Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

Comparative *in vitro* evaluation of various commercial brands of amlodipine besylate tablets marketed in Bangladesh

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ARTICLE HISTORY

Received: 14.01.2016

Accepted: 22.03.2016

Available online: 30.03.2016

Keywords:

Amlodipine, Bangladesh, Dissolution test, In vitro evaluation.

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ABSTRACT

Choosing the proper drug product is getting complicated for health professionals and patients due to the existence of abundant generic brands in local drug market. The study was intended to evaluate the different physical parameters of generic amlodipine besylate tablet from different manufacturers using *in vitro* tests in order to minimize health risk factors and maximize the safety of local people. Six brands (**A, B, C, D, E** and **F**) of amlodipine besylate tablets (5 mg) marketed in Bangladesh were evaluated for eight *in vitro* tests including both official and unofficial viz. diameter test, thickness test, hardness test, friability test, uniformity of weight, disintegration test, dissolution test and assay. Dissolution study revealed brand **B** (99.87%) was the fastest and brand **D** (87.19%) was the slowest in terms of drug release. Using a validated UV spectrophotometric method assay value was recorded within 92% to 98.70%. Such study serves as a good pointer for assessment of in vitro parameters of commercially available products which may be advantageous for future formulation development studies.

INTRODUCTION

Post market medicines monitoring serves as a confidential tool to judge the quality, therapeutic efficacy and safety of medicine [1]. Improvement of existing regulations and product development can be accelerated with the help of information obtained from such monitoring [2]. In this research physical parameters of commercially available amlodipine besylate tablets were evaluated. Amlodipine besylate (ADB) is a long acting dihydropyridine calcium antagonist which is broadly used for the treatment of cardiovascular diseases [3-5] specifically in high blood pressure, certain types of angina, and coronary heart failure with the recommended dosage in between 2.5 to 10 mg once daily. The drug works by inhibiting the trap membrane influx of calcium ions into vascular smooth muscle and cardiac muscle [6]. Among two sterioisomers [R(+), S(-)], the (-) isomer has been reported to be more active than the (+) isomer [3, 7]. Amlodipine ($C_{20}H_{25}N_2O_5Cl$), chemically 3-ethyl 5-methyl-2-[(2-aminoethoxymethyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridinedicarboxylate [8], is a white crystalline powder with a molecular weight of 567.1. It is soluble in water and sparingly soluble in ethanol. It is extensively (about 90%) converted to inactive metabolites via hepatic metabolism with 10% of the parent compound and 60% of the metabolites excreted in the urine [9].According to the Biopharmaceutical Classification System (BCS) drug substances are classified to four classes upon their solubility and permeability [10-13]. Amlodipine falls under the BCS Class I, rapidly soluble and highly permeable drugs. Biowaivers were granted for BCS Class I drugs by FDA and WHO [14-16].It is a common psychology that drug products manufactured by top pharmaceutical companies are better in comparison with the products manufactured by small scale companies. Moreover, no such evaluation on amlodipine besylate of the local market was carried out before. These facts directed our interest to assess the quality of some commercially available amlodipine besylate tablets in the Bangladeshi market with special emphasis on disintegration and dissolution study due to their mammoth significance in predicting bioavailability and product quality.

MATERIALS AND METHODS

Materials

Drug: Standard of amlodipine besylate was a kind gift from ACI Pharmaceuticals Ltd, Bangladesh.

Dosage form: Amlodipine besylate tablets (5 mg) from six different brands were purchased from local drug store of Dhanmondi, Dhaka city. The samples were properly checked for their manufacturing license numbers, batch numbers, production and expiry dates. They were randomly coded as A, B, C, D, E, F and stored properly.

Solvents and reagents: Potassium dihydrogen phosphate (Lot No: P21010D, Daejung Chemicals & Metals Co. Ltd.) and sodium hydroxide (Batch No: PA344CB01, Qualikems Fine Chem Pvt. Ltd.) were of analytical-reagent grade and obtained from South Korea and India respectively. Purified water was used during the study.

