ABSTRACT

The sale and use of substandard drugs is a major health problem in many developing countries where strict drug control legislation does not exist. The study investigates pharmaceutical quality of different brands of cefixime 400mg capsules marketed in Karachi (Pakistan). Compendial standards and government regulations require that all drug products, whether prescription or OTC products, meet strict standards of identity, potency, and purity. Each type of dosage form requires careful study of the physical and chemical properties of drug substances to achieve a stable and effective product. The different brands were subjected to various tests like uniformity of weight, diameter, length, disintegration, dissolution and chemical assays. The susceptibility test of drug was also carried out using agar dilution method along different isolates obtained from health care set ups. All the 6 brands had satisfactory drug content, antimicrobial activity and all passed the USP drug release test. The six brands are physically and chemically equivalent and could be interchanged. The study reinforces the need for constant monitoring of proprietary products of the same generic drugs to ensure quality and consequent efficacy with pharmacoeconomy, which would help in the selection of the most appropriate brand in terms of Pharmacoeconomy and quality.

Keywords: Cefixime; Comparative evaluation; Physiochemical property; Pharmacoeconomy; Antibiotics; Study of Pharmaceutical quality; Cephalosporin.

INTRODUCTION

Marketing of poor quality drugs is of concern in developing countries and has been widely reported1-4. In some cases, expired drugs have been intentionally given to patients due to economic reasons or lack of adequate drug information and education4. In a recent study in Sudan, a number of drugs used in the country such as ergometrine, lignocaine, adrenaline, suxamethonium and ampicillin were shown to degrade rapidly under tropical conditions1. The Caribbean Regional Drug Testing Laboratory found that between 10 and 50% of the samples from different countries in the region did not comply with test requirements during 1988-91. Similarly, the Drug Quality Control Laboratory in Daru, Kenya, found that about 45% of the locally manufactured drugs and 31% of those imported during 1983-86 did not meet test requirements1. The increase in the number of generic drug products from multiple sources has placed people involved in the delivery of health care in a position of having to select one from among several seemingly equivalent products. For instance, in 1975 approximately 9% of all prescription drugs dispensed in the United States were generic versions5. This figure5 rose to 20% in 1984 and 40% in 1991. Over 80% of the approximately 10,000 prescription drugs available, in 1990, were obtained from more than one source and variable clinical responses were documented due to two or more drug manufacturers who supplied these dosage forms6. These variable responses may be due to formulation ingredients employed, methods of handling, packaging and storage and even the rigors of in-process quality control. Thus, there is need to determine their pharmaceutical and therapeutic equivalence in order to ensure interchangeability7. However, many developing countries do not have an effective means of monitoring the quality of generic drug products in the market. This results in widespread distribution of substandard and/or counterfeit drug products. It was in view of this fact that the World Health Organization issued guidelines for global standard and requirements for the registration, assessment, marketing, authorization and quality control of generic pharmaceutical products8. Preliminary physicochemical assessment of the products is very important and in vitro dissolution testing can be a valuable predictor of the in vivo bioavailability and bioequivalence of oral solid dosage forms9. Cefixime, a broad spectrum, bactericidal, β-lactamase stable, third generation cephalosporin, is a semi synthetic first orally active and effective antibiotic10-11. Years of extensive investigations on cefixime have

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indicated that it is an excellent orally active antibiotic with similar antibacterial activity and resistance to \( \beta \)-lactamase as the third generation parenteral cephalosporins\(^{12-13} \). Cefixime has excellent biological properties, displaying potent antibacterial activity against a wide range of Gram-positive bacteria and gram-negative bacteria, highly stable towards \( \beta \)-lactamases and long acting efficacy\(^{12} \). Memon et al.\(^{14} \) reported cefixime as a safe, effective, and cheaper oral option for the treatment of multidrug-resistance.

In the present study the physico-chemical equivalence and antimicrobial activity of six different commercial brands of cefixime capsules sourced from retail pharmacies of Karachi was determined using in vitro method. This preliminary study is aimed to obtain baseline data towards the establishment of bioequivalence of the capsules.

