

In-vitro bioequivalence, physicochemical and economic benefits study for marketed innovator and generic ciprofloxacin hydrochloride tablets in Saudi Arabia

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ABSTRACT

The objective of the present study was to compare and evaluate the economic benefits and *in-vitro* bioequivalence of different marketed generic ciprofloxacin tablets against the innovator tablet formulation that are present in the local market of Saudi Arabia. The comparative bioequivalence and physicochemical study of five ciprofloxacin marketed tablets were performed through the assessment of the uniformity of weight, hardness, disintegration, dissolution, and content assay of the products. In order to compare the dissolution profiles of all generic tablet formulations and the innovator, a model independent approach of similarity factor (f_2) and difference factor (f_1) was employed in the *in vitro* dissolution studies. Deviations were noted in two generic products, these deviations caused significant differences in disintegration time and dissolution profile in only one of the generic products. All tested generic products passed USP monograph dissolution testing except one product which failed to pass both similarity factor (f_2) and difference factor (f_1) tests required by FDA bioequivalence testing. The majority of generic products in Saudi Arabia which had lower prices showed comparable quality to innovator.

INTRODUCTION

Ciprofloxacin is a synthetic fluoroquinolone derivative with broad spectrum antibacterial activity. It is widely used in the treatment of lower respiratory tract infections, urinary tract infections, skin and soft tissue infections, bacterial diarrhea, bone and joint infections, and in surgical prophylaxis (Mandell *et al.*, 2002). Ciprofloxacin is considered to be the drug of choice by most physicians in most of the cases. This caused continuous increase in demand and accordingly the need to increase supply of generic products of ciprofloxacin in the market. It is a general psychology that the quality of generic products is considered poor when compared to leading innovator products available in the market (Adegbolagun *et al.*, 2007).

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World Health Organization (WHO) has continuously supported the use of generic drug products, aiming to improve the overall health care system (Dunne *et al.*, 2013). The generic substitution can be considered when a generic product of an innovator drug contains identical amounts of the same active ingredient in the same dose, same dosage form and route of administration together with meeting standards for strength, purity, quality, and identity (Meredith *et al.*, 2003).

The generic products are usually much cheaper than its innovator as generic manufacturers do not have similar investment costs for the development of a new drug. In order to substitute the innovator with generics while keeping therapeutic efficacy, dissolution testing can be used as a tool to distinguish between acceptable and unacceptable drug products (Shahnaz Usman *et al.*, 2014). It can be used as a sign for bioequivalence; dissolution testing is a practical and economic approach that can be used when there are limitations for performing *in vivo* studies (Meredith *et al.*, 2003).

In the present study, *in vitro* bioequivalence of some generic ciprofloxacin HCl tablets (500) mg were investigated in comparison to an innovator product of ciprofloxacin HCl tablets (500 mg) to justify the quality of generic substitution of ciprofloxacin generic products in the Saudi Arabia market. Some of general quality assessments testes for tablets were also determined to access similarity between innovator brand and the different generic substitutions.

MATERIAL AND METHODS

Material

Ciprofloxacin HCl, USP (United States Pharmacopoeia, 2012) was kindly donated from Jamjoom pharmaceuticals. Brand (innovator) and four generic ciprofloxacin 500 mg tablet products were randomly collected and purchased from Community pharmacies in Madina, Saudi Arabia. Reagents utilized including hydrochloric acid, potassium dihydrogen phosphate, and sodium hydroxide were of analytical grade.

Price deviation and product label information

The percentage price differential of four generic brands in reference to innovator was calculated using proposed formula by Akinleye *et al.* (Akinleye *et al.*, 2012)

$$\% \text{ price differential} = \frac{(\text{Price of innovator} - \text{Price of generic})}{\text{Price of innovator}} \times 100$$

Label information including manufacture date, expiry date and price per 10 tablets were compared for generic and innovator products to study if price correlates with quality of product. The innovator and generic products were purchased from market provided that they have a minimum 6 month before expiry date.

Physicochemical Parameters and moisture content measurement

Tablet dimensions (length, width and/or diameter) and hardness were measured using tablet hardness tester (Tablet hardness tester TBH 125, Erweka, Germany) using six tablets from each of the studied products, the average and standard deviation were recorded for the studied innovator and the four generic products. Disintegration and friability testing for studied tablets were performed using disintegration tester (Disintegration tester model, Pharma Test, Germany) and tablet friability tester (Friability tester model, Pharma Test, Germany) respectively according to USP.