Methods

Determination of diameter and thickness: 20 tablets from 6 brands were taken and both the diameter and thickness of the tablets was measured with an electronic digital caliper (MEGA Digital Clipper) in order to determine the average diameter and thickness.

Hardness test: The crushing strength (KgF) was determined with an Automatic Tablet Hardness Tester (8M, Dr Schleuniger, Switzerland). The force applied to the edge of the tablet was gradually increased by moving the screw knob forward until the tablet was broken. Ten tablets were randomly selected from each brand and the pressure at which each tablet crushed was recorded.

Friability test: Ten tablets from each brand were weighed and subjected to abrasion by employing a Veego friabilator (VFT-2, India) which was operated at 25 RPM for 4 minutes. The Friabilator was made of a plastic chamber divided into two parts. During each revolution the tablets were fallen from a distance of six inches to undergo shock. After 100 revolutions the tablets were again weighed. The loss in weight indicated the friability.

Determination of uniformity of weight: 20 tablets from each of the 6 brands were weighed individually with an analytical weighing balance (AY-200, Shimadzu, Japan). The average weight for each brand was determined as well as the percentage deviation from the mean value were calculated using the formula given by Banker and Anderson [17].

Disintegration test: Six tablets from each brand were employed for the test in distilled water at 37 ± 0.5 °C using a Tablet Disintegration Tester (Model: VDT-2, Veego, India). As stated by Alderborn [18], the disintegration time (DT) was taken as the time when no particle remained on the basket of the system.

Dissolution test: The dissolution test was undertaken using Tablet Dissolution Tester (TDT-08L, Electrolab, India) in 6 replicates for each brand involving USP apparatus-II (paddle) at 75 RPM. The dissolution medium was 900 ml of phosphate buffer (P^H 6.8) which was maintained at 37 ± 0.5 °C. In all the experiments, 10 ml of dissolution sample was withdrawn at 0, 10, 20, 30, 40, 50 and 60 min and replaced with equal volume to maintain an ideal sink condition. Samples were filtered and from the filtrate 1 ml solution was taken and diluted with 99 ml phosphate buffer to make the final volume of 100 ml. The solution was then assayed by UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan) at 239 nm. To determine the concentration of sample, help from the standard curve of pure API (Figure 1) was taken. Using the $Y = mX + C$ equation, sample concentration was calculated.

Assay: Twenty tablets from each brand were weighed and finely powdered. The powder equivalent to 20 mg of amlodipine besylate was taken and dissolved in phosphate buffer (P^H 6.8). Flasks were subjected to sonication to dissolve the powdered material. Then the solution was filtered**.** The filtrate was suitably diluted. After that absorbance values were measured at the maximum wavelength (λ_{max}) of these concentrations using a UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan). Maximum wavelength (λ_{max}) was obtained by scanning samples from 200 to 400 nm and it was found 239 nm.

RESULTS

From the data mentioned in Table 1, it has been found that among six brands brand-D had highest average diameter (8.54 mm) whereas brand-A had lowest average diameter (6.04 mm). The average thickness of Brand A, B, C, D, E, F were found to be 2.01 mm, followed by 3.95 mm, 2.36 mm, 4.06 mm, 2.95 mm and 3.18 mm respectively as shown in Table 1. So, brand D had the highest average thickness of 4.06 mm and brand A had the lowest average thickness which is 2.01 mm. According to Table 1, brand-B had maximum hardness of 4.5 kgF whereas brand-D had the lowest hardness of 2.1 kgF among the six brands. As shown in Table 1, three brands have percent friability below 1%. Among six brands, brand-C showed maximum friability (1.47%) whereas brand-D showed minimum friability (0.67%)., As depicted in Table 1, brand B showed the highest deviation, two tablets crossed the limit but none of them crossed the double limit of 15%. And brand A showed least deviation among all the six brands.