**EXPERIMENTAL**

### Instrumentation

The analysis of cefixime content in their dosage form was done by a Shimadzu HPLC system, equipped with LC-10 AT VP pump and SPD-10 a VP UV-VIS detector. Chromatographic system was integrated via Shimadzu model CBM-102 Communication Bus Module to P-III computer loaded with Shimadzu Class-VP software for data acquisition and mathematical calculations. C\(_{18}\) Shim-pack CLC–ODS Column (6mmID \( \times \) 15 cm), protected by octa decyl silane guard column (Pre-column), manual injector fitted with a Loop -20\( \mu \)l. Analytical balance, dissolution test apparatus (Erweka GmbH), UV spectrophotometer (Parken ELMER; \( \varnothing \)-20, Model 1601), disintegration test apparatus (Erweka GmbH, Heusenstamm, Germany), sonicator, pH meter, micropipettes (200 - 1000\( \mu \)l), incubator (Heraeus, Typ B-6, Germany), hot oven (EHRET, D -2800 Breman, Germany), autoclave (OSK 8869-D, Autoclave AC -220V50HZ), Laminar flow (ESCO), wire loop, burner, test tube holder and vortex.

### Materials and reagents

Reference cefixime was a kind gift from Hilton Pharma (Private) Limited. Six different brands of cefixime were obtained from retail pharmacies of Karachi (Pakistan) market. All reagents were of HPLC grade. Methanol, sodium hydrogen phosphate and orthophosphoric acid 85% (Merck, Germany). Mueller–Hinton broth, Mueller–Hinton agar, standard organisms [\( E. \ coli, \) ATCC = 25922; \( S. \ aureus, \) ATCC = 25923], clinical isolates (\( E. \ coli, \) and \( S. \ aureus \)), dihydrate barium chloride, sulphuric acid, sterile cotton rolls and HPLC grade distilled water were freshly obtained to prepare mobile phase and dilutions.

### Chromatographic Condition

The sample solutions were introduced into the system by injector with a 20-\( \mu \)l loop. The flow rate was 1ml/min. Chromatograms were recorded at 288nm.

### Spectrophotometric condition

Base line was adjusted to zero by using blank solvent (0.05M Potassium phosphate buffer). Standard and test sample were analyzed (the observed result was based on three average readings).

### PHYSICOCHEMICAL PARAMETERS

Drug cost and quality are the major component of the total cost of the National Health Services (NHS) which is constantly rising. As the resources of the NHS are limited, so it is the need of time to keep eye on the quality and cost of the drugs that are available in the markets. There are a number of companies that manufactures cefixime capsules. The label information of six different brands of capsule are presented in Table 1

#### Table 1: Label Information of Cefixime Capsule (400mg)

<table>
<thead>
<tr>
<th>S #</th>
<th>BRAND NAME</th>
<th>MANUFACTURE NO</th>
<th>DATE OF PACK</th>
<th>PACK YEARS</th>
<th>MANUFACTURE CODE</th>
<th>MANUFACTURE DATE</th>
<th>CODE NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CEF 1</td>
<td>1234</td>
<td>10/2001</td>
<td>2008</td>
<td>001</td>
<td>000001</td>
<td>1234</td>
</tr>
<tr>
<td>2</td>
<td>CEF 2</td>
<td>5678</td>
<td>15/2001</td>
<td>2009</td>
<td>002</td>
<td>000002</td>
<td>5678</td>
</tr>
<tr>
<td>3</td>
<td>CEF 3</td>
<td>9102</td>
<td>20/2001</td>
<td>2010</td>
<td>003</td>
<td>000003</td>
<td>9102</td>
</tr>
<tr>
<td>4</td>
<td>CEF 4</td>
<td>3456</td>
<td>25/2001</td>
<td>2011</td>
<td>004</td>
<td>000004</td>
<td>3456</td>
</tr>
<tr>
<td>5</td>
<td>CEF 5</td>
<td>7890</td>
<td>30/2001</td>
<td>2012</td>
<td>005</td>
<td>000005</td>
<td>7890</td>
</tr>
<tr>
<td>6</td>
<td>CEF 6</td>
<td>1012</td>
<td>35/2001</td>
<td>2013</td>
<td>006</td>
<td>000006</td>
<td>1012</td>
</tr>
</tbody>
</table>