Moisture content was measured after grinding tablets in a mortar and accurately weighing 1 gm in aluminum plates (W1). Samples were tested at 75°C for 30 minutes using Halogen moisture analyzer (Halogen moisture analyzer, HB43-S, Mettler Toledo, Germany).

Assay for drug potency of studied tablet products was performed by analyzing three tablets from each brand by dissolving one tablet in 0.01N HCl. This was followed by filtering,

diluting sample and measuring spectrophotometrically at 276nm (UV-Vis spectrophotometer, SP-3000 plus, Optima Tokyo, Japan).

Differential Scanning Calorimetry (DSC) for studying drug-excipients compatibility

Incompatibility between drug and excipients can alter the stability and bioavailability thereby, affecting their safety and/or efficacy. DSC (Differential scanning calorimetry, Maia DSC 200 F3, Netzsch, Germany) was used as a tool for screening compatibility of excipients used in the different generic products with ciprofloxacin as well as studying similarity of excipients used to excipients used by innovator.

Two tablets were crushed from each of the studied products. 10 mg of each sample was sealed in aluminum crucible and tested using a heating rate of 10 ° C/min at a temperature of 0-360°C.

Fourier transformation Infrared spectroscopy (FTIR) for studying similarity of excipients between innovator and studied generic products

Spectroscopic analysis was carried out with FTIR-total reflectance method (Fourier transformation infrared spectroscopy, IR Affinity-1, Shimadzu, Japan) to investigate similarity of excipients between the brand and generic products. Spectroscopic analysis was performed for pure ciprofloxacin HCl, crushed innovator tablet, and crushed generic product tablets.

Comparative Dissolution profile

Dissolution test of ciprofloxacin tablets was performed according to USP ciprofloxacin tablet monograph dissolution method for innovator and the four generic products. Dissolution test was carried out using dissolution tester equipped with USP paddle (apparatus-2) operated at 50 rpm (Dissolution tester, DT 600, Erweka, Germany). The dissolution medium is 900 ml 0.01N HCl kept at 37±0.5°C. Six tablets from each product were tested. 5ml sample were withdrawn at 5, 10, 15, 20, 30 min, and the volumes withdrawn, replaced with fresh dissolution medium. Samples were then filtered, diluted and measured spectrophotometrically at 276nm (UV-Vis spectrophotometer, SP-3000 plus, Optima Tokyo, Japan).

Dissolution profile data analysis

Dissolution comparison methods recommended by FDA (United States Food and Drug Administration) guidance for immediate release solid dosage forms were used in this study (Guidance for Industry: Immediate Release Solid oral Dosage Forms, 1995). A simple model independent approach including difference factor (f1) and a similarity factor (f2) were used to compare dissolution profiles between innovator and the four generic products.

The difference factor (f1) calculates the percent (%) difference between the two curves at each time point and is a measurement of the relative error between the two curves

(Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms, 1997):

$$f1 = \{ [\sum_{t=1}^n |Rt - Tt|] / [\sum_{t=1}^n Rt] \} \cdot 100$$

where (n) is the number of time interval points, (Rt) is the dissolution value of the innovator at time (t), and (Tt) is the dissolution value of the generic product under test at time (t).

The similarity factor (f2) is a logarithmic reciprocal square root transformation of the sum of squared error. It is a measurement of the similarity in the percent (%) dissolution between the two curves (Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms, 1997).

$$f2 = 50 \cdot \log \{ [1 + (1/n) \sum_{t=1}^n (Rt - Tt)^2]^{-0.5} \cdot 100 \}$$

Curves can be considered similar when (f1) values are close to 0, and (f2) values are close to 100. (f1) values from (0-15) and (f2) values from (50-100) ensure similarity or bioequivalence of the two curves and the performance of the product under test and innovator product (Guidance for Industry: Immediate Release Solid oral Dosage Forms, 1995).

RESULTS AND DISCUSSION

Price deviation and product label information

Product label information and price percentage differences for the innovator and four generic ciprofloxacin marketed products are presented in Table (1). Reviewing the manufacture date and expiry date for the marketed products showed large differences in labeled expiry date period. The longest expiry date period was 4 years and it belonged to the innovator and one of the generic products (Cipro-4). The other three generic products showed variability in expiry date period varying from 2 years to 3 years. These differences in expiry date period might give an indication that most of the generic products do not have the same stability achieved by the innovator product.