Table 1 shows that brand C took maximum time of 1.66 minute and brand A took the minimum time of 0.22 minute to disintegrate. Intra-brand (within a brand) dissolution profile in Figure 2 and inter-brand (brand to brand) dissolution profile in Figure 3 reveals that brand B showed maximum % of drug release (99.87%) whereas brand D showed minimum % of drug release (87.19%) in 60 minutes. Table 1 illustrates that the active content of all the brands were in between 92% (brand-D) and 98.70% (brand-A).

DISCUSSION

By monitoring the diameter and thickness of the tablets at regular intervals, potential problems relating to tablet weight and hence content uniformity can be detected at an early stage [19]. Whereas with increasing thickness, there is a decrease in hardness due to compression force, on the other hand with decreasing thickness there is an increase in hardness, so tablets of the same batch having lower thickness show greater hardness than the tablets having higher thickness. In consideration of average diameter and thickness the variation was found satisfactory for all brands.

Hardness has impact on disintegration. If the tablet is hard then it cannot disintegrate within the specified time and if the tablet is soft then it becomes hard to withstand the handling during coating or packaging. Therefore, adequate tablet hardness and resistance to powdering and friability are necessary requisites for consumer acceptance [20]. Oral tablets normally have a hardness of 4 to 8 or 10 kg. In general, if the tablet hardness is too high, disintegration test is performed before rejecting the batch. And if the disintegration is within limit, the batch is usually accepted [19]. Here, only one brand was within the range but since the hardness test is an unofficial test [21] and later their disintegration time was found satisfactory, the batches were considered as of good quality.

Friability assessment reveals good mechanical strength of the tablets [22]. The compendial specification for friability is not more than 1% [23]. Friability test is influenced by internal factors like the moisture content of tablet granules and finished tablets. Moisture at low and acceptable level acts as a binder. As the hardness of the tablets is increased gradually there is a notable decrease in the percent friability in all formulations. The possible

Table 1. : Summary of the quality control tests undertaken on different brands of ADB tablets

Brand code	Diameter (mm)*	Thickness (mm)*	Hardness (KgF)*	Friability (%)	Weight deviation (mg)	DT (min)*	% Drug content
A	6.04 ± 0.01	2.01 ± 0.04	2.5 ± 0.02	1.13	169.8 ± 4.01	0.22 ± 0.03	98.70
B	8.05 ± 0.03	3.95 ± 0.03	4.5 ± 0.27	0.92	252.5 ± 8.31	1.17 ± 0.06	95.00
C	8.11 ± 0.04	2.36 ± 0.05	3.3 ± 0.14	1.47	150.4 ± 5.63	1.66 ± 0.12	96.80
D	8.54 ± 0.14	4.06 ± 0.06	2.1 ± 0.12	0.67	189 ± 5.98	1.26 ± 0.25	92.00
E	8.25 ± 0.03	2.95 ± 0.07	2.87 ± 0.11	0.98	170 ± 6.45	0.53 ± 0.14	96.00
F	7.86 ± 0.04	3.18 ± 0.09	3.4 ± 0.28	1.06	166 ± 7.16	0.39 ± 0.05	97.00

*Values are expressed as mean ± SD

Table 2. : Dissolution profile of six brands of ADB tablets (values expressed as mean ± SD).

Time (min)	% Drug release					
	Brand A	Brand B	Brand C	Brand D	Brand E	Brand F
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
10	55.56 ± 4.35	48.74 ± 2.53	45.00 ± 2.52	52.63 ± 4.65	49.53 ± 1.63	46.29 ± 5.55
20	66.55 ± 0.87	65.00 ± 1.57	54.56 ± 1.94	61.89 ± 2.19	58.56 ± 1.84	57.13 ± 1.58
30	69.76 ± 1.84	78.97 ± 1.22	69.79 ± 3.24	75.98 ± 1.02	67.18 ± 2.08	66.19 ± 7.98
40	78.89 ± 3.74	88.87 ± 5.52	78.87 ± 1.78	86.48 ± 3.09	79.56 ± 1.06	77.89 ± 3.72
50	92.00 ± 2.52	94.00 ± 1.24	83.35 ± 2.14	92.12 ± 1.94	85.13 ± 1.49	86.23 ± 3.76
60	98.56 ± 1.37	99.87 ± 1.39	95.95 ± 3.17	87.19 ± 1.53	97.00 ± 2.31	97.00 ± 1.84

