Comparative bioavailability study of three brand products of diclofenac sodium 50mg oral tablets in healthy volunteers

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ABSTRACT

***Background*: Poor quality medicines could be substandard, degraded or counterfeit medicines. One of the measures used to detect poor quality drugs is comparison with a reference product taking pharmacokinetic end points in consideration.**

***Objectives*: To compare the bioavailability of three brand products of diclofenac sodium 50mg oral tablets in healthy volunteers. One of these brands is locally manufactured. The other two are from known foreign drug manufacturers.**

***Methods*: A randomized, three-way, cross-over bioavailability study was performed on 10 apparently healthy male volunteers. Each received successively, a single oral tablet of 50 mg diclofenac sodium from the three sources with a washout interval of one week. Blood samples were taken until six hours after drug administration and analyzed using HPLC system (Agilant, model 1200-USA, with an Agilent 1200 variable wavelength detector and a Zorbax Eclipse XBD-C18 column). Quantitation was achieved by measurement of the peak height ratios of the drug to ibuprofen as internal standard. The amount of diclofenac sodium in each of the three drug products was also measured in vitro.**

***Results*: Maximum plasma concentration (C_{max}) was significantly different among the three tablets with the main difference was between the locally manufactured tablet and the two other tablets (0.72 µg/ml for the locally manufactured product compared with 1.55 and 1.24 µg/ml for the reference products). Time to reach maximum concentration (T_{max}) showed no significant difference between the three brands of diclofenac sodium tablets. The area under the curve differs significantly between the three products. The area under plasma concentration-time curve (AUC) of the locally manufactured product represented 64.7% and 74% of the AUC of the two reference brands. The plasma elimination half life ($t_{½}$) differs among the three different types of products but these differences ran short of statistical significance ($P<0.058$). *In vitro* assay of the amount of diclofenac sodium in each tablet showed no significant differences between the three types of tablets.**

***Conclusion*: The locally manufactured enteric coated diclofenac tablet is not interchangeable with the two reference foreign brand products, although they contain approximately the same amount of diclofenac sodium. It is speculated that differences in bioavailability and in peak drug levels might be attributed to pharmaceutical factors such as the rate of disintegration and dissolution which can be affected by the types of additives and the coating materials in each tablet.**

دراسة التوافر الحياتي المقارن لثلاثة منتجات تجارية لأقراص الدايكلوفيناك صوديوم ٥٠ ملغم في المتطوعين الأصحاء

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خلفية الدراسة: إن الأدوية ذات النوعية الرديئة يمكن أن تكون غير قياسية أو متحللة أو مزيفة . وإحدى الوسائل المستعملة للكشف عن الأدوية ذات النوعية الرديئة هي المقارنة مع أدوية مرجعية مع الأخذ بقياسات حرائك الأدوية بعين الاعتبار.

الهدف من الدراسة: لمقارنه التوافر الحياتي لثلاثة منتجات تجارية للداكلوفيناك صوديوم في المتطوعين الأصحاء. احد هذه المنتجات مصنعه محليا" والاثنين الآخرين من شركات أجنبية معروفة.

الطرائق: تم إجراء دراسة عشوائية تعابرية ثلاثية على ١٠ متطوعا" من الذكور الأصحاء. كل واحد منهم اخذ وبشكل متتالي، قرصا" واحدا" ٥٠ ملغم من الدايكلوفيناك صوديوم عن طريق الفم من مصادر ثلاثة مع فترة تنظيف لأسبوع واحد. وتم اخذ عينات الدم على مدى ست ساعات بعد إعطاء الدواء وتم تحليله باستعمال نظام الكروماتوغرافي السائل عالي الكفاءة (نوع اجلنت ١٢٠٠، من الولايات المتحدة مع كاشف متغير الطول ألموجي وعمود من نوع زوبراكس C18). واعتمدت القياسات الكمية باستعمال نسبة ارتفاع القمة للدايكلوفيناك مقارنة مع الابوبروفين كمقياس داخلي. كما تم قياس كمية الدايكلوفيناك صوديوم في كل نوع من الأقراص الثلاثة خارج الجسم.