#### Weight variation

The volume and complexity of modern medicines are increasing day by day. The need is to compare the pharmaceutical, chemical and therapeutical efficacy of medicines with their cost. Capsules are the solid dosage forms containing the drug and usually filler(s), enclosed in a hard or soft gelatin shell. The gelatin shell readily ruptures and dissolves following oral administration, and in most cases the drug is released from capsules faster than from tablets\(^{15} \). The dose uniformity of tablets/capsules can be determined by weight variation. Weight variation requirements may be applied where the product is a liquid-filled soft capsule, or where the product to be tested contains 50mg or more of a single active ingredient comprising 50% or more, by weight, of the dosage form unit.

#### Length and diameter

BP-2002\(^{16} \) introduced a standard for capsules length and diameter to reduce patient confusion over generic equipments. Length and diameters of the capsules were also collected.

#### Disintegration

Disintegrations are required to break up tablets, capsules and granules into primary powder particles in order to increase surface area of the drugs exposed to gastrointestinal fluids. The disintegration test was carried out by using Erweka ZT 3 Disintegrator. A 1000ml beaker was filled with distilled water (approx. 900ml), equilibrated to 37\( \pm \)0.5\(^\circ\)C. Six capsules from each brand were subjected to the test. Time required for the last capsule to disintegrate was recorded.
Dissolution
During dissolution test the cumulative amount of drug that passed into the solution was studied as a function of time. The test described the rate of release of the drug from the dosage form. Dissolution test was carried out by using an Erweka dissolution instrument. Paddle method (apparatus-2; USP-27) was used at 50 rpm. Potassium phosphate buffer 0.05M (900ml), pH-7.0, was poured into the vessel and equilibrated to 37±0.5 ºC. Six capsules from each brand were tested. 5ml of aliquot was withdrawn at the intervals of 15, 30 and 45 min, and the volumes withdrawn, replaced with fresh dissolution medium. The sample was filtered, using Whatman filter paper and 2ml of filtrate was further diluted as working solution. The absorbance was measured at 288 nm.

Standard Preparation for dissolution
44 mg (on dried basis) of cefixime standard powder was dissolved in 100 ml of 0.05M potassium phosphate buffer (pH-7.0) and the final concentration was made of 8.8µg/ml.

Assay
Analysis of drug potency in capsules comprises the following steps:

Preparation of standard solution for HPLC
100 mg of cefixime standard was weighed and dissolved in 50 ml volumetric flask with few ml of methanol and the volume was made up with mobile phase obtaining concentration equivalent to 0.2mg/ml.

Preparation of Mobile Phase
Mobile phase consisted of 12.5mM KH₂PO₄ buffer solution and methanol (65:35v/v, pH adjusted at 2.75 with 85% orthophosphoric acid). Prior to delivering into the system it was filtered through 0.45µm filter and degassed using a sonicator. The method used for the analysis of cefixime in capsules was first developed and validated.

Preparation of Sample Solution
20 capsules were weighed accurately and ground to a fine powder. 500mg of ground powder i.e. equivalent to average weight of capsule was transferred to a volumetric flask (250ml), dissolved in mobile phase and shaken for about 10 minutes and then filtered. Filtrate solution was further diluted in the mobile phase and the concentration of working sample solution made equivalent to 0.2mg/ml.

ANTIBACTERIAL ACTIVITY
In the present study the resistance pattern and comparative activities of cefixime standard with different commercial brands (available in the local market) were determined using agar dilution method. The organisms used were:

- E.coli (ATCC-25922).
- Five isolates of E.coli of different sources.
- S. aureus (ATCC - 25923), and.
- Five isolates of S. aureus of different sources.