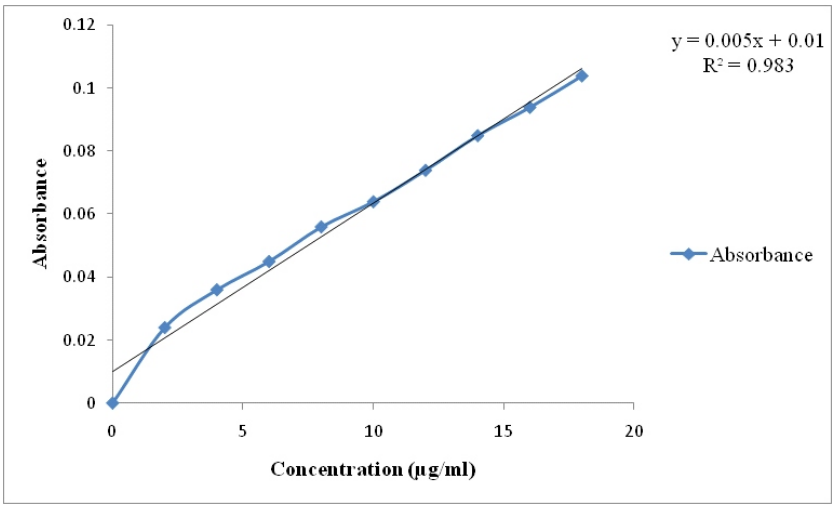

Figure 1. : Standard Curve of Amlodipine Besylate.

