EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 8
Issue: 4
101-106
Year: 2021

A COMPARATIVE STUDY OF THE QUALITY OF DIFFERENT BRANDS OF AMITRIPTYLINE TABLETS AVAILABLE IN SOME NIGERIAN MARKETS

Ukwueze S.E.[1*], Andrew-Kekii L.P.[1], Nwoke E.A.[1] and Odigie J.[2]

[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Rivers State, Nigeria.
[2]Department of Pharmacology and Toxicology, Faculty of Basic Medical Sciences, University of Port Harcourt, Rivers State, Nigeria.

***Corresponding Author: Dr. Ukwueze S.E.**
Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Rivers State, Nigeria.

Article Received on 04/02/2021 **Article Revised on 24/02/2021** **Article Accepted on 14/03/2021**

ABSTRACT
In recent times, generic brands of psychotropic medications have flooded the Nigerian market, thus, decreasing the cost of managing mental health disorders. However, the World Health Organization (WHO) had estimated that 30 % of the medicines in circulation in low- and middle-income countries, like Nigeria, are substandard and counterfeit. Poor quality medicine poses serious public challenge to end-users globally. The objective of this study was to evaluate the degree of compliance of different brands of amitriptyline, an antidepressant commonly used in Nigeria, with quality and safety standards. Generic brands of amitriptyline were sourced from pharmacies in south-south states of Nigeria and analyzed for uniformity of weight, hardness, friability, disintegration, and dissolution profiles. The content of active ingredients and similarity factor (F) of the dissolution profiles for the tablets were also determined. All the tests were done according to USP (2009) and BP (2016) specifications. Amitriptyline content was determined at a wavelength of 245 nm using UV-spectrophotometer. The innovator brand (R1) was applied as the primary standard. All the brands, except R1, failed the hardness test (< 4 kg/f) and the content of active ingredients assessment (< 90%). However, they all passed the friability test (< 1.0%), disintegration test (< 10 min) and dissolution test (> 80% at 30 min). Only one brand failed the similarity factor test (< 50). This study suggests that not all brands of amitriptyline available in Nigerian market meet the stringent quality and safety standards necessary to ensure efficacy and safety of the patient. This also makes outcome unpredictable when brands are switched.

KEYWORDS: Amitriptyline, Substandard, Counterfeit, Pharmacopoeia, Medicines, Similarity factor.

1.0 INTRODUCTION
The problem of substandard or counterfeit medicine has become a global issue. It has defied most existing international and national borders. Even countries with the strictest drug regulation framework have to face this problem such as the incidence of anticancer drug Avastin® in USA in 2012, erectile dysfunction drugs, Viagra® and Cialis®, in United Kingdom in 2012 and Truvada® drugs for the treatment of HIV/AIDS in UK in 2011.[1] However, this problem occurs more in developing countries.[2] The World Health Organization (WHO) estimated that 30% of the medicines in circulation in low- and middle-income countries are substandard and counterfeit due to very weak policies, regulation and policy enforcement systems for medicine. It flourishes mainly as a result of the huge profits to be made. Poor quality medicine poses serious public health challenge to end-users globally.[3] Antidepressants have become one of the major culprits for counterfeiters due to its increasing use in the relief of symptoms of depression, treatment of chronic pains and improvement of sleep.[2]

Amitriptyline hydrochloride is an antidepressant with sedative effects which acts by inhibiting the membrane pump mechanism responsible for uptake of serotonin and norepinephrine in serotonergic and adrenergic neurons.[4] Pharmacologically, this leads to the potentiation of neuronal activity since reuptake of these biogenic amines is important physiologically in terminating neuronal transmitting activity. This interference with reuptake of norepinephrine and/or serotonin underlies the basic mechanism of the antidepressant activity of amitriptyline.[4]

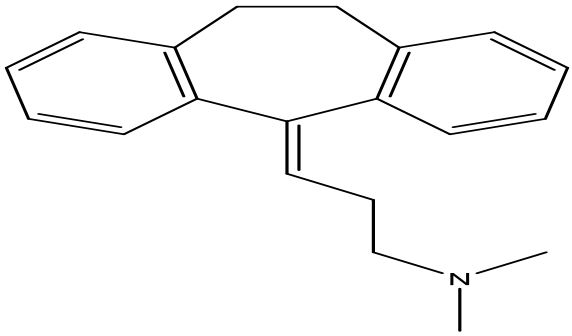

Fig 1: Structure of Amitriptyline.

