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***In vitro* Evaluation of Some Different Brands of Alprazolam Tablets**

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Abstract: Alprazolam belongs to a group of medicines called benzodiazepines. These medicines are thought to work by their action on brain chemicals. Alprazolam has sedative effects and is used to treat: (i) Anxiety and (ii) Panic attacks. The results obtained in this research by various HPLC analyses and other physicochemical tests have shown that alprazolam tablets manufactured by Dr. Abidi and Poursina Pharmaceutical Companies of Iran have the standard limits acceptable by the internationally well known Pharmacopoeia such as USP and can satisfy the needs of patients quite well and are quite comparable with Xanax[®] of USA and Apo-Alpraz[®] of Canada.

Keywords: Alprazolam, Xanax, Apo-Alpraz[®], Benzodiazepine.

Introduction

Alprazolam is a triazolobenzodiazepine, that is, a benzodiazepine with a triazolo-ring attached to its structure (Figure 1). The chemical name of alprazolam is 8-chloro-1-methyl-6-phenyl-4H-s-triazolo [4,3- α] [1,4] benzodiazepine. Alprazolam is a white crystalline powder, which is soluble in methanol or ethanol but which has no appreciable solubility in water at physiological pH. It is a short-acting drug in the benzodiazepine class used to treat anxiety disorders and as an adjunctive treatment for depression. Anxiety or tension associated with the normal stress of everyday life usually does not require treatment with medicines. Alprazolam was invented by Pfizer and is marketed under the trade name Xanax¹. Alprazolam is a benzodiazepine which affects chemicals in the brain that may

become unbalanced and cause anxiety and is most commonly used to relieve anxiety, nervousness, and tension associated with anxiety disorders. It is also used to treat panic disorders. Clinically, all benzodiazepines cause a dose-related central nervous system depressant activity varying from mild impairment of task performance to hypnosis². Alprazolam may also be used for purposes other than those listed here. This medication should be swallowed whole and should not be crushed or chewed. Following oral administration, Alprazolam is readily absorbed. Peak concentrations in the plasma occur in 1 to 2 h following administration. Alprazolam and its metabolites are excreted primarily in the urine. This medication may cause dependence, especially if it has been used regularly for an extended period of time, or if it has been used in high doses. Withdrawal after long-term treatment should be done slowly over a period of weeks (or even months) to avoid serious withdrawal symptoms such as agitation, panic attacks, rebound anxiety, muscle cramps and seizures. Side effects caused by Xanax/Alprazolam tablet may be generally observed at the beginning of therapy and usually disappear when the anxiety medication is properly administered for some period³.

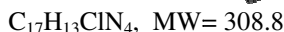
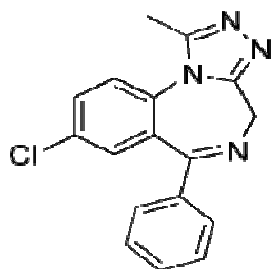


Figure 1. 8-Chloro-1-methyl-6-phenyl-4H-1,2,4-triazolo [4,3- α][1,4]benzodiazepine

Experimental

All the chemicals used were purchased from Merck Company. Alprazolam tablets (0.5 mg) were all purchased (those produced in Iran and those imported from abroad) from domestic pharmaceutical markets in Iran. Alprazolam standard powder was donated by Dr. Abidi's 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 (Type; TB24m 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.

Measuring the hardness of alprazolam tablets (Hardness test)

On the basis of the method reported in USP Pharmacopoeia⁷, 10 tablets of each of the four types used in this research (by Dr. Abidi and Poursina Pharmaceutical Companies of Iran, Xanax[®] of USA and Apo-Alpraz[®] of Canada) were taken separately. The degree of hardness of each type was measured by the following procedure:

Each tablet was placed on the lower anvil of the instrument and the anvil was adjusted so that the tablet just touched the upper test anvil. The instrument was switched on, 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/cm². As soon as the tablet started to break, the pointer stopped. The results are given in Table 1.