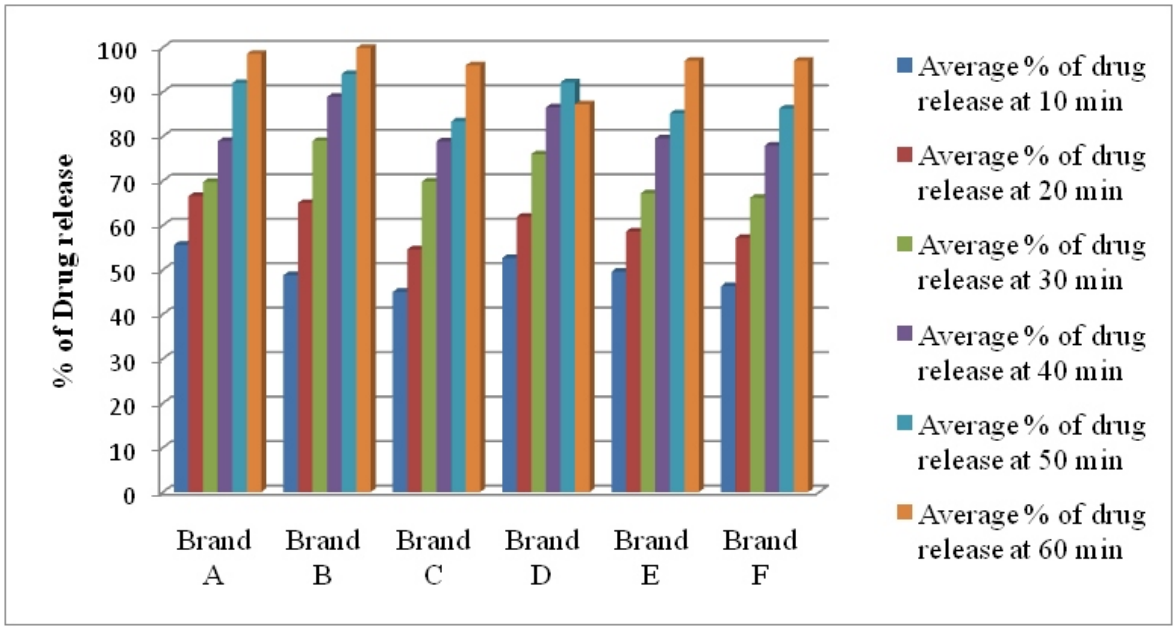

Figure 2: Intra brand Dissolution Profile of Six Brands of ADB Tablets

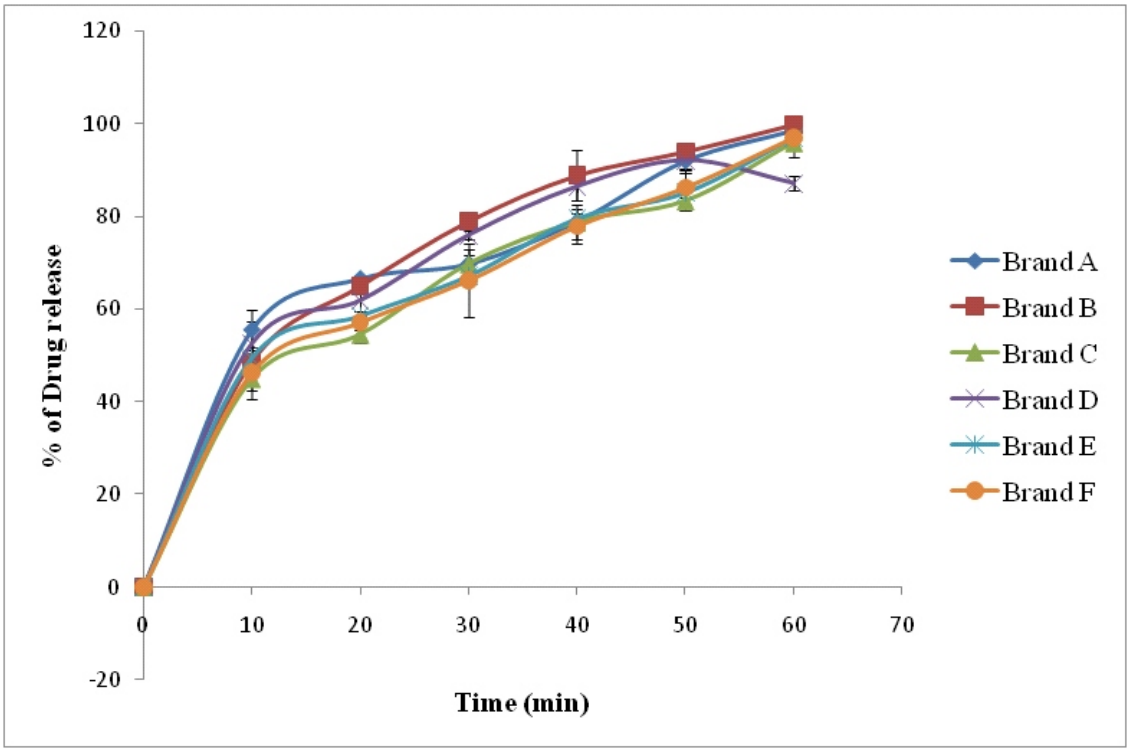

Figure 3: Inter brand Dissolution Profile of Six Brands of ADB Tablets. Vertical bars represent mean ± SD

reason for this result may be that at high compressional force the granules are packed strongly together and there is low degree of crumbling during friability. So harder the tablets less will be the percent friability and vice versa [20]. Here, three brands (B, D and E) have percent friability below 1% which indicates tablets from other three brands (A, C and F) may face difficulty during storage or transportation.

Weight variation does serve as a pointer to good manufacturing practices (GMP) maintained by the manufacturers as well as amount of active pharmaceutical ingredient (API) contained in the formulation [23]. The weight variation for all the tablets used in this study showed compliance with the official specifications of USP. Since all the brands have average weight in between 130 - 324 mg. Therefore, not more than 2 tablets should differ from the average weight by more than 7.5% and none should deviate by 15% of average weight.