Overdose of this drug as a result of accident, medical error or poor dosage form quality can leads to cardiac dysrhythmias, severe hypotension, convulsions, and CNS depression, including coma, changes in the electrocardiogram particularly in QRS axis or width, prolonged PR interval, ST-T wave changes, ventricular tachycardia and fibrillation, impaired myocardial contractility. It can also cause confusion, disturbed concentration, transient visual hallucinations, dilated pupils, disorders of ocular motility, agitation, hyperactive reflexes, stupor, drowsiness, muscle rigidity, vomiting, hypothermia, hyperpyrexia as well as death; therefore, there is an urgent need to strictly regulate the quality of amitriptyline in circulation to ensure that they comply to standard specifications in order to prevent drug toxicity and promote predictability of therapeutic outcome in patients. This study was, thus, aimed at evaluating the degree of compliance to quality and safety standards by different brands of Amitriptyline sold in Nigeria.

2.0 MATERIALS AND METHOD

2.1 Sample Collection: Four brands of amitriptyline 25mg tablets (coded A1 – A3) and a reference sample (coded R1) used in this study were purchased from retail pharmacy outlets in south-south states of Nigeria. These were stored under appropriate conditions and tested within their expiration dates.

2.2 Instruments/Reagents used in the study

Jenway 6405 UV/Vis Spectrophotometer, Acculab analytical weighing balance, Dissolution tester (DT 600 High head), Erweka disintegration tester (ZT122, serial No: 125397.Oa96; ZT x 20 series), Erweka Friabulator and Monsanto Hardness Tester were used in this study. Methanol, 5% NaOH, Distilled water, NaOH Pellets and Potassium dihydrogen phosphate were also used. All reagents were of analytical grade.

2.3 Preliminary test

2.3.1 General Appearance

Organoleptic analysis was performed on each sample. This included shape, colour and coating type of the different brands of amitriptyline tablets were examined and recorded.

2.3.1 Packaging and Labeling Inspection: The labeling on the primary and secondary packages of the tablets was properly examined for the following details: name and strength of active ingredient, batch number, brand name, manufacture date and expiry date were determined.

2.4 *In Vitro* Official Tests

2.4.1 Uniformity of Weight

Twenty tablets from each brand of amitriptyline were selected and weighed with Acculab® analytical balance ALC210.4 (Germany) individually. The determinations were done in triplicate. The weights were recorded and the mean, standard deviation, and percentage standard deviation were calculated.

$$\text{Weight Variation} = \frac{I_w - A_w}{A_w} \times 100\%$$

Where I_w = individual weight of tablets
A_w = average weight of tablets.

2.4.2 Disintegration Test

The disintegration test for the different brands of amitriptyline was carried out according to the method described in the BP.[5] A 700 ml of distilled water was placed into the beaker in the disintegration apparatus (Erweka disintegration machine, Germany). The temperature of immersion fluid was maintained at 37^{o}C. Six tablets were randomly selected from each brand of amitriptyline, one tablet was placed in each of the six tubes and the tubes were immersed into the fluid. The disintegration time was recorded and average time as well as percentage deviation was calculated.

2.4.3 Preparation of standard stock solution:

A 10 ml volume of concentrated hydrochloric acid was dissolved in 500 ml of distilled water and made up to 1000 ml with distilled water. A 20 mg amitriptyline tablet (secondary standard) was dissolved in 20 ml of distilled water in a beaker and filtered. The filtrate obtained was used as stock solution to obtain concentrations of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 µg/mL solutions.

2.4.4 Determination of maximum wavelength (λ_{max}) of absorption

An aliquot from the stock solution was scanned in the UV-Visible spectrophotometer at different wavelengths to determine the maximum wavelength of absorption. Maximum wavelength obtained was 245 nm.

2.4.5 Determination of standard calibration curve

The serial dilutions (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 µg/ml) obtained from amitriptyline stock solution were passed through the UV-Visible spectrophotometer and their absorbance was read at 245 nm. A plot of concentration against absorbance was made and it's coefficient of determination (r^2) was determined.

2.4.5 Assay of Content of Active Ingredients

Ten tablets from each brand of amitriptyline were weighed and crushed to powder in a mortar. An average

weight of the tablets of amitriptyline was weighed, transferred into a 100 ml volumetric flask and dissolved with 100 ml of 0.1 N hydrochloric Acid. A 0.05 ml of the solution was measured and transferred to 10ml volumetric flask separately; 0.1 N HCl was added to make up to 10 ml volume. Aliquot volume of the solution was diluted to get a concentration of 20 µg/ml. The average absorbance of the sample solution after two determinations at 245 nm against a blank solvent obtained was recorded. The percentage drug content was calculated for each batch.

