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In vitro Analysis and Data Comparison of Market Brands of Ciprofloxacin, Ofloxacin and Levofloxacin

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Abstract. In the evaluation of three different groups of 12 brands of locally manufactured Quinolone tablets available in the market i.e. ciprofloxacin HCl, ofloxacin and levofloxacin hemihydrate, it was found that composition of active ingredients were within the range of pharmacoepial limits but their disintegration time and rate of dissolution were different, some being very close to the lower pharmacoepial limit. One product was substandard having high disintegration time and very low rate of dissolution.

Keywords: flouroquinolone, ciprofloxacin, ofloxacin, levofloxacin

Introduction

The spread of fake and substandard drugs is a major problem in both the developed and the developing countries. The existence of counterfeit and substandard drugs particularly antibiotics and antiparasitic agents (Frankish, 2003; WHO, 1999; Wondemagegnehu, 1999; Shakoor *et al*., 1998; Anon, 1993) have been increasingly reported in the developing countries where drug regulations are ineffective (Newton *et al*., 2001; WHO, 1999). Drugs that treat serious diseases such as malaria, tuberculosis, AIDS or other infections are more often the object of counterfeit (Ahmad 2004; Pincock, 2003). Consistent with the recommendations of the WHO (1999) the World Health Organization launched the International Medical Products Anti-Counterfeiting Taskforce (IMPACT) in February 2006, to stop the production and trading of fake medicines.

In Pakistan, although Drug Regularity Authority and drug laws are in function, the problem exits and the extent of problem is not known due to unavailability of data. Aim of the present study is to evaluate various market brands of antiinfective drugs to provide a data base as a ground for better implementation of drug laws in the country. Fluoroquinolones are synthetic anti-bacterial agents derived from the first pyrridone-beta-carboxylic derivative, nalidixic acid (Bryskier and Lowther, 2002). Fourteen brands of three types of quinolone derivatives are the focus of the present evaluation study.

Materials and Methods

Three types of quinolone derivatives were selected for the study: ciprofloxacin, ofloxacin and levofloxacin. Four brands of each type i.e. total of twelve samples were collected from local market and reference standards of USP grade were used. After identification, the drugs were analyzed for their disintegration time, dissolution rate and assay by spectrophotometer and HPLC.

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Identification of active ingredient. E1% solution of the ingredients were prepared in 0.1M HCl and absorbance was measured by UV-Vis spectrophotomer Perkin Elmer Lambda 35. $\ddot{e}_{max}$ for ciprofloxacin was 276 nm and for ofloxacin and levofloxacin, it was 294 nm.

I. Evaluation of Ciprofloxacin

Disintegration time. Test was carried out according to the specifications of British Pharmacoepoeia (2007).

Dissolution test. Test was carried out according to United States Phamacopoeia (2004) with 900 ml of 0.01N HCl in each basket; temperature was adjusted at 37±1°C. The rotation of the paddle was adjusted at 50 rpm and the run time of the apparatus was 30 min.

Sample preparation. Sample was drawn after 30 min, filtered and cooled down to room temperature. 9 ml of this solution was taken in a 100 ml flask and diluted with 0.01N HCl to 25μg/ml concentration.

Standard preparation. For preparation of the working standard, 291 mg ciprofloxacin HCl, equivalent to 250 mg ciprofloxacin, was weighed in 100 ml volumetric flask and dissolved in 0.01N HCI till the volume was up to the mark. 1ml of this solution was further diluted up to 100 ml with 0.01N HCl (25 μg/ml).

Absorbance of the standard and the sample solution was measured at 276 nm taking 0.01N HCl as blank.

Assay by UV: ***Standard preparation.*** For preparation of working standard, ciprofloxacin HCl, 116.4 mg equivalent to 100 mg of ciprofloxacin, was weighed in 100 ml volumetric flask, dissolved in 0.1 N HCl till the volume was upto the mark. 1 ml of this solution was diluted upto 100 ml with the same solvent for preparing a solution of 0.3 mg/ml concentration.

Sample preparation. Twenty (20) tablets were weighed and ground. The powder equivalent to 100 mg of ciprofloxacin was taken in 100 ml volumetric flask and dissolved in 0.1 N HCl. The solution was fltered and diluted. 1 ml of this solution was diluted up to 100 ml with 0.1 N HCl.

The absorbance of the sample and the standard were measured at 276nm taking 0.1N HCl as blank.

Assay by HPLC. The assay was carried out by the method of United States Pharmacopoeia (2004) using high performance liquid chromatography. A Schimadzu LC-9 was used with UV detector set at 278 nm having ODS column (25 cm × 4.6 mm). Temperature was adjusted at 30 ± 1 °C.

