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In vitro evaluation of Atenolol tablets produced in Iran with the Atenolol tablets imported from abroad and comparison between them

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ABSTRACT

In vitro evaluation on three types of atenolol tablets produced by three Iranian Pharmaceutical companies (Exir, Darou Pakhsh and Tolid Darou) and three types of atenolol tablest produced by foreign companies (Tenormin® of England, Apo-Atenolol of Canada and Hipres® of India) were carried out. Results obtained in this research have shown that tablets produced in Iran by Exir and Darou Pakhsh Pharmaceutical companies have the minimum standard limits which are acceptable by the internationally well known Pharmacopoeia such as USP, but Atenolol tablets produced by Tolid Darou company, at least in the case of releasing of active ingredient in the desired time, lacks the standard limits. The three imported types of tablets have the internationally acceptable standard limits.

Keywords: Atenolol, Tenormin®, 4 -[2'-hydroxy-3'-[(1- methylethyl) amino] propoxy]-benzeneacetamide, beta-blockers.

INTRODUCTION

Atenolol (Tenormin®) belongs to a group of medicines called beta-blockers. Beta-blockers affect the heart and circulation (blood flow through arteries and veins) and reduce the workload on the heart and help it to beat more regularly. Atenolol controls, but does not cure, high blood pressure (hypertension). Atenolol also relieves chest pain (angina), and can be helpful after a heart attack. Sometimes atenolol is used to help prevent migraine headaches.[1] Atenolol is effective at reducing blood pressure and maintaining this reduction for at least 24 hours after a single oral dose. This encourages patient compliance. Atenolol may also be used for purposes other than those listed above. All clinical and physiological effects of beta blockade are no longer present 72 hours after cessation of therapy. Atenolol is not completely absorbed from the gastrointestinal tract, its oral bioavailability being of the order 50-60%. It is approximately 5% bound to plasma proteins. The plasma half-life of atenolol is about 6 hours. However, the duration of therapeutic effect is much longer than this, allowing once daily dosing. Maximum plasma concentrations are reached within two to four hours after a single oral dose and doses of 50mg and 100mg produce mean peak plasma concentrations of approximately 300 and 700 ng/mL, respectively. Approximately 10% of atenolol is metabolised in man. In summary atenolol can be used for the following purposes: [2]

1. Control of hypertension
2. For mild to moderate hypertension
3. Long term management of angina pectoris
4. Control of cardiac dysrhythmias
5. Myocardial Infarction.

Atenolol (Tenormin®), a synthetic, beta[1]-selective (cardioselective) adrenoreceptor blocking agent, may be chemically described as benzeneacetamide, 4 -[2'-hydroxy-3'-[(1-methylethyl) amino] propoxy]-. The molecular and structural formulas (Fig. -1) are:

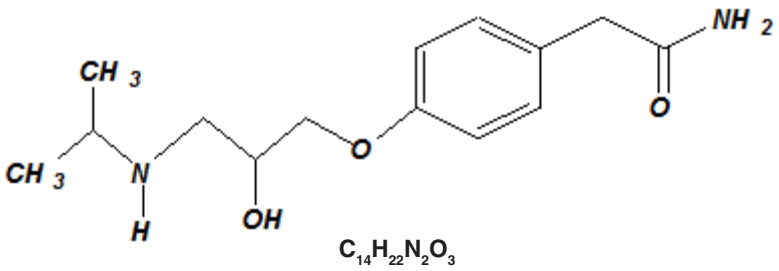

Fig. - 1

Atenolol (free base) has a molecular weight of 266. It is a relatively polar hydrophilic compound with a water solubility of 26.5 mg/mL at 37°C and a log partition coefficient (octanol/water) of 0.23. It is freely soluble in 1N HCl (300 mg/mL at 25°C) and less soluble in chloroform (3 mg/mL at 25°C).[3]

EXPERIMENTAL

All the chemicals used were purchased from Merck Company. Atenolol tablets (100 mg) were all purchased (those produced in Iran and those imported from abroad) from domestic pharmaceutical markets in Iran. Standard and working powders of atenolol tablets were gift of Exir pharmaceutical company of Iran. High Performance Liquid Chromatography tests were performed on a HPLC (JASCO, Japan, with Liquid Pump 880-PU; UV-Visible detector (870-UV); the instrument was equipped with an Interface (from Knauer company of Germany) and soft ware program ECW2000 version 2.05. Millipore membranes (0.45) made in Germany, were used. Friability Tester (TA3R; ERWEKA, Germany), Hardness Tester (TB24; ERWEKA, Germany), Disintegration Apparatus (ERWEKA, Germany), Dissolution Apparatus (ERWEKA, DT800, Germany) were used for the measurement of friability, hardness, disintegration time and dissolution tests, respectively. Analytical balance (Sartorius 2434, Germany) was used for measuring the variation of the weights of the tablets. UV spectra were recorded using a JASCO UV-VIS. 7850.