The percentage price differences showed that the price of ciprofloxacin innovator is higher than all generic products in Saudi market with differences varying from 38.6% to 67.3%. (Cipro-4) had the lowest percentage price difference from innovator of

38.6%, the closest price to innovator. While (Cipro-5) had the highest price difference from innovator of 76.3%, the lowest price between the studied generic products.

Physicochemical Parameters

Dimensions, shape, color and packaging description for studied ciprofloxacin tablet products are presented in table (2). The studied products have oblong shape and have close dimensions to the innovator except for one product (Cipro-3) which was round. This might represent a difficulty for patients to swallow (Cipro-3) as the tablet is quite big. Color for all ciprofloxacin products under study were white except for one product (Cipro-4) which was coated in blue. Tablet color might have a positive or negative psychological effect on patients (Augsburger *et al.*, 2008), so change of this color without a reference might have a negative effect on patients. All tested ciprofloxacin tablets were scored except (Cipro-2) product. This kind of scoring is usually used to give half dose if needed, and so (Cipro-2) product would deprive patients from taking half dose 250 mg if required. The packaging materials of the products under study are transparent packaging material except for (Cipro-3) which has an opaque packing material. Weight variation, hardness, disintegration, friability, moisture content, and content percent of the tested products are presented in Table (3). Weights of generic products were close to innovator product approximately 770 mg except for (Cipro-4) which had weight of approximately 830 mg. This means that (Cipro-4) contains approximately 63mg excess inactive ingredients which might have negative effect in retarding disintegration time (Al Ameri *et al.*, 2012). (Cipro-2) product tablets also showed deviation from innovator weight. It had approximately 77 mg lower weight than innovator.

Hardness, friability, moisture content and assay results for all studied products were similar to the innovator. Disintegration time results for the generic products were all similar to innovator giving approximately 1 minute disintegration time except for (Cipro-4) tablet which had approximate disintegration time of 6 minutes. This 5 fold increase in disintegration might have a negative impact on dissolution results for tablets and it might be attributed to using different manufacturing technique like using wet granulation instead of direct compression, using different excipients, and using larger amount of excipients as seen in table (3).

Table 1: Label information and percentage price differences for five different ciprofloxacin tablet (500 mg) products marketed in Saudi Arabia.

Product code	Mfg. date	Exp. Date	Price/10 units	% price differences with innovator
Cipro -1 (innovator)	1/2014	1/2018	101 SR	-
Cipro -2	5/2013	5/2015	50 SR	50.5
Cipro -3	7/2014	7/2017	37 SR	63.3
Cipro -4	5/2012	12/2016	62 SR	38.6
Cipro -5	1/2013	1/20116	33 SR	67.3

Table 2: Ciprofloxacin Products dimensions, description and packaging characteristics.

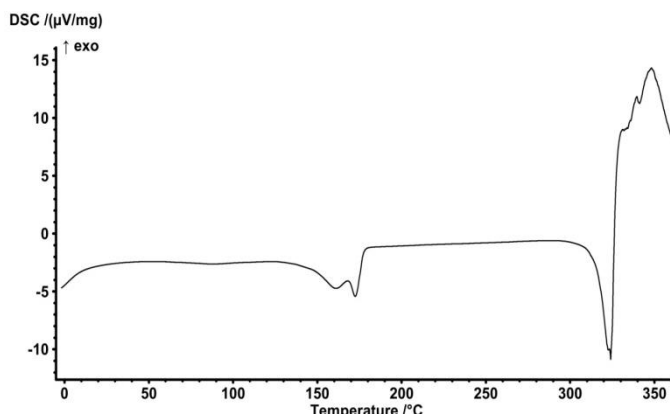
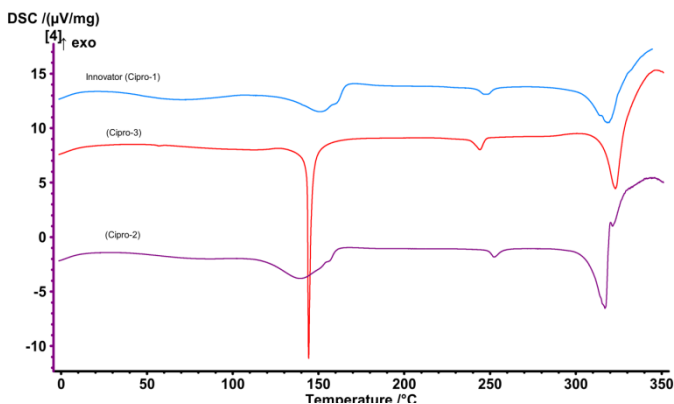
Product code	Length (mm)	Width (mm)	Shape	Color	Scoring	Packaging
Cipro -1 (Innovator)	18.18	8.12	Oblong	White	scored	Transparent
Cipro -2	19.36	6.96	Oblong	White	Not Scored	Transparent
Cipro -3	13.23	13.23	Round	White	scored	Opaque
Cipro -4	18.16	8.88	Oblong	Blue	scored	Transparent
Cipro -5	18.06	8.05	Oblong	White	scored	Transparent