Table 1. Results obtained from the hardness test of alprazolam tablets (in Kg/cm²)

Manufacturer	Minimum hardness	Maximum hardness	Average hardness	Standard Deviation (SD)	RSD
Dr. Abidi of Iran	1.50	2.5	2.05	0.3	14.6
Poursina of Iran	3.25	4.75	4.00	0.5	12.5
Xanax [®] (Upjohn) of USA	8.00	10.25	8.90	0.86	9.6
Apo-Alpraz [®] of Canada	3.75	6.00	4.90	0.66	13.4

Measuring the friability of alprazolam tablets (Friability test)

On the basis of the methods reported in USP Pharmacopoeia⁵, 20 tablets from each of the four types of tablets 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 calculated⁶, the results are given in Table 2.

Table 2. Results obtained from friability measurement

Manufacturer	%Friability
Dr. Abidi of Iran	0.38
Poursina of Iran	0.15
Xanax [®] (Upjohn) of USA	0.00
Apo-Alpraz [®] of Canada	0.00

Measuring the disintegration time of alprazolam 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 minimum disintegration time, was the time the first tablet started to disintegrate and the maximum disintegration time was the time the last tablet started to disintegrate. The results are given in Table 3. According to the general rule, the six coated tablets in distilled water must disintegrate in a period of up to 15 minutes^{4,5}.

Table 3. Results obtained from disintegration time measurement (min)

Manufacturer	Minimum	Maximum	Average	Standard Deviation (SD)	RSD
Dr. Abidi of Iran	1.91	2.58	2.23	0.24	10.7
Poursina of Iran	0.25	0.35	0.29	0.04	13.8
Xanax [®] (Upjohn) of USA	1.08	1.41	1.23	0.11	8.9
Apo-Alpraz [®] of Canada	0.21	0.28	0.25	0.02	8.0

Measurement of weight variations

20 tablets of each of the four types of the tablets were chosen randomly and weighed with a precise analytical balance. According to the authentic references the acceptable range should be within 92.5-107.5% of the weight of the middle one⁶. The results are given in Table 4.

Table 4. Results obtained from weight variation measurement (mg)

Manufacturer	Minimum weight	Maximum Weight	Average Weight	Standard Deviation (SD)	RSD
Dr. Abidi of Iran	129	132	130.1	0.83	0.63
Poursina of Iran	129	131	130.15	0.58	0.44
Xanax [®] (Upjohn) of USA	129	131	130.1	0.45	0.34
Apo-Alpraz [®] of Canada	129	131	130.15	0.49	0.37

Preparation of the stock solution

10 mg of the alprazolam standard powder was weighted precisely and transferred to a 100 mL volumetric flask. A solvent mixture of methanol:water (9:1 V/V) was added to the flask and made the volume exactly to 100 mL. Therefore, a 0.1mg/mL or 100 µg/mL of the active ingredient was made. 1 mL of this solution was taken with microsyringe and transferred into a 100 mL volumetric flask and made the volume exactly to 100 mL with the above mentioned solvent mixture. Therefore, the final concentration of 1 µg/mL was obtained and used for the preparation of various concentration solutions necessary for plotting the calibration curve.

Preparation of the standard solutions

For plotting the calibration curve, concentrations of 0.2, 0.4, 0.6, 0.8 and 1µg/mL were needed. From the above mentioned stock solution, 2, 4, 6, 8 and 10 mL were taken and each one was placed in an individual 10 mL volumetric flask, then made the volumes exactly to 10 mL by adding the solvent mixture of methanol:water (9:1 V/V) to each of the flasks. Therefore, solutions with concentrations of 0.2, 0.4, 0.6, 0.8 and 1µg/mL were obtained which would be used for plotting the calibration curve and injection into the HPLC instrument.

Determination of λ_{max} of alprazolam standard powder

The UV spectrum of alprazolam standard powder in methanol:water (9:1 V/V) was taken. The λ_{max} was determined as 254 nm.