As disintegration plays an important role in a tablet's dissolution before the active drug substance is finally released from the tablet's structure into the body. Therefore type, concentration, and efficiency of disintegrates to a large extent affects the dissolution [24]. BP specifies that uncoated tablets should disintegrate within 15 minute which is 30 minute in case of USP [21]. Here, all the brands met the official criteria.

Dissolution profile (Table 2) of all the investigated brands was found within the limit. The evaluation showed that almost all the 6 brands dissolved 100% within 60 minutes indicating that the release pattern of drugs were same although the brands were manufactured by different companies using different excipients in different ratio but on the basis of releasing factor they can be used interchangeably.

Analysis of drug potency in tablets indicates the presence of drug in dosage form and their stability [25]. The result indicates there was no significant variation in content of active moiety in their dosage form among the six companies and all are within the USP specification of 100 ± 10%.

CONCLUSION

In the current industrial practice, to compare with the multi brand generic molecules and to provide enough therapeutic activity of the dosage form, *in-vitro* tests play a significant role. The presented data exhibits that all six brands of amlodipine besylate tablets included in this study seem to have good overall quality with sufficient dissolution rate and adequate potency. This study illustrates the current scenario of different quality parameters of drug products manufactured by local companies. It is a general psychology that the drug products manufactured by mid or small level companies may be poor as compared to leading companies available in the market. But this investigation will help to change the view of the people.

ACKNOWLEDGEMENT

Authors are grateful to their parental institute for providing the necessary facilities to accomplish the research work.