Stepwise considerable points were:
1. Preparation of the Mcfarland Standard
2. Preparation of Inoculums
3. Preparation of Antibiotic Stock Solution

Suitable ranges of antibiotic concentrations were selected for the organisms to be tested.

Standard antibiotics powder (Reference powder of cefixime and different brands) weighed accurately for the preparation of stock solutions by using the following formula:

$$W = \frac{1000}{P} \times V \times C$$

Where,
- $W$ = Weight of antibiotic to be dissolved in volume (v).
- $P$ = Potency given by the manufacturer.
- $V$ = Volume required.
- $C$ = Final concentration of solution.

After preparing the stock solution, serial dilutions of varying concentrations, usually doubling concentration (0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, and 64 µg/ml), were prepared. Equal volumes of inoculums were added in each.

RESULT
Many drugs that are manufactured in developing countries are implicated to be substandard. Price fluctuation in societies where there is no regulatory control has been a severe problem related to the quality of the drug. The variation in the price has been observed from as much as 32 to 55 PKR per unit (Fig.1) while there was no significant variation in the quality of the tested drugs. Hence it may be suggested that the most economic available drug should be used when the pharmaceutical outcomes are promising too. This would lead to more pharmaco-economic practices for the common people in the third world countries like us.

Comparative study of various formulations is a pronounced concept used in therapeutic disciplines. It provides understanding in the comparative difference of quality control test / parameters of cefixime and the effect of these differences on the release of drug from the dosage form. The present study made attempt to check the weight variation, diameter, length, disintegration time and dissolution, chemical assay, of the six different brands of cefixime 400mg capsules.

Price Fluctuation
The price fluctuation of the studied brands is depicted in Fig. 1.
Weight variation
Table 2 shows weight variation of capsules with their standard deviation, average diameter of six brands with their standard deviation and average length of the different brands of cefixime capsules. The Fig. 2 represents the variation in the length of the capsule shell size that plays an important role in the compliance of patients. It reveals that results are highly significant with the limits given ±7.5%.

Table 2. Physical parameters of six different brands of cefixime 400mg capsules

<table>
<thead>
<tr>
<th>Brand</th>
<th>Weight variation</th>
<th>Average diameter</th>
<th>Average length</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEF-1</td>
<td>518.75 ± 11.51</td>
<td>7.3 ± 3</td>
<td></td>
</tr>
<tr>
<td>CEF-2</td>
<td>575.3 ± 3.3</td>
<td>7.3 ± 3</td>
<td>3.1 ± 3</td>
</tr>
<tr>
<td>CEF-3</td>
<td>365 ± 3.3</td>
<td>7.3 ± 3</td>
<td>3.1 ± 3</td>
</tr>
<tr>
<td>CEF-4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEF-5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEF-6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Disintegration
The disintegration tests serve as a component in the overall quality control of capsule manufacturing. Disintegration test was conducted on the six different brands of cefixime 400mg capsule. In the present investigation all the six capsules of each brand showed disintegration within the range of 5.273 – 13.66 minutes (Fig. 3). The BP-2002 stipulates a disintegration time of not more than 15 min for uncoated tablets/capsules. Product CEF-6 has shown a maximum average disintegration time about 13.66 ± 1.128 minutes and product code CEF-4 has shown minimum disintegration time about 5.273 ± 0.472 minutes, which is good as compared to other products because as early the tablet/capsule break as rapidly it goes under dissolution.

Dissolution
Dissolution test is an extremely valuable tool in ensuring the quality of a drug product. Generally product-to-product variation is due to the formulation factors such as particle size, compression force, excessive amount of lubricant etc. Thus dissolution test are very effective in discriminating between and within batches of drug product(s). Dissolution testing is the most important way to study, under in vitro conditions, the release of a drug from a solid dosage form and thus represents an important tool to assess factors that affect the bioavailability of a drug from a solid preparation. It is not possible for any manufacturer to check the bioavailability of all batch of any product. In this situation the dissolution test acts as an important mean.

The present investigation showed that all the brands dissolved within limit (Table 3) and the statistical evaluation (ANOVA) of dissolution test indicated no significant variation between and within different brands of cefixime capsules (Table 4).