Plotting the standard calibration curve

For plotting the standard curve, five times and each time 20 μL 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 the assurance of 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 (Figure 2). On the basis of the calibration curve (Figure 2), the unknown samples were injected into the HPLC instrument and the chromatograms were recorded, then the amounts of the unknown samples were determined.⁶

Table 5. HPLC data obtained from the injection of samples prepared from alprazolam standard powder with given concentrations

Concentration $\mu\text{g/mL}$	Retention time (t_R) min.	Height, mv	Area, mv*min
0.2	1.633	11.77 \pm 0.18	0.98 \pm 0.050
0.4	1.633	23.57 \pm 0.19	1.97 \pm 0.032
0.6	1.633	34 \pm 0.45	2.85 \pm 0.030
0.8	1.633	46.01 \pm 0.16	3.72 \pm 0.045
1.0	1.633	56.7 \pm 0.29	4.67 \pm 0.030

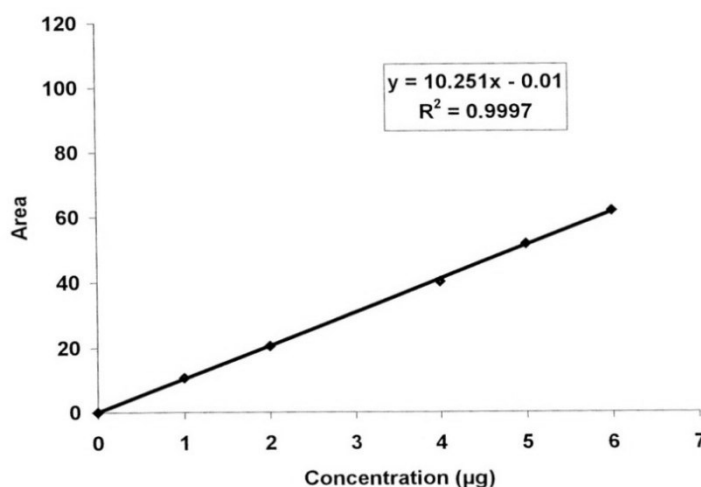


Figure 2. Calibration curve of alprazolam standard powder

Measurement of the active ingredient (Assay)

20 tablets from each of the four types of the alprazolam tablets were taken separately, then weighed precisely and powdered in a porcelain mortar and pestle. 5mg of the powder from each set of the tablets was taken and dissolved separately in a mixture of methanol:water (9:1 V/V), then filtered each on a separate Millipore filter disc. Each of the filtered solutions was transferred to a 50 mL volumetric flask separately and made the volume to 50 mL by adding more of the above mentioned solvent mixture. Finally, 1mL from each of the

individual flask was taken and placed into a 100 mL volumetric flask separately and made the volumes to 100 mL by adding more of the solvent mixture. Therefore, a solution of 1µg/mL for each of the four types of the tablets was made. Then, five times and each time 20 µL from each of these solutions was injected into the HPLC instrument.⁷The results are given in Table 6.

Table 6. Determination of the active ingredient (assay) (µg/mL)

Manufacturer	Average Assay, mg	Standard Deviation (SD)	%RS D	%Label
Dr. Abidi of Iran	0.494	0.0051	1.03	98.8
Poursina of Iran	0.510	0.0036	0.7	102
Xanax [®] (Upjohn) of USA	0.515	0.0039	0.76	103
Apo-Alpraz [®] of Canada	0.501	0.0048	0.96	100

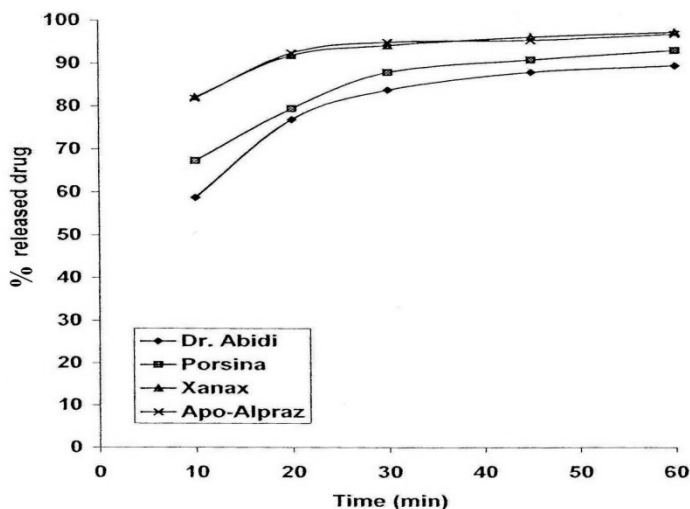


Figure 3. Comparison of the curves of the average percentage released of the active ingredient of alprazolam tablets at various times.