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النتائج: اختلف التركيز الأعلى بشكل معتد بين الأقراص الثلاثة وكان الاختلاف الرئيس بين القرص المصنع محليا" والقرصين الآخرين (٠.٧٢ مايكروغرام/مل للمنتج المصنع محليا" مقارنة ب ١.٥٥ و١.٢٤ مايكروغرام/مل للمنتجين المرجعيين على التوالي). إما الوقت اللازم للوصول للتركيز الأعلى فلم يكن هناك فرقا" معتدا" بين المنتجات الثلاثة للدايكلوفيناك صوديوم. وكانت المساحة تحت المنحنى مختلفة وبشكل معتد بين المنتجات الثلاثة. فالمساحة تحت المنحنى للمنتج المصنع محليا" يوازي ٦٤.٧% و ٧٤% من المساحة تحت المنحنى للمنتجين المرجعين الاثنين. واختلف عمر النصف بين أنواع الأقراص لكن هذا الاختلاف كان على حافة الاعتداد الإحصائي ($P<0.58$) .ولم يظهر قياس كمية الدايكلوفيناك صوديوم في كل قرص اختلافا" مهما" بين أنواع الأقراص الثلاثة.

الاستنتاج: إن القرص المغلف للأمعاء والمصنع محليا" ليس بديلا" عن المنتجين التجاريين المرجعين على الرغم من إن الأقراص الثلاثة تحتوي على كميات متقاربة من الدايكلوفيناك. ويمكن أن نخمن بأن الاختلاف في التوافر الحياتي وفي التركيز الأعلى يمكن إن يعزى للخواص الصيدلانية مثل التفتت والذوبان والتي قد تتأثر بأنواع المضافات ومواد التغليف لكل منتج.

INTRODUCTION

A drug is considered substandard when the composition and ingredients of a drug product do not conform with the correct scientific specifications.[1] Such a drug can be ineffective leading to exacerbation of the patient's condition, or toxic, and both can be fatal. Substandard products may be caused by negligence, human error, insufficient human and financial resources or counterfeiting. Therefore, counterfeit (fake) medicines are part of a wider term called substandard pharmaceuticals. Counterfeit medicines are **"deliberately" and "fraudulently"** mislabeled with respect to identity and/or source and may include products with fake packaging, with wrong ingredients, without active ingredients or with insufficient active ingredients.[1,2] Apart from pharmaceutical legislations, good manufacturing practices (GMP) and national drug regulatory capacity, bioequivalence and comparative bioavailability studies can be performed to ensure good quality of drugs and to detect substandard drugs. For two orally administered drug products to be bioequivalent, the active drug ingredient in the test product must exhibit the same rate and extent of absorption as the reference drug product if administered in the same dose, in the same amount of active substance(s) and in the same dosage form.[3-5] Bioavailability and bioequivalence studies are now widely used for the assessment of both brand-name as well as generic drugs.[6] In addition to approving these types of drugs, bioequivalence studies are used to reduce the cost of medications by introducing into the drug markets of generic equivalents of brand-name drugs and to ensure interchangeability of the products.[7] In the present study, we have tested the bioequivalence of a locally manufactured diclofenac sodium enteric-coated 50mg oral tablet with two similar tablets from known international sources as reference products.

SUBJECTS AND METHODS

The study was carried out in 10 apparently healthy volunteers who provided their written informed consent to participate in the study prior to enrolment, and were free to withdraw at any time during the study. The study was performed during the period October 2011 to July 2012 and was approved by the Ethical Committee of the College and by the Council of College of Medicine, University of Basrah.

Subjects

The study population consisted of 10 adult male subjects. Their mean ± SD for body mass index (BMI) was 24.6±4, age 24.9±5.2 years, weight 75.5±14.6 kg and height was 174.9±4.6 cm.

Inclusion and exclusion criteria

Subjects were males, age between 20 to 50 years, with no associated diseases. During the week before the study, no repeated use of drugs, particularly NSAIDs was permitted. Exclusion criteria were history of drug allergy, any associated disease and repeated drug use. Any contraindication to the use of NSAIDs (such as

peptic ulcer, bleeding disorders, hypersensitivity reactions, liver disease, ….) should be excluded.

Design

This study was an open-label, single-dose, crossover, comparative bioavailability study that assessed the three tablet formulations of diclofenac sodium under fasting conditions, with a washout period of seven days between the three periods. The volunteers were given each of the three study drug products after an overnight fast. They received single doses of the three tablet formulations of diclofenac sodium 50 mg, two formulations were enteric coated and one Lactab (also resistant to gastric juices) from three manufacturing sources; one from inside Iraq and the other two from known international manufacturing companies.