2.4.6 Dissolution Test

The dissolution test for the different brands of amitriptyline tablets were carried out according to United States Pharmacopoeia[6] using Erweka dissolution apparatus Germany (paddle type). The 0.1 N hydrochloric acid (900 ml) was placed in each of the vessels of the dissolution apparatus and the medium was maintained at 37°C. The paddles were rotated at a rotational speed of 50 rpm. A tablet from each brand was placed in the vessel containing 0.1 N hydrochloric acid and the dissolution apparatus was operated for 30 min. A 5 ml of dissolution medium was withdrawn using a pipette for each brand at 5, 10, 15, 20, 25, and 30 min intervals and replaced immediately with 5 ml of 0.1N hydrochloric acid after each withdrawal. The withdrawn samples were filtered and assayed using UV-Visible spectrophotometer at 245 nm to determine the release of amitriptyline from the tablets.

2.5 *In Vitro* Unofficial Tests:

2.5.1 Hardness/Crushing Strength Test

Ten tablets were randomly selected from each brand of amitriptyline. One tablet was placed between the jaws of a hardness tester and adjusted by pushing forward the movable jaw inside, turning the plunger clockwise. The value on the scale that coincides with the pointer was noted and pressure applied till the tablet breaks. The value on the scale was recorded. The procedure was repeated for all tablets.

2.5.2 Friability test

Ten tablets are selected at random. Each batch of ten tablets was weighed. The tablets were placed in the friabilator and rotated for 4 min at 25 revolutions per minute (rpm). The tablets were removed, de-dusted and reweighed.

2.5.3 Calculation of fit factor (F)

a.) Difference factor

The difference factor (f1) calculates the percent % difference between the two curves at each time point and is a measurement of the relative error between the two curves: the standard range is between 0 to 15.

$$f_1 = \left\{\frac{\sum_{t=1}^{n}|R_t - T_t|}{\sum_{t=1}^{n} R_t}\right\} \times 100$$

Where:
n is the number of time points,
R is the dissolution value of the reference (pre-change) batch at time t, and
T is the dissolution value of the test (post-change) batch at time t

b.) Similarity factor

The similarity factor (f2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent % dissolution between the curves. F2 acceptable range falls between 50 to 100.

Fit factor was calculated using the formula; F2

$$f_2 = 50\, log\left\{\left(1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right)^{-0.5} \times 100\right\}$$

Where:
n = number of time points,
R_t =dissolution value of reference product at time t
T_t =dissolution value of the test product at time t.

3.0 RESULTS

Table 1: Result of the labeling and Inspection Test.

S/N	*Brand*	*Batch No*	*Label claim (mg)*	*Manufacturing date*	*Expiry date*	*Country of origin*
1	*A1*	*AMT601*	*25*	*09/2016*	*08/2019*	*India*
2	*A2*	*AF52706*	*25*	*07/2017*	*07/2020*	*India*
3	*A3*	*APIH0137*	*25*	*05/2016*	*04/2020*	*India*
4	*R1*	*16197717*	*25*	*12/2017*	*01/2020*	*UK*

Table 2: Results of the general appearance for the tested brands of Amitriptyline.

Product code	Coating type	Colour	Dosage form
A1	Film coated	White	Tablet
A2	Film coated	Yellow	Tablet
A3	Film coated	White	Tablet
R1	Film coated	White	Tablet

Table 3: Results of weight variation, hardness, % friability, disintegration and % content.

Sample	*Weight variation mean (mg)± SEM*	*Hardness (Kg/f)*	*% friability*	*Disintegration Mean (min)/ % Dissolution*	*% content*	*F2 (vs R1)*
A1	*129.5 ± 5.86*	*2.24*	*0.01*	*5.5 / 98.9*	*84.48*	*34.19*
A2	*131.0 ± 4.22*	*2.15*	*0.13*	*4.9 / 80.7*	*85.72*	*62.43*
A3	*158.0 ± 5.28*	*1.20*	*0.01*	*3.6 / 99.5*	*85.72*	*65.76*
R1	*130.0 ± 4.75*	5.23	0.25	*5.0 / 93.5*	*100*	-
Official Specification	*≤ 5-7.5 (USP)*	*4-8 (USP)*	*<1 (USP)*	*5-30 / 75% (USP)*	*95-105 (USP)*	*> 50 (FDA)*