The mobile phase was prepared by mixing 0.025 M phosphoric acid of pH 3.0 and acetonitrile (87:13). Flow rate was kept at 1.5 ml/min.

Standard preparation. For preparation of working standard, 349.2 mg of ciprofloxacin HCl, equivalent to 300 mg of ciprofloxacin, was weighed in 100 ml volumetric flask, dissolved in water and the volume was made upto the mark. 10 ml of this solution was diluted to 100 ml with the same solvent to prepare a solution of 0.3 mg/ml.

Sample preparation. Twenty (20) tablets were weighed and ground. The powder equivalent to 5 tablets was taken in 500 ml volumetric flask, about 400 ml water was added and sonicated for 20 min. The volume was made upto the mark with water; 10 ml of this solution was diluted to 100 ml with the same solvent to prepare a solution of 0.25 mg/ml.

Ciprofloxacin contents expressed in mg/tablet were calculated using the following formula:

$$\text{Amount of ciprofloxacin (mg)} = \frac{331.35 \times C \times L \times ru}{367.81 \times D \times rs}$$

where:

331.35 = Molecular weight of ciprofloxacin HCl.
367.81 = Molecular weight of anhydrous ciprofloxacin HCl.
C = Concentration (mg/ml) of USP grade ciprofloxacin HCl RS in the prepared standard.
L = Labelled amount (mg) of ciprofloxacin in each tablet.
D = Concentration (mg/ml) of ciprofloxacin in the prepared sample.
ru = Ciprofloxacin peak response obtained from the prepared sample.
rs = Ciprofloxacin peak response obtained from the prepared standard.

II. Evaluation of Ofloxacin

Disintegration time. Test was carried out according to specifications of British Pharmacoepoeia (2007).

Dissolution test. Test was carried out according to USP (2004) with 1000 ml of 0.1N HCl in each basket; temperature was kept at 37±1°C. Rotation of the paddle was adjusted at 50 rpm; run time of the apparatus was 30 min.

Sample preparation. Sample was drawn after 30 min, filtered and cooled down to room temperature; 10 ml of this solution was taken in 100 ml volumetric flask and diluted with 0.1 N HCl to get a solution of 20 µg/ml concentration.

Standard preparation. For preparing reference standard, ofloxacin equivalent to 200 mg was weighed in 100 ml volumetric flask, dissolved in 0.01 N HCl and the volume was made upto the mark; 1 ml of this solution was diluted with the same solvent to get a solution of 20 µg/ml concentration.

Absorbance of the standard and the sample solution was measured at 294 nm, taking 0.1 N HCl as blank.

Assay by UV. ***Standard preparation.*** For preparing working standard, ofloxacin equivalent to 100.0 mg was weighed in 100 ml volumetric flask, dissolved in 0.1 N HCl and the volume was made up to the mark; 1 ml of this solution was diluted to 100 ml with the same solvent.

Sample preparation. Twenty (20) tablets were weighed and ground. Powder equivalent to 100 mg of ofloxacin was taken in 100 ml volumetric flask, dissolved in 0.1N HCl and the volume was made upto the mark. The solution was filtered and 1 ml of this solution was diluted up to 100 ml with the same solvent.

Absorbance of the sample and the standard was measured at 294 nm taking 0.1 N HCl as blank.

Assay by HPLC. The assay was carried out by the method of USP (2004) using high performance liquid chromatography. A Schimadzu LC-9 was used with UV detector set at 294 nm having ODS column (25 cm × 4.6 mm), temperature was adjusted at 35 ± 1°C.

The mobile phase used was mixture of dodecyl sodium sulphate (0.24% aqueous solution), acetonitrile, and glacial acetic acid (580:400:20) with flow rate of 1.5 ml/min.

Standard preparation. For preparing working standard, ofloxacin, equivalent to 60 mg, was weighed in 100 ml

volumetric flask, dissolved in 0.05 N HCI and the volume was made upto the mark; 10 ml of this solution was diluted to 100 ml with the same solvent so as to prepare a solution of 0.06 mg/ml.

Sample preparation. Twenty (20) tablets were weighed and ground. Powder equivalent to 60 mg of ofloxacin was taken in 100 ml volumetric flask, dissolved in 0.05 N HCI and the volume was made up to 100 ml; 10 ml of this solution was diluted to 100 ml with the same solvent so as to prepare a solution of 0.06 mg/ml.

Percentage amount of ofloxacin expressed in mg/tablet was calculated by the following formula.