Blood sampling

Drugs were administered with 150 ml water starting at 08.00 h after an overnight fast with the last meal taken before 24.00 h. Intake of food was delayed for 3h after drug administration. Peripheral venous blood samples were taken immediately before, at 30 min and at 1,1.5, 2, 2.5, 3, 4 and 6 hours after drug administration. During each session, an indwelling catheter was inserted into a forearm vein. Samples were collected in EDTA tubes and immediately centrifuged. Plasma was separated and frozen at -15°C until further analysis.

Determination of diclofenac in human plasma

Reagents and Chemicals

Diclofenac sodium and buprofen pure powder were kindly supplied by SDI, Samara, Iraq. Methanol, isopropyl alcohol and water (HPLC grade) were purchased from Carbon Group, Belfast, Northern Ireland. Orthophosphoric acid and hexane were supplied from Scharlau group, India.

Sample preparation

In a 10 ml test tube, 50 µl of internal standard solution (containing ibuprofen 2.5 µg) were added to 0.5ml of plasma. Acidification with 1 ml of 1M orthophosphoric acid was followed by extraction with 5ml of a mixture of hexane and isopropyl alcohol (90:10); then centrifugation, recovery of the organic layer, evaporation to dryness and dissolution of the dry residue in 100µL of the mobile phase.

Apparatus and HPLC Conditions

The apparatus was an Agilent HPLC system model 1200 (USA), consisting of an Agilent Isocratic Pump-G1310A, a 20µl manual injection loop (Rheodyne 7725i manual injector), a computerized system controller (Chemstation), and an Agilent 1200 variable wavelength detector G1314B. Chromatographic separation was performed using a Zorbax Eclipse XBD- C18 (4.6×150mm, 5µm) column.

The chromatographic parameters were given in Table-1.

Table 1. ***Chromatographic Conditions.***

Mobile phase	**Methanol: water (70:30 v/v)**
pH	3.5 adjusted with Orthophosphoric acid
Flow rate	1.0 mL min^{-1}
Injection volume	20 µL
Elution type	Isocractic
Detection	276 nm
Temperature	25 ^{o}C

Quantitation was achieved by measurement of the peak height ratios of the drug to the internal standard. The intraday coefficients of variation (%CV) of all internal standard samples was 9.42%. The lowest value on the calibration curve was the lower limit of quantitation 100ng/mL. Preparation of standard stock solutions. A standard stock solution of diclofenac sodium (5000 µg/ml) was prepared by dissolving 50 mg of drug pure powder in a 100-ml volumetric flask with methanol. A series of standard solutions at concentrations of 0.25, 0.5, 1, 2, 4, 8 µg/ml were prepared by further dilution of the standard solution in methanol to obtain different working solutions. Stock of

internal standard solution (2500 µg/ml) of ibuprofen was prepared in methanol.

Preparation of Calibration curve

To 0.5 ml of blank plasma, 50 µl of diclofenac standard solutions at concentrations of 0.25, 0.5, 1, 2, 4, 8 µg/ml and 50 µl of internal standard were added. To the resulting solutions, 1 ml of 1 M orthophosphoric acid, and 5 ml of a mixture of hexane and isopropyl alcohol (90:10) were added, vortexed and centrifuged at 3000 g for 5 min. The supernatant was separated and evaporated to dryness. The residue was reconstituted with 100 µl of mobile phase and 50 µl aliquot of the resulting solution was injected to HPLC. The calibration curves were obtained by plotting peak height ratio versus concentration. All peaks were integrated by using software Chemstation.