Table 3. Percent Dissolution (Mean ± SD) of the six brands of Cefixime capsules

<table>
<thead>
<tr>
<th>Mu MM of MMN solutions</th>
<th>Percent of dissolution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WMN</td>
</tr>
</tbody>
</table>
**Table 4. ANOVA for percent of dissolution (%) of different brands of cefixime**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>F</th>
<th>p-value</th>
<th>F critical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Within Groups</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

**Chemical Assay**

The assay content of cefixime capsules (Table 5) showed that the active content of all the brands were between 95% and 105% of the label. The results indicated that although different manufacturers formulated different brands by different methods of formulation but all were under the BP/USP specifications. There were no significant difference in the release pattern of cefixime from different brands (Table 5), for the analysis of active content the method used was first developed and validated. The precision and accuracy of the method is shown in Fig. 4a and 4b (standard and brand) indicates no interfering peak of excipient. Statistical analysis was also conducted using one-way analysis of variance (ANOVA). The statistical evaluation indicated that there was no significant variation between and within different brands of cefixime capsules (Table 6).

**Table 5. Content assays of Cefixime capsules by HPLC**

<table>
<thead>
<tr>
<th>Assay</th>
<th>CEF 1</th>
<th>CEF 2</th>
<th>CEF 3</th>
<th>CEF 4</th>
<th>CEF 5</th>
<th>CEF 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area 1</td>
<td>90.1%</td>
<td>102.2%</td>
<td>90.6%</td>
<td>101.5%</td>
<td>101.2%</td>
<td>99.2%</td>
</tr>
<tr>
<td>Area 2</td>
<td>90.5%</td>
<td>101.5%</td>
<td>90.6%</td>
<td>101.2%</td>
<td>101.4%</td>
<td>99.3%</td>
</tr>
<tr>
<td>Area 3</td>
<td>90.7%</td>
<td>100.2%</td>
<td>90.7%</td>
<td>100.2%</td>
<td>100.2%</td>
<td>98.4%</td>
</tr>
<tr>
<td>S/VI</td>
<td>258.22</td>
<td>300.42</td>
<td>269.32</td>
<td>301.71</td>
<td>250.52</td>
<td>250.56</td>
</tr>
<tr>
<td>S/MD</td>
<td>54.63</td>
<td>63.27</td>
<td>58.77</td>
<td>61.02</td>
<td>59.75</td>
<td>59.20</td>
</tr>
<tr>
<td>SD</td>
<td>0.125</td>
<td>0.116</td>
<td>0.261</td>
<td>0.062</td>
<td>0.102</td>
<td>0.125</td>
</tr>
</tbody>
</table>

**Antimicrobial Susceptibility Test**

Resistance of common pathogens to Antimicrobial agents has emerged as one of the most important problems in the field of infectious diseases. As a matter of microbial evaluation of different brands of cefixime, broth micro-dilution method was employed against E. coli and S. aureus. The experiment was carried out on standardized cultures and isolates. The results were observed visually and spectrophotometrically (6 = 546nm). Among E. coli, the reported MIC for standard cefixime was in the range of 0.25-1mg/ml against ATCC # 25922 that was observed in the same range. Whereas the standard cefixime value against clinical isolates of E. coli has shown MICs ranging between 2 – 32 µg/ml (Fig: 5).

The MICs of different brands of cefixime against standard E. coli, ATCC # 25922 showed MICs ranging 1–2 µg/ml, five brands showed MICs of 1µg/ml and one brand showed MICs of 2µg/ml. The same brands were evaluated against clinical isolate of E. coli and showed MICs range of 8 – 64 µg/ml (Fig: 5). Single factor ANOVA was applied on the MICs of cefixime reference and on different brands capsule against E. coli. The ANOVA showed insignificant variation of inhibitory concentration range among standard and brands of cefixime.