Percentage of ofloxacin = (ru/rs) × 100,

where:

ru = Ofloxacin peak response obtained from the sample preparation and
rs = Ofloxacin peak response obtained from the standard preparation.

III. Evaluation of Levofloxacin

Disintegration time. Test was carried out according to the specifications of British Pharmacopoeia (2007) using one tablet.

Dissolution test. Dissolution test was carried out by the same procedure as used for ofloxacin tablets.

Sample preparation. Sample was drawn after 30 min, filtered and cooled down to room temperature; 08 ml of this solution was diluted with 0.1N HCl in a 100 ml flask to get a solution of 20 µg/ml concentration.

Standard preparation. For reference standard, levofloxacin equivalent to 250 mg was weighed in 100 ml volumetric flask, dissolved and the volume was made upto the mark with 0.01 N HCl; 0.8 ml of this solution was further diluted with 0.1 N HCl to get a solution of 20 µg/ml concentration.

Absorbance of the standard and the sample solution were measured at 294 nm taking 0.1 N HCl.

Assay by UV. *Standard preparation.* For preparing working standard, levofloxacin equivalent to 100.0 mg was weighed in a 100 ml volumetric flask, dissolved in 0.1N HCl and the volume was made up to the mark; 1 ml of this solution was diluted to 100 ml with the same solvent.

Sample preparation. Twenty (20) tablets were weighed and ground. Powder equivalent to 100 mg of levofloxacin was taken in 100 ml volumetric flask and dissolved in 0.1 N HCl. The solution was filtered and 1ml of this solution was diluted with 0.1 N HCl upto 100 ml.

Absorbance of the sample and the standard were measured at 294 nm taking 0.1 N HCl as blank.

Assay by HPLC. The assay was carried out by high performance liquid chromatography. A Schimadzu LC-9 was used with UV detector set at 294 nm having ODS Column (25cm × 4.6 mm) and temperature was adjusted at 35 ± 1°C.

The mobile phase used was mixture of dodecyl sodium sulphate (0.24% aqueous solution), acetonitrile, and glacial acetic acid (580:400:20) with flow rate of 1.5 ml/min.

Standard preparation. For preparing the working standard, levofloxacin equivalent to 60 mg was weighed in 100 ml volumetric flask, dissolved in 0.05 N hydrochloric acid and the volume was made upto the mark; 10 ml of this solution was diluted to 100 ml with the same solvent to prepare a solution of 0.06 mg/ml.

Sample preparation. Twenty (20) tablets were weighed and ground. Powder equivalent to 60 mg levofloxacin was taken in 100 ml volumetric flask, dissolved in 0.05 N HCl and the volume was made upto the mark; 10 ml of this solution was diluted to 100 ml with the same solvent to prepare a solution of 0.06 mg/ml.

For expressing the percentage amount of levofloxacin in mg/tablet, formula is the same as used for ofloxacin.

Results and Discussion

For evaluation of four brands of ciprofloxacin, ofloxacin and levofloxacin each, only tablet form of the drugs was selected. Physical and chemical analysis and *in vitro* bioavailability through dissolution were carried out; results are reported in Tables 1-3.

Table 1. Analysis of ciprofloxacin tablets

Formulations	Assay by UV-Vis spectrophotometer (%)	Assay by HPLC (%)	Dissolution assay (%)	Disintegration time (min)
C1	100.62	98.59	88.38	9-10
C2	102.01	103.15	85.316	5-6
C3	96.74	97.15	99.31	2-3
C4	103.10	101.80	95.89	3-4

Table 2. Analysis of ofloxacin tablets

Formulations	Assay by UV-Vis spectro-photometer (%)	Assay by HPLC (%)	Dissolution assay (%)	Disintegration time (min)
O1	97.29	97.68	96.80	0 - 1
O2	96.30	95.84	93.93	10 - 11
O3	96.59	95.76	81.91	2 - 4
O4	98.90	98.12	89.75	11 - 12

Table 3. Analysis of levofloxacin tablets

Formulations	Assay by UV-Vis spectro-photometer (%)	Assay by HPLC (%)	Dissolution assay (%)	Disintegration time (min)
L1	102.71	102.23	102.71	2-3
L2	98.61	100.14	97.51	9-10
L3	97.30	96. 87	24.18	34-35
L4	99.92	99.50	100.11	5-6

Physical tests of all the samples show the physical stability of compounds in different formulations. UV absorption of the sample and the reference standard of ciprofloxacin showed absorption $ë_{max}$ at 276 nm and that of ofloxacin and levofloxacin showed $ë_{max}$ at 294. It was found that the active compounds in all the samples were chemically stable and there were no significant impurities present in the tablets.