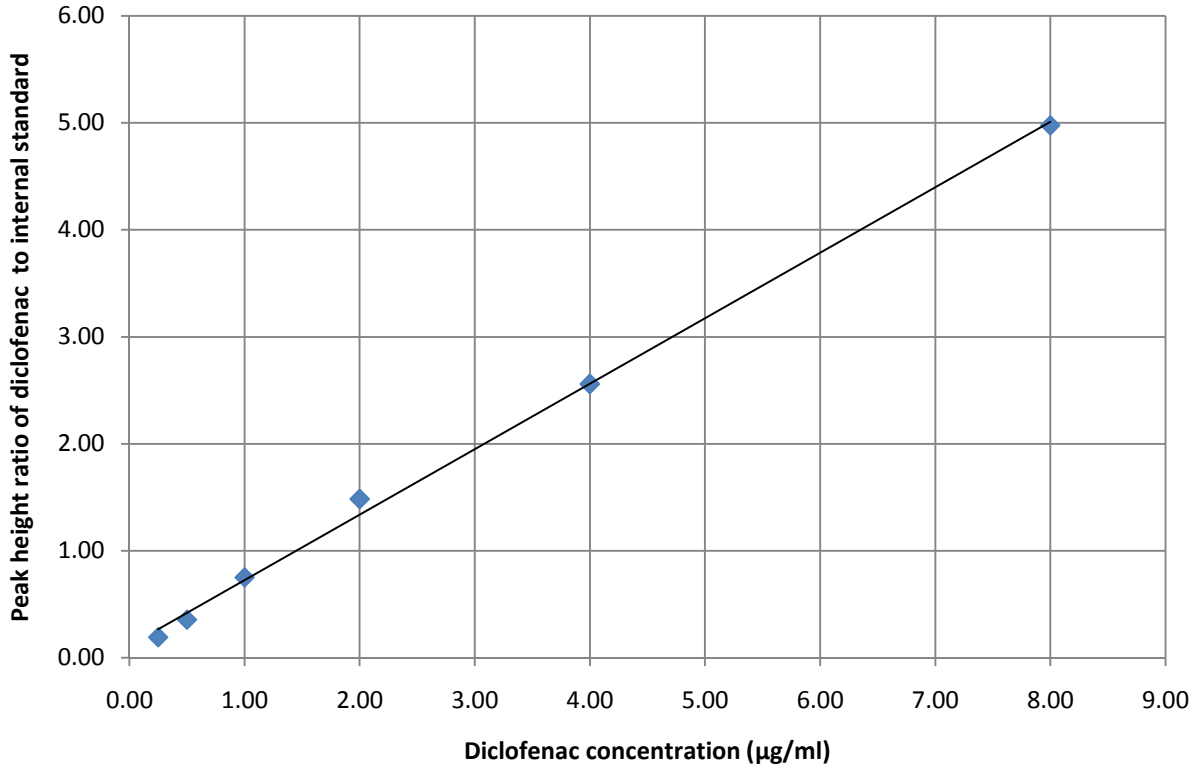

Fig1. The calibration of CURVE diclofenac sodium.

The amount of diclofenac sodium in each of the three drug products was measured in vitro using the method described by Kasperek.[8] Ten tablets of each brand product of diclofenac sodium were crushed into fine powder. One tenth of the powder weight was put in a 50ml volumetric flask. The powder was dissolved in 35 ml of methanol (HPLC grade) and the flask was kept for 15 minutes in an ultrasonic waterbath. The volume was then completed to 50ml with methanol (HPLC grade) and filtered using Whattman filter paper 0.46µm. Five ml of the filtrate were transferred to a volumetric flask and completed to 50 ml with methanol (HPLC grade). Fifty µl of the final dilution were injected into the HPLC device.

Data Analysis

The pharmacokinetic parameters measured include the observed maximum plasma concentration (Cmax), and time to reach Cmax (Tmax). They were obtained directly from the raw data. The area under the plasma concentration-time curve from 0 to 6 hours (AUC_{0-6}) was estimated by the linear trapezoidal rule. The terminal half-life, $t_{1/2}$, was

calculated as 0.693/k, where k denotes the elimination rate constant (the time constant of the terminal slope). Comparison between different pharmacokinetic parameters were determined using analysis of variance (ANOVA) with post hoc and Mann – Whitney tests. A difference among parameters was considered statistically significant for p value of 0.05 and less.

RESULTS

Chromatography

A reverse-phase high-pressure liquid chromatographic method based on that described by Emami[9] was used to determine the bioavailability of diclofenac sodium after administration of the three different formulations. (Figure-2), shows the chromatogram of blank plasma (A) spiked with diclofenac sodium, blank plasma spiked with ibuprofen (B) as internal standard and diclofenac sodium at concentration of 4 µg/ml with ibuprofen (C). All chromatograms were free from interferences at the retention times of diclofenac or internal standard. Both compounds eluted as completely resolved peaks and no peak tailing was noticed enabling the use of peak height ratio in the calculation of the calibration curve. An optimum flow rate of 1.5 ml/min for the mobile phase resulted in the retention times of 5.3 min for diclofenac sodium and 6.3 min for ibuprofen (the internal standard).