1. Measuring the hardness of atenolol tablets (Hardness test)

On the basis of the method reported in USP Pharmacopoeia,[4] 10 tablets of each of the six types used in this research (Exir, Darou Phakhsh, Todid Darou, Tenormin® of England, Apo-Atenolol of Canada and Hipres® of India) were taken separately.

The degree of hardness of each type was measured by the following procedure

Each tablet was placed on the lower anvil and the anvil was adjusted so that the tablet just touched the upper test anvil. A suspended motor driven weight moved along a rail, which slowly and uniformly transmitted pressure to the tablet. A pointer moving along the scale provided the breaking strength value in kilograms. The results are given in Table 1.

Table - 1: Results obtained from the hardness test of atenolol tablets (in Kg/cm^2)

Manufacturer	Minimum hardness	Maximum hardness	Average hardness	Standard Deviation (SD)	RSD
Exir, Iran	4.1	5.2	4.62	0.31	6.7
Darou Pakhsh, Iran	4.2	5.1	4.70	0.27	5.7
Tolid Darou, Iran	4.8	6.5	5.55	0.56	10
Tenormin, England	4.19	4.9	4.48	0.25	5.5
Apo-Atenolol, Canada	4.5	5.5	4.90	0.29	5.9
Hipres, India	4.9	6.3	5.58	0.49	8.7

2. Measuring the friability of atenolol tablets (Friability test)

On the basis of the method reported in USP Pharmacopoeia,[4,5] 20 tablets of each of the six types used in this research were weighed separately. Each set of the tablets were placed simultaneously in the Friability Tester instrument. The instrument was set on 25 rpm for 2 minutes. After this, the tablets were removed and weighted again. Friability percentages of the tablets were calculated, the results are given in Table 2.

Table - 2: Results obtained from friability measurement

Manufacturer	%Friability
Exir, Iran	0.18
Darou Pakhsh, Iran	0.17
Tolid Darou, Iran	0.13
Tenormin, England	0.23
Apo-Atenolol, Canada	0.25
Hipres, India	0.14

3. Measuring the disintegration time of atenolol tablets

The instrument is equipped with a basket containing 6 open ended tubes with the length of 7.5-8 cm and a diameter of 2.15 cm. A 10 mesh stainless steel sieve is placed under the tubes. The basket was placed in a 1 liter beaker containing distilled water with temperature of 37±2 °C. The basket was moved upward and downward 28-32 times per minute to a height of 5-6 cm in water. Each time, six tablets were taken randomly from each type of the tablets and to each open ended glass tube, one tablet was placed and covered with a special plastic sheet. Then, the instrument was turned on and disintegration time of each tablet was recorded. The minimum, maximum and average disintegration time of each tablet from each type of the tablets were determined and recorded. The results are given in table 3. According to the general rule, all the six coated tablets in distilled water must disintegrate in a period up to 60 minutes.[6]

4. Measurement of weight variations

20 tablets of each of the six types of the

Table - 3: Results obtained from disintegration time measurement

Manufacturer	Minimum disintegration time (min)	Maximum disintegration time (min)	Average disintegration time (min)	Standard Deviation (SD)	RSD
Exir, Iran	18	21	19.1	0.98	5.1
Darou Pakhsh, Iran	17	20	18.8	0.98	5.2
Tolid Darou, Iran	22	26	24.6	1.75	7.1
Tenormin, England	19	21	20	0.63	3.1
Apo-Atenolol, Canada	18	20	19	0.63	3.3
Hipres, India	21	25	22.8	1.47	6.4

Table - 4: Results obtained from weight variation measurement (in mg)

Manufacturer	Minimum weight	Maximum weight	Average weight	Standard Deviation (SD)	RSD
Exir, Iran	360	365	361.0	2.23	0.61
Darou Pakhsh, Iran	360	365	361.3	2.16	0.59
Tolid Darou, Iran	355	360	357.4	2.50	0.70
Tenormin, England	360	365	360.83	2.04	0.56
Apo-Atenolol, Canada	360	365	361.4	2.01	0.55
Hipres, India	360	365	361.5	2.16	0.59