Dissolution rate measurement

According to the reported conditions on alprazolam monograph in USP Pharmacopoeia,⁵ a solution of phosphate buffer with pH of 6 ± 0.1 was made. 500 mL of this buffer solution was used as the dissolution medium. Measurements were made by a basket type dissolution instrument with 100 rpm. 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 in the medium after 30 minutes. In this research, 6 tablets from each type of the tablets were placed individually in the special basket 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 10, 20, 30, 45 and 60 minutes, 5 mL aliquot was taken from the dissolution medium and filtered on Millipore filter discs. Meanwhile, after the removal of the 5 mL aliquot, each time 5mL of the buffer solution was added to the dissolution medium to make the volume

to 500mL. Finally, 20 µL from each sample was injected separately into the HPLC instrument and the extent of dissolution was determined as the percentage released of the active ingredient. By dividing the 5 mg of the possible active ingredient of an alprazolam tablet (0.5 mg) to the total volume of the dissolution medium (500 mL), concentration of 1µg/mL was obtained. This concentration was considered as 100% drug release. By dividing the concentrations obtained from HPLC data (the figure written down the amount column of the peak report as the concentration of the injected sample, Figures 4 and 5) for each individual tablet at the specified times to 1, the percentage release for each individual tablet was calculated. The results of the average percentage released of the active ingredient at various times and the dissolution profile for each type of the tablets are given in Table 8 and Figure 3, respectively.^{5,7}

Table 7. Results obtained from the content uniformity test of alprazolam tablets (mg)

Manufacturer	Min. Active ingredient	Max.Active ingredient	Average Weight	Standard Deviation (SD)	RSD
Dr. Abidi of Iran	0.483	0.506	0.492	0.074	1.50
Poursina of Iran	0.475	0.519	0.506	0.016	3.16
Xanax® (Upjohn) of USA	0.497	0.520	0.509	0.010	1.96
Apo-Alpraz® of Canada	0.490	0.509	0.497	0.0067	1.35

Table 8. Average of the percentage released of active ingredient of alprazolam tablets at various times.

