frac{\text{Standard Deviation}}{\text{mean of Aup readings}} * 100\%$$

These Data were arranged according to the higher recovery % or higher weight of the samples as shown in Table (6).

Table (6): Data represent the weight of Diclofenac error (%) , recovery companies, (%) and RSD of the four samples from different pharmaceutical sources

No.	Drug source	Weight of Diclofenac of each sample (mg)	Error (%)	Recovery (%)	RSD (%) or CV
1	Voltadin (SDI)	27.7125	0.7085	110.85	0.862
2	DIVON (EROS Parma/ INDIA)	25.8125	3.25	103.325	0.8
3	OPTIFENAC (Ajanta Pharma/ INDIA)	26.37	5.5	105.48	0.95
4	Voltagin (MEDI CHEMI/INDIA)	25.525	2.1	102.1	0.75

Conclusion

From this study we can conclude the followings:

1- All of the tested tablets were within the range of maximum (%) difference allowed.

2- The quantitative analysis was performed using HPLC with external standard method. The recoveries were close to the highest upper limit (110%) with acceptable accuracy and precision.

3- The results indicate that Diclofenac sodium is accepted within the normal range percentage (90%- 110 %) according to the u.s.p. XXIV.

4- The HPLC quantitative analysis is fast and accurate for Diclofenac analysis and can be used for routine work.

5- From the comparison of the results obtained from the tested four samples, it was found that Voltadin (SDI- IRAQ) is the most potent one. The result showed that all of the four samples are within the allowed percentage of the 3 range (90%- 110%) according to the u.s.p. XXIV.

References

1- Martindale. (1996). The Extra Pharmacopoeia, 31st edition. Evaluated information on the world's drugs and medicines from Royal Pharmaceutical Society of Great Britain.

2- Wikipedia. (2007). the free encyclopedia. Diclofenac.

3- Robert E. Willet. (ed.). (2004). Analgesic agents. In: Wilson and Gisvold's Textboolk of Organic Medicinal and Pharmaceutical Chemistry, 11th edition. Lippincott, Williams, and Wilkins. Baltimore, p 759.

4- Umit Ubeyt Inan, Ercument Bozkurt, Faruk Ozurk, Samet Ermis, Sevim Yaman. (2006). Effect of Diclofenac on prevention of posterior capsule opacification in human eyes. Can J Ophthalmol: vol. 41, No. 5.

5- Mark J. Courtney, Dilhan Cabraal. (2001). Tramadol vs. Diclofenac for posttonsillectomy analgesia. Arch Otolaryngeal Head Neck Surg, vol. 127.

6- British Pharmacopoeia. (2003). CD-ROM.

7- USP/NF. (2002). The United States Pharmacopoeia and the National Formulary, 25th Edition, CD-ROM.

8- L. Lachman, H.A. Lieberman, J.L. Kanig. (1986). The Theory and Practice of Industrial Pharmacy, LEA and Febiger Philadelphia PUB, 3rd Edition, p 300.

تحديد كمية المادة الفعالة لحبوب ديكلوفيناك الصوديوم المصنعة من شركات دوائية عدة والمتوفرة في الاسواق الصيدلانية العراقية

كوكب يعقوب ساعور نجيب بطرس السبع
قسم الكيمياء الصيدلانية
كلية الصيدلة – جامعة بغداد

الخلاصة

إن الديكلوفيناك صوديوم هو من مجموعة الأدوية المضادة للإلتهابات غير الستيرويدية والتي تستخدم في علاج الحالات المرضية التي تتضمن إلتهاب الأنسجة الحيوية .

في هذه الدراسة تمّ استخدام نظام (التحليل الكروماتوغرافي العالي الكفاءة للسوائل) لتحديد الكمية الدقيقة للمادة الفعالة (الديكلوفيناك صوديوم) في العينات الاربعة من شركات دوائية مختلفة والمتوفرة في السوق الصيدلاني العراقي ومقارنتها مع النسب المقررة حسب دستور الأدوية البريطاني .

وقد جرت الدراسة بتشكيل منحني التحديد الناتج من اسخدام محاليل قياسية بتراكيز مختلفة من الديكلوفيناك صوديوم وتحقن هذه المحاليل في نظام (التحليل الكروماتوغرافي العالي الكفاءة للسوائل) لتعطي قراءات مختلفة لمساحات ما تحت القمة ومن معرفة التراكيز ومساحة كل واحد يمكننا رسم المنحني الذي يتبع معادلة الخط المستقيم، واستخلص الديكلوفيناك صوديوم من القرص لكل عينة وبعد ذلك حقن في نفس الجهاز لمعرفة مساحة ما تحت القمة ومن المساحة الناتجة يمكن معرفة تركيز المادة الفعالة في القرص بواسطة معادلة الخط المستقيم الناتج.