Table - 5: HPLC data obtained from the injection of samples prepared from atenolol standard powder with given concentrations

Retention time (t_R) (min.)	Height (mv)	Area (mv*min)	Concentration (µg/mL)
4.883 ± 0.02	219.83 ± 2.87	39 ± 1.47	40
4.883 ± 0.02	323.45 ± 3.04	59 ± 1.63	60
5.067 ± 0.03	419.27 ± 3.24	78 ± 1.58	80
5.067 ± 0.04	498.39 ± 3.41	98 ± 1.63	100
5.067 ± 0.05	610.56 ± 3.62	117 ± 1.69	120

Table - 6: HPLC data obtained from the injection of samples prepared from atenolol working powder with given concentrations

Retention time (t_R) (min.)	Height (mv)	Area (mv*min)	Concentration (µg/mL)
4.885 ± 0.01	222.38 ± 0.89	40 ± 0.44	40
4.885 ± 0.01	326.65 ± 1.12	60 ± 0.51	60
4.890 ± 0.01	423.29 ± 1.38	79 ± 0.56	80
4.895 ± 0.02	503.86 ± 1.71	99 ± 0.64	100
4.902 ± 0.02	615.14 ± 1.96	118 ± 0.71	120

Table - 7: Determination of the active ingredient (assay) (mg)

Manufacturer	Average Assay (mg)	Standard Deviation (SD)	RSD	%Label
Exir, Iran	97.5	1.4	1.43	97.5
Darou Pakhsh, Iran	97.0	1.58	1.62	97
Tolid Darou, Iran	96.0	2.23	2.32	96
Tenormin, England	100.2	1.3	1.29	100.2
Apo-Atenolol, Canada	101.8	1.3	1.27	101.8
Hipres, India	97.4	1.45	1.48	97.4

Table - 8: Average of the percentage released of active ingredient of atenolol tablets at various times.

Manufacturer	5 min.	10 min.	15 min.	20 min.	30 min.	45 min.	60 min
Exir, Iran	31.6±4.07	43.9±3.87	61.3±3.42	74.5±3.28	83.8±2.96	91.2±2.35	95.1±1.98
Darou Pakhsh, Iran	30.2±4.56	41.4±4.11	59.5±3.82	71.8±3.44	81.2±3.15	89.7±2.42	94.1±2.18
Tolid Darou, Iran	28.3±5.93	36.5±5.42	55.2±4.95	64.4±4.38	76.9±3.89	86.7±3.25	89.8±3.06
Tenormin, England	35.4±3.24	47.2±2.86	68.8±2.35	80.5±1.97	86.6±1.69	93.7±1.35	97.1±1.28
Apo-Atenolol, Canada	37.4±3.07	49.1±2.73	69.7±2.26	81.2±1.85	88.3±1.61	94.8±1.21	98.2±1.14
Hipres, India	32.4±4.01	43.8±3.63	63.1±3.35	74.6±3.19	84.1±2.84	92.2±1.92	95.5±1.81