Table 3: Physicochemical parameters of five different brands of ciprofloxacin tablets 500mg.

Product code	weight variation (mg)	Hardness (N)	Friability (%)	Disintegration Time (min.)	moisture content (%)	Assay (%)
Cipro -1(Innovator)	766.9 ± 3.3	23.3	0.02	1.15	2.35	95.6
Cipro -2	689.5 ± 3.5	26.6	0.05	1.10	2.18	97.5
Cipro -3	767.8 ± 3.1	24.8	0.05	1.38	2.06	95.9
Cipro -4	829.7 ± 10.1	24.1	0.03	6.10	1.75	95.9
Cipro -5	781.1 ± 9.4	23.9	0.03	1.33	1.85	96.1

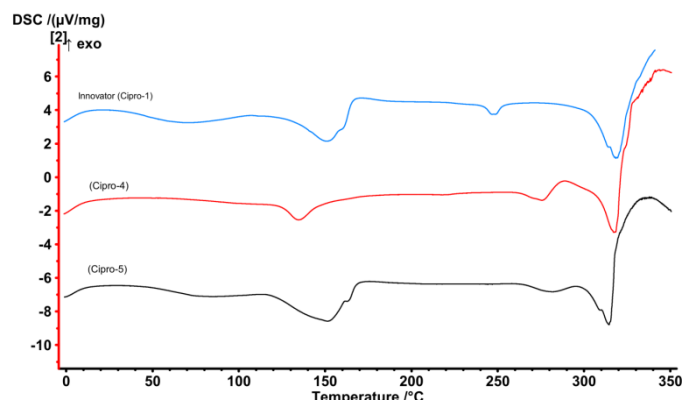
Studying compatibility of excipients using Differential Scanning Calorimetry

DSC thermograms for pure ciprofloxacin HCl monohydrate, innovator (Cipro-1) and studied generic products are presented in figure (1), figure (2) and figure (3). Pure ciprofloxacin HCl showed two endothermic peaks, the first is a broad endothermic peak at 150°C which represent the dehydration peak and the second endothermic peak at 325°C indicating ciprofloxacin HCl melting peak.

**Fig. 1:** DSC thermogram for ciprofloxacin HCl monohydrate**Fig. 2:** DSC thermograms in sequence from upper for Cipro-1 (Innovator), Cipro-3, and Cipro-2.

These results were in accordance with Dillen *et al.* research work (Dillen *et al.*, 2004). Innovator product (Cipro-1) thermograms showed three endothermic peaks, two of them are similar to pure ciprofloxacin HCl. However, a third peak at 250°C appeared which might be due to one of the excipients. Generic products (Cipro-2), and (Cipro-5) thermograms showed three

endothermic peaks which are very close to those of innovator. On the other hand, product (Cipro-4) thermogram showed very broad endothermic peak of dehydration which might signify different tablet preparation technique like granulation with water or hydroalcoholic solutions together with ciprofloxacin melting peak and excipient melting peak. Moreover, product (Cipro-3) showed three endothermic peaks but the second endothermic peak at 150°C was very sharp and long.