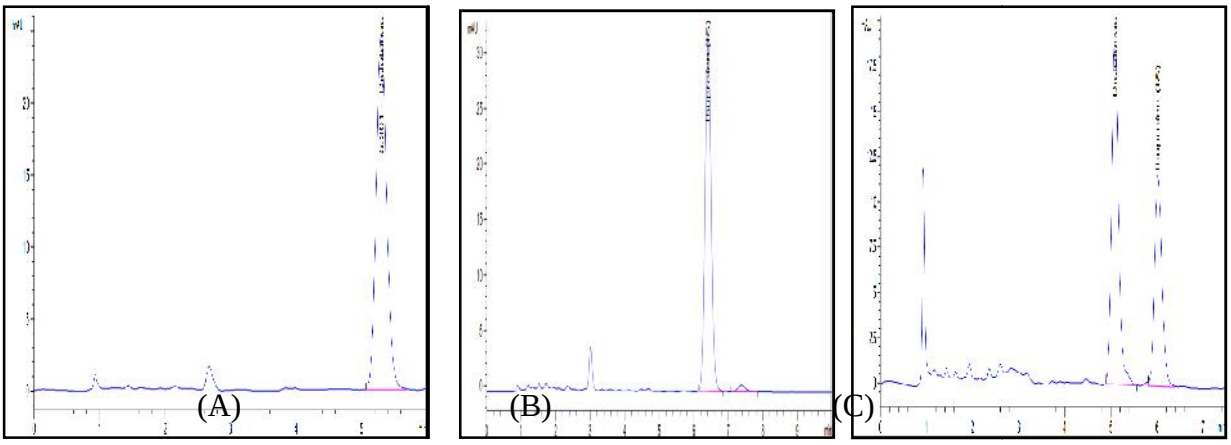

Fig 2. The chromatogram of blank plasma spiked with diclofenac sodium (A), blank plasma spiked with ibuprofen as internal standard (B) and Diclofenac sodium at a concentration of 4 µg/ml with ibuprofen (C). The retention times of 5.3 minutes for diclofenac sodium and 6.3 minutes for ibuprofen.

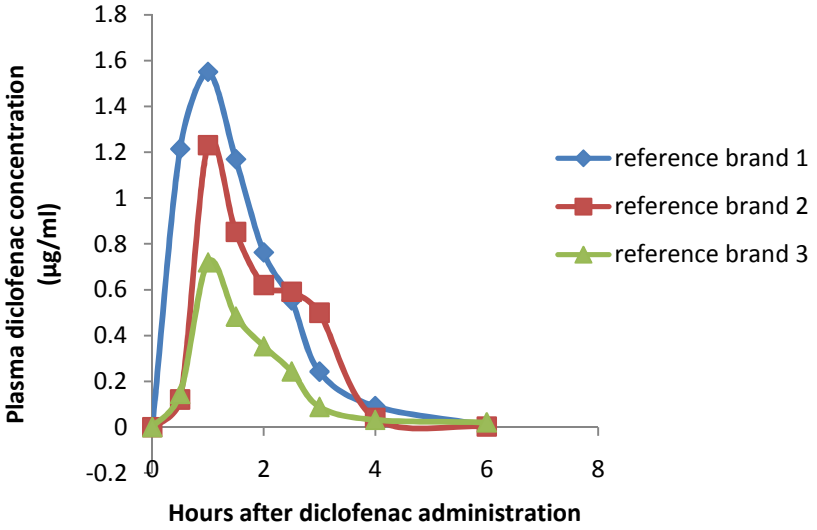

Fig 3. Plasma concentration versus time curve after administration of 50mg diclofenac sodium of the locally manufactured product and two reference brand products in healthy volunteers (Each point represents the mean of n = 10).

The mean pharmacokinetic parameters are presented in (Table-1). Analysis of variance (ANOVA) demonstrates that Cmax was significantly different among the tablets with the main difference found between the locally manufactured tablet and the two other tablets (0.72 compared with 1.55 and 1.24 µg/ml). The difference in the Cmax between the two foreign brand products is not statistically significant. Time to maximum concentration (Tmax) shows no significant difference among the three brands of diclofenac tablets with mean times of 2, 1.70, and 2.18 hours for the local and the two foreign brand products respectively. The area under the curve differs significantly between the three brand products; mainly between the locally manufactured tablet product and other two reference brands (0.97 µg ml^{-1} h for the local and 1.50, 1.31 µg ml^{-1} h for the foreign brand products respectively). The $t_{½}$ differs among the different types of tablet products but this difference did not reach a statistical significance.