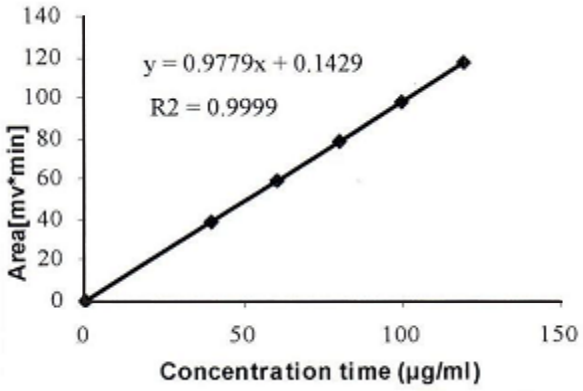

Fig. - 2: Calibration curve of atenolol standard powder

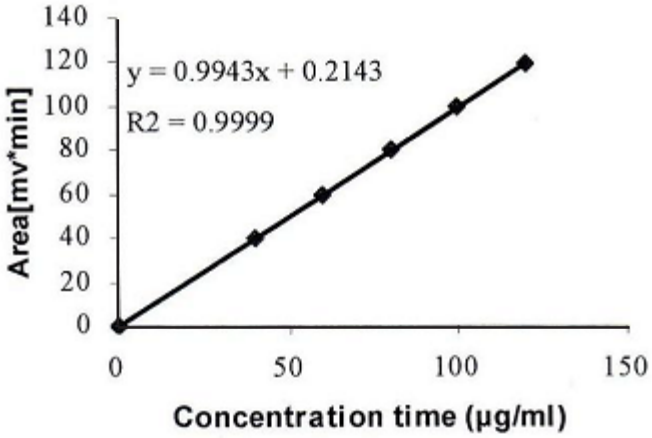

Fig. - 3: Calibration curve of atenolol working powder

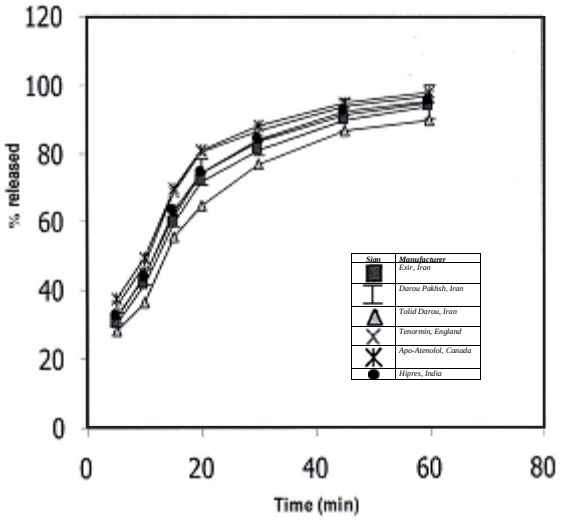

Fig. - 4: Comparison of the curves of the average percentage released of the active ingredient of atenolol tablets at various times.

tablets were chosen randomly and weighed with a precise analytical balance. The acceptable range should be within 95-105% of the weight of the middle one.[5] The results are given in Table 4.

5. Preparation of the stock solution

100 mg of the atenolol standard powder was weighed precisely and transferred to a 100 mL volumetric flask. A solvent mixture of methanol:phosphate buffer solution (3:7 V/V) was added to the flask and made the volume exactly to 100 mL. Therefore, a 1mg/mL or 1000 mg/mL of the active ingredient was made and used for the preparation of various concentration solutions necessary for plotting the calibration curve.

6. Preparation of the standard solutions

For plotting the calibration curve, concentrations of 40, 60, 80, 100 and 120 mg/mL were needed. From the stock solution, 0.4, 0.6, 0.8, 1.0, and 1.2 mL were taken and placed each in an individual 10 mL volumetric flasks, then made the volumes to 10 mL exactly by adding more of the solvent mixture of methanol:phosphate buffer solution (3:7 V/V) to each individual flask. The same procedure was carried out for the preparation of the standard solution from the working standard powder of atenolol.

7. Determination of λ_{max} of atenolol standard powder

The UV spectrum of atenolol standard powder in methanol:phosphate buffer solution (3:7 V/V) was taken. The λ_{max} was determined as 223.5 nm (A=0.7205).

8. Plotting the standard curve

For plotting the standard curve, five times and each time 20 mL from each of the standard solutions prepared in **(6)** was injected into the HPLC instrument from the lowest to the highest concentrations. The chromatograms and the relevant data such as peak area, peak height, retention time, etc. were recorded and saved as Peak – Report tables in the soft ware program (Table 5). For assuring the accuracy and precision of the measurement method, the whole procedures for plotting the calibration curve were repeated three times within a day and twice between two consecutive days. Then, the calibration curve was plotted (Fig. 2). The same procedure was done on the working powder and the results are given in table 6 and the calibration curve was plotted (Fig. 3). On the basis of the calibration curve (Fig. 2), the unknown samples were injected into the HPLC instrument and the chromatograms were recorded, then the amounts of the unknown samples were determined.

9. Measurement of the active ingredient (Assay)

10 tablets from each of the six types of the atenolol tablets were taken separately, then weighed precisely and powdered in a porcelain mortar and pestle. 100 mg of this powder was taken and dissolved in a mixture of methanol:phosphate buffer solution (3:7 V/V), then filtred on Millipore filters. Each of the filtred solution was transferred to a 100 mL volumetric flask separately and made the volume to 100 mL by adding more of the above mentioned solvent mixture. Finally, 1 ml from each flask was taken and placed in a 10 mL volumetric flask separately and made the volume to 10 mL by adding the solvent mixture. Then, five times and each time 20 mL from each of these solutions were injected into the HPLC instrument. The results are given in Table 7.