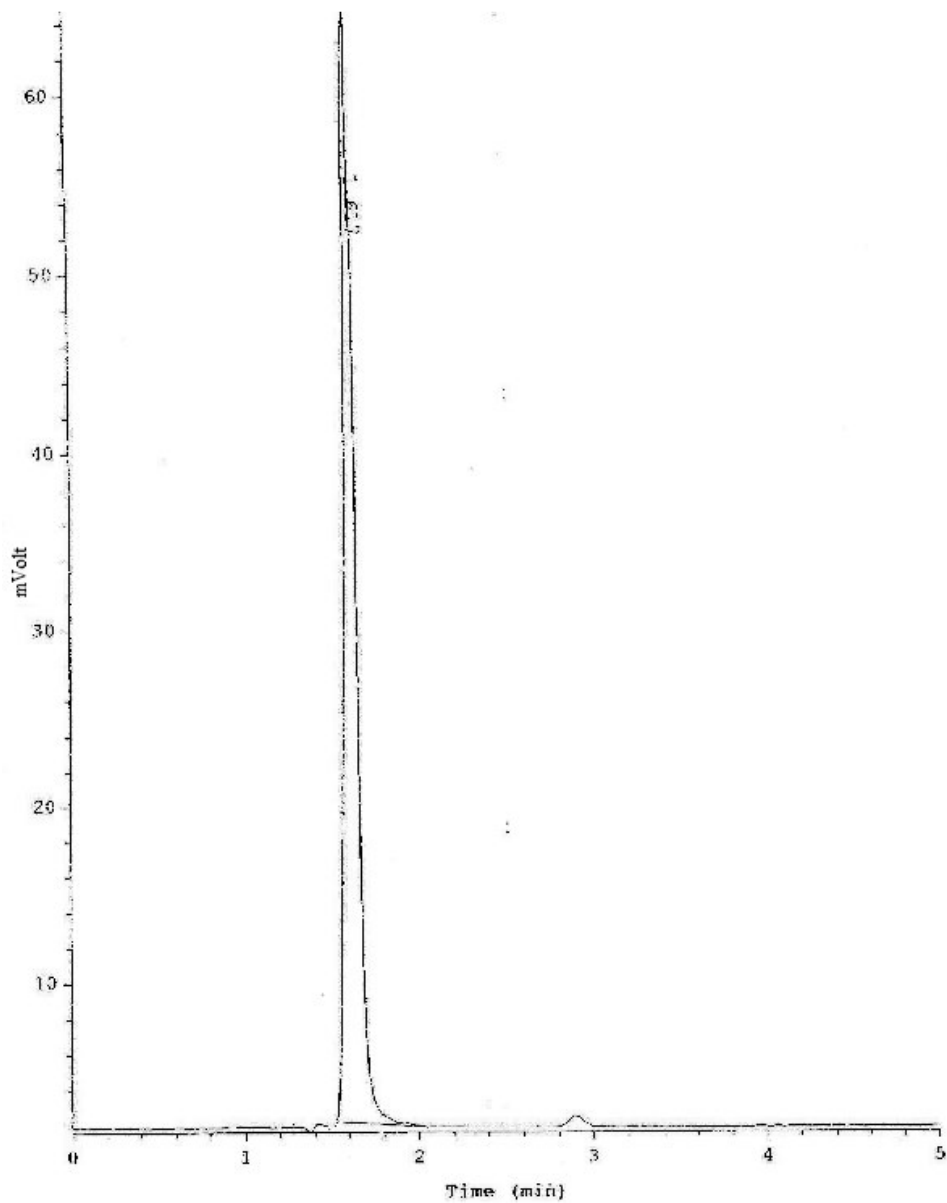
Manufac-turer	0 min.	10 min.	20 min.	30 min.	45 min.	60 min
Dr. Abidi of Iran	0	58.7±6.6	76.8±3.9	83.7±2.31	87.8±1.47	89.3±2.1
Poursina of Iran	0	67.3±5.9	79.3±2.3	87.8±1.85	90.7±1.4	92.9±1.2
Xanax® (Upjohn)	0	82.1±4.38	91.8±3.31	94.1±1.9	96.0±1.38	97.1±1.33
Apo-Alpraz® of Canada	0	81.8±4.07	92.3±2.73	94.9±2.4	95.3±1.36	96.7±1.34

Measurement of content uniformity

10 tablets from each type of the tablets used in this research were placed in separate 50 mL volumetric flasks. The tablets were dissolved in methanol:water (9:1 V/V) solvent mixture and made the volume to 50 mL by adding more of the solvent mixture. 1 mL from each of these solutions were placed in separate 10 mL volumetric flasks and made the volume to 50 mL by adding more of the solvent mixture. By doing this, the desired concentrations were obtained. The results⁷ are given in Table 7.