REFERENCES

1. Hailu GS, Gutema GB, Hishe HZ, Ali YS, Asfaw AA. Comparative In Vitro Bioequivalence Evaluation of Different Brands of Amoxicillin Capsules Marketed in Tigray, Ethiopia. Int. J. Pharm. Sci. Nanotech. 2013; 6(1): 1966-1971.
2. Chandrasekaran AR, Yi HC, Yang CAC, Cheang LW, LS Ping. Post-marketing in vitro equivalency evaluation of paracetamol tablets in Kedah, Malaysia. Int. J. Pharm. Sci. Nanotech. 2011; 4(2): 1403-1407.
3. Ohmori M, Arakawa M, Harda K, Takasali H, Hifumi S, Miyamori I, Fujimura A. Stereoselective pharmacokinetics of amlodipine besylate in elderly hypertensive patients. Am. J. Ther. 2003; 10(1): 2931.
4. Mathew M, Bernard S, Kumar KLS, Girija KN. Development and validation of a high performance thin layer chromatographic method for the simultaneous estimation of Atorvastatin calcium and Amlodipine besylate as the bulk drugs and in the tablet dosage form. Asian J. Pharm. Hea. Sci. 2014; 4(2): 986-991.
5. Dai SY, Qiu ST, Wu W, Fu CM. Development and validation of an RP-HPLC method for simultaneous determination of Ramipril and Amlodipine in tablets. J. Pharm. Anal. 2013; 3(6): 440446.
6. Shaikh SA, Shaikh SS, Shahi SR, Shookur MA, Reddy LK, Padalkar AN, Thube M. Formulation and Evaluation of S-(-)-Amlodipine Besylate and Nebivolol Hydrochloride Tablets. J. Adv. Pharm. Technol. Res. 2010; 1(2): 199-206.
7. Laufen H, Leitold M. Enantioselective disposition of oral amlodipine in healthy volunteers. Chirality. 1994; 6(7): 531536.
8. Luksa J, Josic D, Kremser M, Kopitar Z, Milutinovic S. Pharmacokinetics behaviour of R-(+)- and S-(-)-amlodipine after single enatiomer administration. J. Chromatogr. Biomed. Sci. Appl. 1997; 703(1-2): 185193.
9. Bhardwaj V, Shukla V, Goyal N, Salim M, Sharma PK. Formulation and Evaluation of Fast Disintegrating Sublingual Tablets of Amlodipine Besylate Using Different Superdisintegrants. Int. J. Pharm. Pharm. Sci. 2010; 2(3): 89-92.
10. James RK, Shuvro S, Prince J, Sayeeda FC. Comparative in vitro equivalence evaluation of some Aceclofenac generic tablets marketed in Bangladesh. Pharma Innovation J. 2016; 5(3): 03-07.
11. Khadka P, Ro J, Kim H, Kim I, Kim JT, H Kim; Cho JM, Yun G, Lee J. Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. Asian J. Pharm. Sci. 2014; 9(6): 304-316.
12. Dahan A, Miller JM, Amidon GL. Prediction of Solubility and Permeability Class Membership: Provisional BCS Classification of the World's Top Oral Drugs. AAPS. J. 2009; 11(4): 740-746.
13. Yasir M, Asif M, Kumar A, Aggarval A. Biopharmaceutical Classification System: An Account. Int. J. Pharm.Tech. Res. 2010; 2(3): 1681-1690.
14. Olusola AM, Olubukola OO, Emeka OH, Lilian AE. Equivalence of Two Generic Brands of Amlodipine Besylate Under Biowaiver Conditions. Int. J. Pharm. Pharm. Sci. 2012; 4(2): 265-268.
15. Feroz M, Razvi N, Ghayas S, Anjum F, Ghazal L, Siddiqui SA. Assesment of Pharmaceutical Quality Control and Equivalence of Various Brands of Amlodipine Besylate (5 mg) Tablets Available in the Pakistani market Under Biowaitver Conditions. Int. J. Pharm. Pharm. Sci. 2014; 6(2): 909-913.
16. Sharma D, Jain A, Shrivastava A. Simultaneous estimation of amlodipine besylate and nebivolol hydrochloride in tablet dosage forms by reverse phase-high-performance liquid chromatographic using ultraviolet detection. Pharm. Methods. 2011; 2(1): 9-14.
17. Banker GS, Anderson NR. The Theory and Practice of Industrial Pharmacy. 3rd edition. Lea & Febiger Verghese publishing house, Philadelphia, 1986. pp. 293-303.
18. G Alderborn. The Design and Manufacture of Medicines. 1st edition. Churchill livingstone: Elsevier; Hungary, 1988. pp. 441-481.
19. Bano R, Gauhar S, Naqvi SBS, Mahmood S. Pharmaceutical evaluation of different brands of

Levofloxacin tablets (250 mg) available in local market of Karachi (Pakistan). Int. J. Curr. Pharm. Res. 2011; 3(1): 15-22.

20. Saleem M, Shahin M, Srinivas B, Begum A. Evaluation of tablets by friability apparatus. Int. J. Res. Pharm. Chem. 2014; 4(4): 837-840.
21. Oishi TS, Nimmi I, Islam SMA. Comparative in vitro Bioequivalence Analysis of Some Generic Tablets of Atorvastatin, a BCS Class II Compound. Bangladesh Pharm. J. 2011; 14(1): 61-66.
22. Kumar P, Ganure AL, Subudhi BB, Shukla S, Upadhyay P. Design and comparative in-vitro and in-vivo evaluation of starchacrylate graft copolymer based salbutamol sulphate sustained release tablets. Asian J. Pharm. Sci. 2015; 10(3): 239-246.
23. Nayak AK, Pal D. Comparative in vitro Bioequivalence Analysis of Some Ciprofloxacin HCl Generic Tablets. Int. J. Pharm. Sci. Res. 2010; 1(8): 51-57.
24. Mangal M, Thakral S, Goswami M, Thakur N. Comparative study between various reported disintegration methods for fast dissolving tablets. African J. Basic & Appl. Sci. 2012; 4(4): 106-109.
25. Abbirami V, Sainithya P, Shobana A, Devi DR, Hari BNV. Review on In-vitro Bioeqivalence Studies and its Methodologies. Int. J. Chem.Tech. Res. 2013; 5(5): 2295-2302.