The comparison of MICs of different brands against standard and clinical isolate of E. coli is presented in Fig. 5. The increased MICs of cefixime against clinical isolates of E. coli indicates a budding of resistance of E. coli which is also in confirmation with the study conducted by Nakamura and Takahashi; 2004 and some other factors, that were related with the brands but the pharmaceutical parameters indicated brands to be in accordance to the official specifications.

The reported MICs for standard cefixime was in the range of 4 – 64 µg/ml against S. aureus ATCC # 25922, which was observed in the same range. The MICs for standard cefixime against clinical isolates of S. aureus ranging between 8 –128 µg/ml. The MICs of different brands against standard S. aureus ranging between 8–32µg/ml, three brands showed MICs of 16µg/ml, one brand showed 8µg/ml and two brands showed of 32µg/ml (Fig: 6). The same brands were evaluated against clinical isolate of S. aureus and showed MICs range of 8 – 256 µg/ml (Fig: 6).

**Fig. 4:** a) Chromatogram of Standard b) Chromatogram of Sample

**Fig. 5:** Comparison of MICs against E. coli among standard and different brands.

Table 6. ANOVA single factor for content assay of Cefixime

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>F</th>
<th>p-value</th>
<th>F critical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between batches</td>
<td>28.20</td>
<td>5.27</td>
<td>0.0005</td>
<td>4.39</td>
</tr>
<tr>
<td>Within batches</td>
<td>5.42</td>
<td>5.42</td>
<td>0.0005</td>
<td>4.39</td>
</tr>
<tr>
<td>Total</td>
<td>33.62</td>
<td>11</td>
<td>0.0005</td>
<td>4.39</td>
</tr>
</tbody>
</table>
Single factor ANOVA was applied on the MICs of cefixime against S. aureus. This analysis indicated that there was no significant change of inhibitory concentration range. The increase in the MICs of cefixime against different clinical isolates of S. aureus indicated a growing of resistance in S. aureus. The present results are in confirmation with work of Soussy et al in 198925.

**DISCUSSION**

The specification for pharmaceutical capsule usually includes appearance, weight, length and diameter, disintegration, dissolution, and content assay etc. These specifications are established to ensure that the capsule will have sufficient mechanical strength to withstand packaging, shipping, and handling and are physically and chemically stable to deliver the accurate amount of drug at the desired dissolution rate when consumed by a patient. Any changes in these characteristics may significantly affect the safety and efficacy of the product. Therefore it is very important to keep a check on each and every step during the formulation and manufacturing of a drug product. There are a number of scientists who also evaluated the efficacy and potency of different brands of cefixime26-30 and suggested that these are well tolerated in terms of safety. But none of them evaluated the efficacy and potency of different brands of cefixime in Pakistani environment. Whereas, the Asians have quite different ecological and sociological circumstances and different body physiology, diet habits and economical conditions as compared to others that can produce significant effects on the absorption behavior (bioavailability) of the drug. Table 5 shows the mean percentage of stated content obtained for each of the 6 brands of capsules. All brands met pharmacopoeial specifications with regard to content of active ingredient. All brands passed the dissolution test for the release of cefixime (Table 3). The percentage of cefixime released within 45 minutes was in the range 95.5983 -99.04, which once again was in conformity with USP monograph that requires an amount not less than 80% of the labeled amount of the drug to be released into the medium at 45 minute. In the present study the brand CEF # 6 has shown all the six capsules dissolved within 15minutes but its time of disintegration is greater than all the other five brands that disintegrated within 13.66 minutes (Fig.3).

The antimicrobial action of the different studied brands of cefixime has shown positive results with the required MICs, this result emphasizes on the rationale use of antibiotics in order to keep the efficacy of the antimicrobial agent without causing resistance. A number of research articles29-30 are available which indicates that the physicochemical parameter’s evaluation of different drug substances are required for the achievement of a stable and effective product which may be pharmaceutically equivalent whether the drug is bioequivalent or not in vivo, but to know that if the drug is pharmaceutically equivalent, initially its physicochemical parameters are needed to be evaluated.

There are many researches that emphasize the need to evaluate different brands of the same generic drug in order to screen the quality product available in local market2-31-33.

**ACKNOWLEDGEMENTS**

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