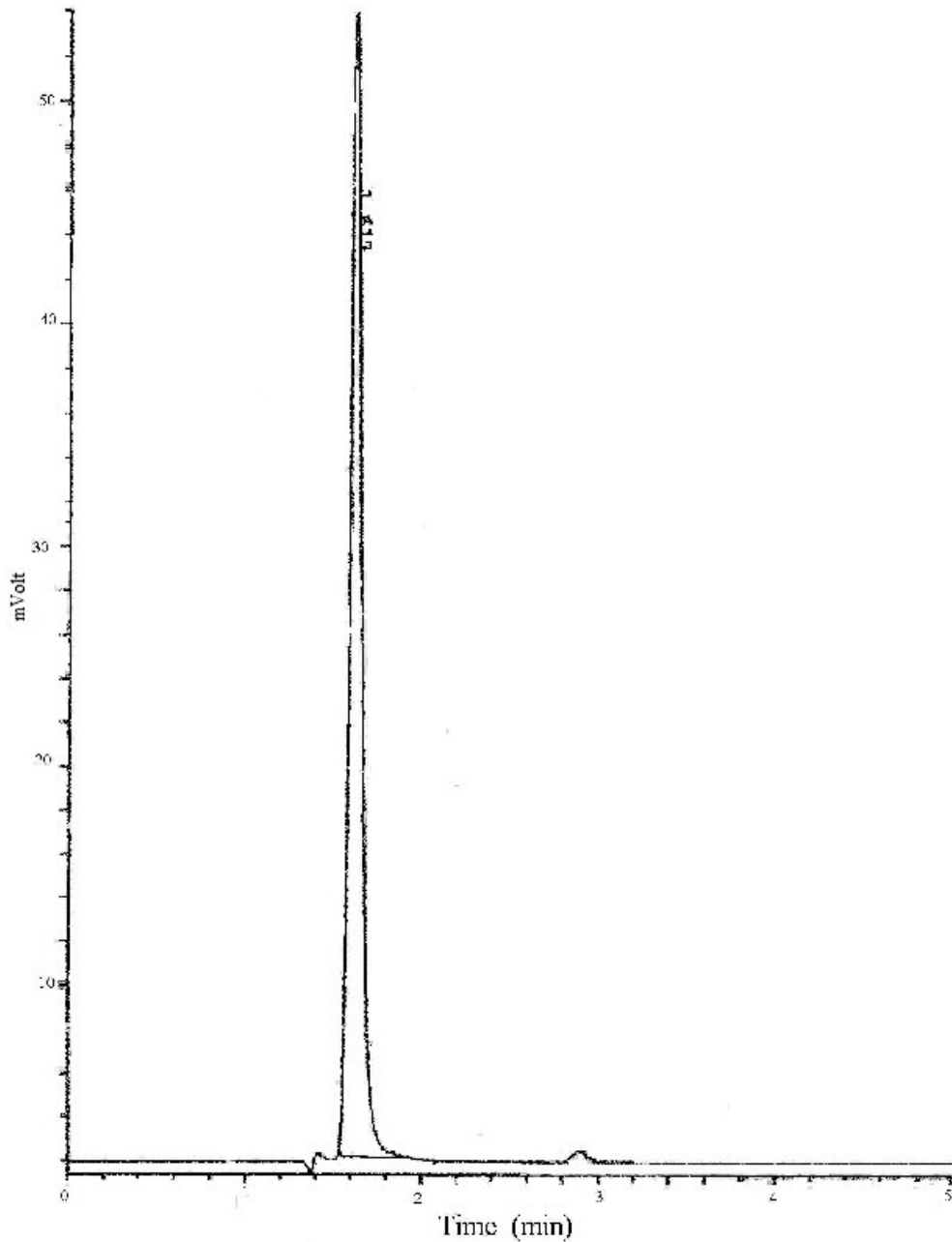
HPLC optimum conditions used for the analyzing the alprazolam tablets

Stationary Phase: Knauer (Germany) Spherimage-80, ODS, 2-5 µm C₁₈ column with 30 cm length, and i.d. 4.5 mm
 Mobile Phase: Methanol:water (9:1 V/V)
 Flow rate: 1.5 mL/min.
 Column Temperature: Room temperature
 λ_{max}= 254 nm, AUFS= 0.01
 Injected volume: 20 µL
 Concentrations of alprazolam standard powder used: 0.2, 0.4, 0.6, 0.8 and 1µg/mL



Ret.time [min]	Start [min]	End [min]	Height mVolt	Area mV*min	Amount	% Area	Width [min]	T-fact.	N-plates	yp	Compound	Units
1.617	1.53	2.05	63.0791	4.95719	1.04516	100.0000	0.070	1.286	2956.2	B	alprazolam	mcg

Figure 4. HPLC Chromatogram and data peak report of alprazolam tablets active ingredient (assay) manufactured by Xanax[®] (Upjohn) of USA.



Ret. Time (min)	Start (min)	End (min)	Height mV	Area mV*min	Amount	% Area	Width (min)	T-fact.	N-plates	Type	Compd	Units
1.617	1.53	2.05	63.0791	4.95719	1.04516	100	0.070	1.286	2956.2	B	Alprazolam	mcg

Figure 5. HPLC Chromatogram and data peak report of alprazolam tablets active ingredient (assay) manufactured by Dr. Abidi Pharmaceutical Company of Iran.