10. Dissolution rate measurement

According to the reported conditions on atenolol monograph in USP Pharmacopoeia, 900 mL distilled water was used as dissolution medium. Dissolution instrument equipped with a Paddle with 50 rpm was used. The medium temperature was set to 37± 0.5°C. According to the USP Pharmacopoeia, 80% of the active ingredient of the tablet should be dissolved after 30 minutes in the medium. In this research, 6 tablets from each type of tablets were placed individually in the special cell of the instrument and the extent of dissolution rate was measured and calculated as percentage of the active ingredient released at various times. After the start of the test, at intervals of 5, 10, 20, 30, 45 and 60 minutes, 5 mL aliquot was taken from the dissolution medium and filtered on Millipore filter discs, then 20 mL of each sample was injected into the HPLC instrument. Meanwhile, after the removal of the 5 mL aliquot, each time 5 mL of the buffer solution was added to the dissolution medium to make the volume to 900 mL. The results of the

average percentage released of the active ingredient at various times and the dissolution profile of each type of tablets are given in Table 8 and Fig. 4.

11. HPLC optimum conditions used for the analyzing the atenolol tablets

Stationary Phase:

Knauer (Germany) Spherimage-80, ODS, 2-5 mm C_{18} column with 30 cm length, and i.d. 4.5 mm

Mobile Phase: Methanol:phosphate buffer solution (3:7 V/V)

Flow rate: 0.6 mL/min.

Column Temperature: Room temperature

λ_{max}= 224 nm

AUFS= 0.01

Injected volume: 20 mL

Concentrations of standard atenolol powder used: 40, 60, 80, 100 and 120 mg/mL.

RESULTS AND DISCUSSION

Regarding the efficacy of atenolol on the one hand and its rapid effect in patients which are dependent upon the quality of the drug on the other hand, the following objectives were carried out in this research on three types of atenolol tablets produced by three Iranian Pharmaceutical companies (Exir, Darou Pakhsh and Tolid Darou and three types of atenolol tablest produced by foreign companies (Tenormin® of England, Apo-Atenolol of Canada and Hipres® of India):

(i) Study and determining the active ingredient of each type of the tablets.

(ii) Study of dissolution rate of each type of the tablets and comparing of them with each other.

(iii) Study of the degree of hardness, friability percentage, disintegration time and weight variations of each type of the tablets and comparing of them with each other.

(iv) Finally, conclusion about the efficacy and quality of these tablets.

Friability percentages of the tablets were calculated using the following formula:

$$\%Friability = [(W_1 - W_2)/ W_1] \times 100$$

where W_1 is the initial weight of the 20 tablets and W_2 is the final weight of the 20 tablets.

For determination of the active ingredient of atenolol tablets and dissolution rates, High Performance Liquid Chromatography (HPLC) which is an advanced, rapid and precise technique was used [C_{18} column, 30 cm, Spherimage-80 ODS2 5 mm stationary phase, methanol:phosphate buffer (3:7 V/V) mobile phase]. Determination of the degree of hardness, friability percentage and disintegration time of the tablets were made by using the corresponding instruments. Weight variations were measured by analytical balance.

The various results obtained in this research have shown that:

1. Atenolol tablets produced in England (Tenormin®) had the lowest, whereas the ones produced by Hipres of India had the highest degree of hardness (Table 1).
2. Atenolol tablets produced by Tolid Darou pharmaceutical company had the lowest and the ones produced by Apo-Atenolol of Canada had the highest friability percentage (Table 2).
3. Atenolol tablets produced by Darou Pakhsh Pharmaceutical Company had the lowest and those produced by Tolid Darou Pharmaceutical Company had the highest disintegration time (Table 3).
4. All of the six types of tablets used in this research were in the acceptable weight limits (Table 4).
5. Atenolol tablets produced by Tolid Darou Pharmaceutical Company had the lowest and the ones produced by Apo-Atenolol of Canada had the highest amount of active ingredient, respectively, but all of the six types of tablets were within the acceptable range (Table 7).
6. All of the tablets except those produced by Tolid Darou, released more than 80% of their active ingredients within 30 minutes according to the standard limit and acceptable range of USP pharmacopoeia. Meanwhile, Tenormin® of England and Apo-Atenolol of Canada released more than 80% of the drug in medium after 20 minutes (Table 8).

Results obtained in this research have shown that tablets produced in Iran by Exir and

Darou Pakhsh Pharmaceutical companies have the minimum standard limits which are acceptable by the internationally well known Pharmacopoeia such as USP, but Atenolol tablets produced by Tolid Darou company, at least in the case of releasing of active ingredient in the desired time, lacks the standard limits. Atenolol tablets produced by the three foreign companies (Tenormin® of England, Apo-Atenolol of Canada and Hipres of India have the internationally acceptable standard limits.

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