**Fig. 3:** DSC thermograms in sequence from upper for Cipro-1 (Innovator), Cipro-4, and Cipro-5.

This peak might be due to using another excipient in this product and not a dehydration peak as the dehydration peak is much smaller in area and broader. DSC thermogram results showed that presence of melting endothermic peak for the ciprofloxacin HCl in studied products' thermograms with no significant shift can indicate absence of incompatibility between ciprofloxacin HCl and used excipients in all products.

It should be noted that the presence of melting endothermic peaks in DSC thermogram of generic products (Cipro-3) and (Cipro-4) which are different from innovator (Cipro-1) might indicate the presence of different excipients or different tablet preparation method which might affect drug dissolution profile (Fusier *et al.*, 2003) or cause problems for patients like allergy or any kind of intolerance (Mumoli *et al.*, 2011).

Studying similarity of excipients using Fourier transform infrared spectroscopy

FTIR spectra for innovator (Cipro-1) and generic products are presented in figure (4) and figure(5). FTIR spectra for the innovator (Cipro-1) seem to coincide with those of the generic

products (Cipro-2), (Cipro-4) and (Cipro-5) which means that the innovator components might be similar to those generic products to a great extent. Generic product (Cipro-3) FTIR spectra did not coincide with that of innovator, this might signify some differences in its components.

These results were in accordance with the DSC thermogram results which showed appearance of a different sharp peak at 150°C.

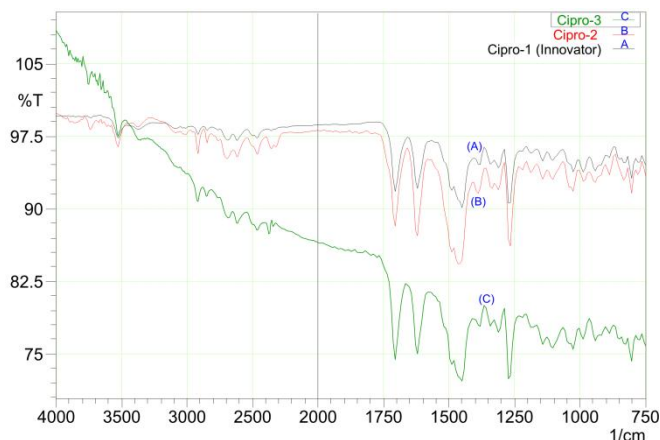


Fig. 4: FTIR spectra for (A)Cipro-1(Innovator) , (B)Cipro-2 labelled, (C) Cipro-3.

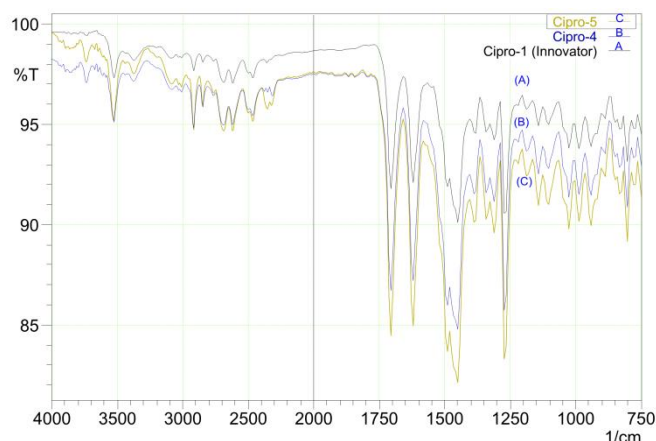


Fig. 5: FTIR spectra in sequence from upper for (A) Cipro-1(Innovator), (B) Cipro-4, (C) Cipro-5.

Comparative Dissolution profile

Dissolution profile of the innovator and four generic products are presented in figure (6). Generic product (Cipro-5) seems to be very close to innovator (cipro-1) in results in all time intervals.

The most obvious difference in dissolution is seen at the 5 minute time interval, where (cipro-2) generic product showed approximately 10% higher release than innovator, drug release 75% on average. (Cipro-3) generic product showed 10% lower drug release than innovator, drug release 65% on average. (Cipro-4) release results were all much lower compared to the innovator.