Results and Discussion

Alprazolam is most commonly used to relieve anxiety, nervousness, and tension associated with anxiety disorders. Alprazolam is also used to treat panic disorders. Regarding the efficacy of alprazolam and its rapid effect in patients, which is dependent largely upon the quality of the drug, it was decided to evaluate in vitro the following objectives between two types of alprazolam tablets produced domestically in Iran by Dr. Abidi and Poursina Pharmaceutical Companies with two types of imported alprazolam tablets produced by foreign Pharmaceutical Companies (Xanax[®] of USA and Apo-Alpraz[®] of Canada) and make a comparison between the quality of these tablets:

1. Investigating and determining the extent of purity of the active ingredient of the imported standard powder.
2. Studying and determining the active ingredient of each type of the tablets.
3. Study the dissolution rate of each type of the tablets.
4. Study the degree of hardness, friability percentage, disintegration time and weight variation and uniformity content of each type of the tablets.
5. Finally, concluding about the efficacy and quality of these tablets.

For the determination of the active ingredient, content uniformity and dissolution rates of alprazolam tablets and standard powder, high performance liquid chromatography (HPLC) which is a rapid and precise technique was used. We used isocratic reversed phase method benefiting a UV-Vis. detector and ECW 2000 software version 1.65 from Knauer Company of Germany.

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

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

Where W_1 is the initial weight of the 20 tablets and W_2 is the final weight of the 20 tablets. The maximum acceptable friability range should be within 0.5-1%, on condition that it does not affect the apparent shape of the tablet.⁶

For the determination of % release of the tablets, the following calculations were done:

- i) Concentration ($\mu\text{g/mL}$) =
$$\frac{\text{Active ingredient of the tablet used (mg)}}{\text{Total volume of the dissolution medium (mL)}}$$
- ii) This concentration was considered as 100% drug release.
- iii) % Release =
$$\frac{\text{The Amount (from the HPLC Peak Report data)}}{\text{Concentration } (\mu\text{g/mL})} \times 100$$

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:

- i) Alprazolam tablets manufactured by Upjohn (Xanax[®]) of USA had the highest whereas those manufactured by Dr. Abidi's pharmaceutical company had the lowest degree of hardness, respectively (Table 1).
- ii) Friability percentages of all four types of the tablets were within the internationally well known pharmacopoeia acceptable range (Table 2).
- iii) Disintegration time of all four types of the tablets were within the expected range and Apo-Alpraz[®] tablets of Canada and Alprazolam of Dr. Abidi's pharmaceutical company had the shortest and the longest disintegration time, respectively (Table 3).

- iv) All of the four types of the tablets used in this research were within the acceptable weight limits (120.54 – 139.75 mg) (Table 4).
- v) Alprazolam tablets manufactured by Upjohn (Xanax[®]) of USA contained the highest (0.515 mg) whereas those manufactured by Dr. Abidi's pharmaceutical company contained the lowest (0.494 mg) active ingredient, respectively, however, all the four types of the tablets were within the internationally well known pharmacopoeia acceptable range (0.45-0.55 mg)(Table 6).
- vi) The various results obtained in this research have shown that Xanax[®] of USA and Apo-Alpraz[®] of Canada released more than 80% of their active ingredient within 10 minutes, whereas alprazolam tablets produced by the two Iranian companies have reached to this level after 30 minutes of their administration, however, the four types of the tablets have reached the standard limits and acceptable range of USP, after 30 minutes (Table 7).
- vii) Results from the content uniformity tests had shown that the active ingredients of the four types of the tablets were within the acceptable range (0.425-0.575 mg) and the Relative Standard Deviation (RSD) of all the tablets was less than 6% (Table 8).

In conclusion the results obtained in this research by various HPLC analyses and other physicochemical tests have shown that alprazolam tablets manufactured by Dr. Abidi and Poursina Pharmaceutical Companies of Iran have the standard limits acceptable by the internationally well known Pharmacopoeia such as USP and can satisfy the needs of patients quite well and are quite comparable with Xanax[®] of USA and Apo-Alpraz[®] of Canada.

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