Table 1. Pharmacokinetic parameters of diclofenac sodium following administration of three different types of tablets

	Reference brand product (1)				Reference brand product (2)				Locally manufactured brand				
	Mean	**SD**	**95% confidence interval**		**Mean**	**SD**	**95% confidence interval**		**Mean**	**SD**	**95% confidence interval**		**P value**
Cmax (µg ml^{-1})	**1.55**	0.61	1.11	1.99	**1.24**	0.42	0.89	1.59	**0.72**	0.37	0.44	1.00	0.004
Tmax (h)	**1.70**	0.67	1.22	2.18	**2.06**	0.68	1.50	2.63	**2.00**	0.61	1.53	2.47	0.455
AUC (µg ml^{-1} h)	**1.50**	0.43	1.19	1.81	**1.31**	0.30	1.06	1.56	**0.97**	0.50	0.59	1.35	0.037
T ½ (h)	**0.48**	0.20	0.33	0.62	**0.93**	0.68	0.36	1.50	**1.37**	1.15	0.49	2.26	0.058

None of our volunteers developed any important adverse effect.

The amount of diclofenac sodium in the locally manufactured tablet was estimated to be 43.95±7.3 mg. This is comparable to the other two reference tablets (47.35±1.3 and 43.4±3.5).

Table 2. The amount of diclofenac sodium in each of the three brand tablets measured twice in vitro.

Experiment	Locally manufactured	Reference -2	Reference -1
Experiment – 1	49.1	45.9	46.4
Experiment -2	38.8	40.9	48.3
Mean ± SD	43.95 ± 7.3	43.4 ± 3.5	47.35± 1.3

DISCUSSION

Counterfeit medicines are estimated to make up more than 10% of the global medicines market and are present in both industrialized and developing countries. In poor countries, a much greater percentage of medicines are thought to be counterfeit or substandard with serious implications on human health.[1,2] In 2007, the WHO estimated that about 30% of the member states have weak regulations or none at all.[10] All samples of ampicillin capsule tested in Senegal in 2002, were found to contain flour only.[11] Nearly 40% of an antimalarial called artesunate in Thailand contained no active ingredient.[12] Therapeutic equivalence and interchangeability of drug products can be ensured by bioequivalence and comparative bioavailability studies.[6] Bioequivalence studies are generally recommended using the following endpoints; pharmacokinetic, pharmacodynamic, clinical, and *in vitro* endpoints. For drug products where drug level can be determined in biological fluids and the drug level is correlated with the clinical effect, pharmacokinetic endpoint bioequivalence studies are preferably conducted.[10] In the present work, bioequivalence studies using pharmacokinetic parameters are used to test the oral bioavailability of enteric-coated diclofenac sodium manufactured inside Iraq compared with a reference diclofenac tablets made by internationally known drug companies. It was found that diclofenac manufactured inside Iraq had lower bioavailability parameters i.e. AUC_{0-6h} and C_{max}. The AUC_{0-6h} is significantly lower (0.97 compared with 1.50 and 1.31 µgl/h for the two reference brand products). The comparative bioavailability of the locally manufactured diclofenac represented 64% and 74% of the two reference brand products respectively. The C_{max} is also significantly lower while the $t_{1/2}$ is longer. The longer half life may reflect slower rate in addition to lower extent of absorption. When diclofenac was measured in each type of the three types of tablet products *in vitro*, they were found to have approximately similar amount of diclofenac sodium (Table-2). Thus, after excluding differences in the amount of diclofenac in each tablet product, the difference in bioavailability and in systemic drug levels may be attributed to pharmaceutical properties such as the rate of disintegration and dissolution which might be affected by the types of additives and the coating materials in each product. Obscuring product differences, variations in gastric and intestinal transit may also be a contributory factor.[13] Hasan et al.[14] found that peak serum level of diclofenac sodium after ingestion of a single enteric-coated tablets by healthy volunteers is 1.04, 1.01 and 1.09 µg/ml at 2, 2.5, and 3 hours after ingestion respectively. The level detected at 6 hours is 0.09 ug/ml which is nearly similar to our results. In the present work, it is difficult to continue taking blood samples after 6 hours because of the inability of the subjects to continue. In the study of Hasan et al,[14] the level at 8 hours after ingestion reaches a low level of 50ng/ml only. Reiss et al. [15] found that the peak plasma level is around 1 µg/ml occurring 2 hours after a single dose of 50mg of enteric coated tablet. Without further confirmatory studies, it is difficult to release the names of the

manufacturers. This seems to be acceptable since Meersch et al.[16] found that in 41% of the bioequivalence studies reviewed, the name of the reference drug was not given. In conclusion, the locally manufactured enteric coated diclofenac tablet is not interchangeable with the two reference brand products, although they contain approximately the same amount of diclofenac. However, the latter two can be interchangeable.

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