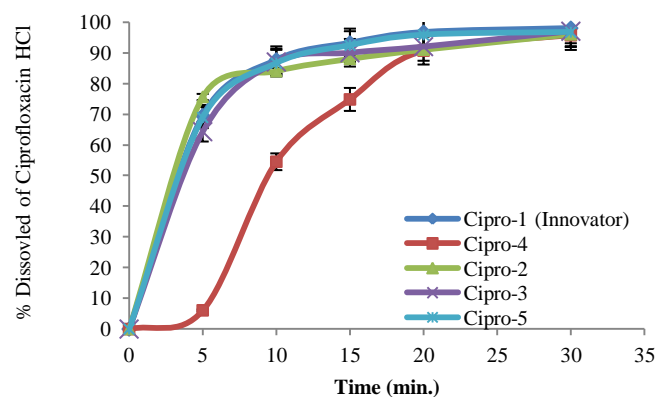


Fig.6: Dissolution rate profile in 900 ml 0.01N HCl for innovator and four generic ciprofloxacin 500 mg tablet products in Saudi Arabia market

Dissolution profile data analysis

Ciprofloxacin HCl is classified according to BCS (biopharmaceutical classification) as class 3 group of drugs (Kyriacos *et al.*, 2009). The minimum requirements for accepting ciprofloxacin immediate release tablet dosage forms are specified by USP ciprofloxacin monograph. It states that the amount of not less than 80% (Q) of the labeled amount of ciprofloxacin dissolved in 30min (United states pharmacopoeia, 2012). All studied products passed this dissolution test limit.

According to the FDA guidance for industry (Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms, 1997), in the dissolution testing of immediate release solid oral dosage forms, the BCS suggests that for class I and in some cases class III drugs 85% dissolution in 0.1N in HCL in 15 min insures that the bioavailability comply with requirement of monograph (Guidance for Industry: Immediate Release Solid oral Dosage Forms, 1995). The innovator (Cipro-1) together with 3 generic products (Cipro-2), (Cipro-3) and (Cipro-5) are passed the stated limit but only one generic product (Cipro-4) did not pass the limit after 15minutes of dissolution as the average drug dissolution was only 75% which is 10% lower than the 85% required for complying to required bioavailability.

In order to compare the dissolution profiles of the studied innovator and generic products, a model independent approach of difference factor (f_1) and similarity factor (f_2) were employed (Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms, 1997). Similarity factor f_2 has been used by FDA and the European medicines evaluation agency (EMA) to compare the similarity of two or more dissolution profiles. For two dissolution profiles to be considered bioequivalent or similar, difference factor (f_1) should be between 0 and 15, while similarity factor (f_2) should be between 50 and 100 (Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms, 1997). The difference factor (f_1) and similarity factor (f_2) values for the different generic products under study with respect to innovator (Cipro-1) are presented in table (4). The calculated values of similarity factor (f_2) were more than 50 and difference factor (f_1) values were less than 15 for studied products except for (Cipro-4). Thus, Cipro-2, Cipro-3 and

Cipro-5 can be considered to be equivalent to the innovator brand. (Cipro-4) generic product had a (f2) value of 23 and (f1) value of 27. Because of the value of (f1) factor is higher than 15 and the (f2) factor is lower than 50, (Cipro-4) is considered to be dissimilar and not bioequivalent to innovator. These results were supported by the disintegration test which showed that product (Cipro-4) had much longer disintegration time relative to innovator product (Cipro-1).

Table 4: Calculated difference factor (f1) and similarity factor (f2) for all generic ciprofloxacin tablets brand in respect to Innovator (Cipro-1).

Studied Factor	Cipro-2	Cipro-3	Cipro-4	cipro-5
Similarity Factor (f2)	61	63	23	66
Difference factor (f1)	5	5	27	4

CONCLUSION

Similarity of generic ciprofloxacin hydrochloride tablet products in Saudi Arabia to innovator was investigated. The physicochemical properties for all these products were studied. Some physical differences were noted in tested generic products like color, tablet scoring, tablet weight and shape. These differences from innovator might have negative impact on psychological therapeutic effect or patient compliance. Deviation in excipients or method of manufacture from innovator was noted in two generic products, these deviations caused significant differences in disintegration time and in dissolution profile in only one of the generic products. All of the tested generic products passed USP monograph dissolution testing and only one product failed to pass both similarity factor (f2) and difference factor (f1) tests required by FDA. This dissimilar generic product showed higher price when compared to other generics. While the majority of studied generic products in Saudi Arabia which had lower prices showed comparable quality to innovator to a great extent. *In-vivo/ In-vitro* correlation is recommended in future studies to extensively evaluate effect of changes in method of manufacturing and excipients on generic products.

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