Quantitative estimation of the active ingredients was also confirmed by HPLC according to the standard procedure of United State Pharmacopoeia (2004). Percentage composition of all the compounds was determined by using reference standards (USP grade) of the same concentration. Retention time of the sample and the reference standard of ciprofloxacin was 7.230 (Fig. 1), of ofloxacin was 9.880 (Fig. 2) and of levofloxacin was 10.570 (Fig. 3). Variations and differences of the active ingredients in all the formulations were within the US pharmacopoeial limit i.e. (90- 110%).

For evaluating the efficacy of the tablets, apart from the amount of drug per tablet, capacity of the tablet to release the drug is to be determined. For this purpose the bioavailability of the tablets was ascertained *in vitro*. A generally accepted maxim for a drug to be readily available to the body is that it must be in the form of solution. For tablets, the first important step towards dissolution is breakdown of the tablet into smaller particles or granules. This disintegration takes place naturally in the stomach.

The disintegration time of all the tablets was determined (Tables 1-3). The standard time for disintegration by the film coated tablet is not more than 30 min (British Pharmacopoeia, 2007).

The disintegration time of ciprofloxacin sample tablets (Table 1) ranged from 2-10 min which complies with the standard values. The disintegration time of ofloxacin tablets (Table 2) ranged from 1-12 min which also complies with the standard values. However, the disintegration time of levofloxacin sample tablets (Table 3) ranged from 2- 35 min which does not follow the standard values. Although the maximum disintegration time is a little above the maximum standard limit of 30 min for film coated tablets, but disintegration time of 35 min is not desirable in life saving medicines.

The disintegration time, however, simply identifies the time required for the tablet to break up under the condition of test and for all particles to pass through a 10-mesh screen. The test offers no assurance that the resultant particles will

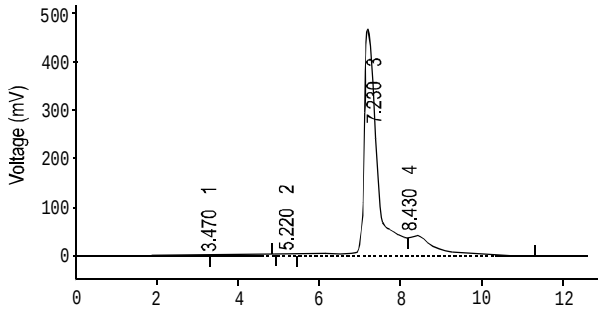

Fig. 1. The HPLC chromatogram of ciprofloxacin.

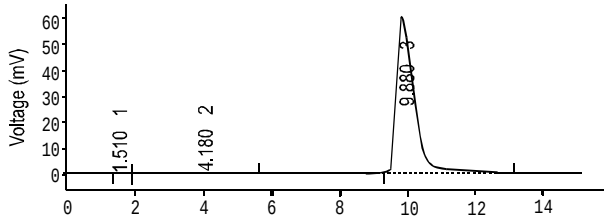

Fig. 2. The HPLC chromatogram of ofloxacin.

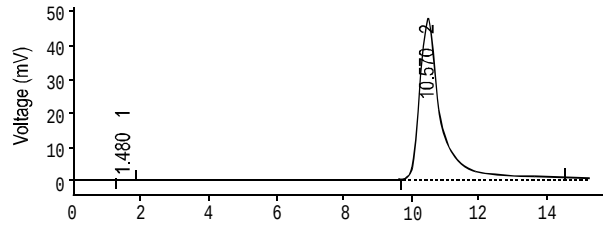

Fig. 3. The HPLC chromatogram of levofloxacin.

release the active ingredient in solution at an appropriate rate. For this purpose the test for the dissolution rate was performed.

The dissolution rate is critically important for the tablets. The standard values for these tablets are not less than 80% (United State Pharamacopoeia, 2004).

The results (Table 1) show that the dissolution rate for the ciprofloxacin tablets ranged from 85-99%, which complies with the standard value. The dissolution rate for ofloxacin tablets (Table 2) ranged from 81-87%. Dissolution rate of 81% though close to the standard values is not a desirable one. Dissolution rate of levofloxacin tablets (Table 3) ranged from 24-102%, which does not comply with the standard value and indicates the substandard criteria of the product. Dissolution rate of 24% is quite below the minimum standard limit. It was observed that the disintegration time also correlated with the dissolution rate.

Thus it may be concluded that the active ingredients in all the tablets were present in appropriate quantities. Products were chemically stable according to their shelf life but the bioavailability of these tablets was considerably different which need to be improved through making improvements in the formulations.

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