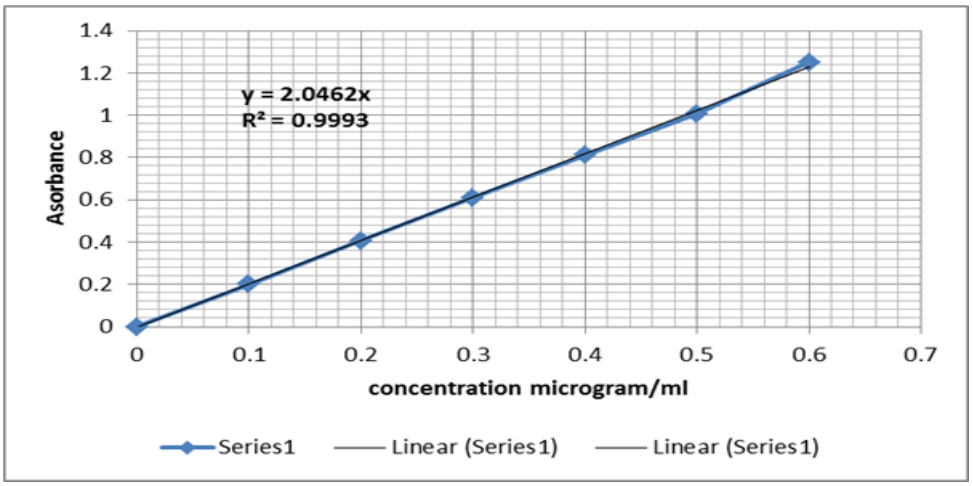

Fig 2: Standard Calibration Curve of Amitriptyline.

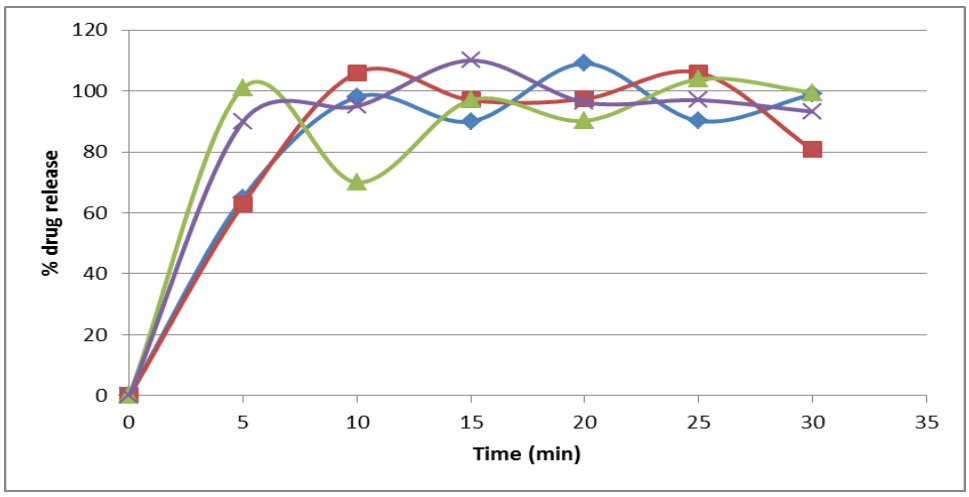

Fig 3: Graph of % drug release against Time of Amitriptyline.

4.0 DISCUSSION

Amitriptyline is a psychotropic drug which acts as anti-depressant for the treatment of depression in patients. Depression is a mental health disorder characterized by persistently depressed mood or loss of interest in activities, causing significant impairment in daily life.[7] Thus, any medication indicated for the treatment of such mental health disorders must comply strictly to laid down guidelines and standard limits. This is especially so among drug generics which are expected to maintain suitable chemical and pharmaceutical equivalence, thereby engendering brand interchangeability with the ultimate aim of reduction in treatment cost.[8]

The different brands of amitriptyline used in this study had suitable appearance, properly labelled and registered with NAFDAC. The samples were all within their expiry dates with batch numbers clearly indicated (Table 1). They were filmed coated and evenly colored (Table 2). The overall appearance of tablets determines consumer acceptability and can affect the level of compliance to the dosage regimen by the patient [9]. Thus, the amitriptyline samples complied with acceptable standards for organoleptic presentations and packaging of pharmaceutical dosage forms.

Hardness is the load required to crush the tablet when placed on its edge. All the brands of amitriptyline tablets

in this study, aside the reference sample, failed the hardness test (Table 3). The USP specification for tablet hardness ranges from 4 to 8 kgf [6]. This showed that most of the tablets in the samples tested might not be able to withstand rigorous treatment in the course of packaging and transportation. Factors affecting the hardness of tablets include compressive force applied during the compression process, the amount of binder and method of granulation.

Friability test is used to evaluate tablet resistance to abrasion. The USP (2009) states that the percentage friability permitted is less than 1%. From the result carried out, all brands passed the friability test [10]. This implied that the samples possess enough robustness to withstand packaging and handling pressures that would make the tablets to maintain the desired weight and content uniformity.

Weight uniformity was done to determine the consistency of the weight of dosage forms (tablets). Determination of percentage standard deviation of tablets and capsules gives an idea of how tablets and capsules vary from each other in a batch. The BP specified that for drugs weighing 80 mg or less, the percentage standard deviation should not be more than 10%.[5] The different brands of tablets used for this study all passed the weight uniformity test. Factors such as the formulation and manufacturing processes influences the weight of tablets in the different brands of a generic drug.[11] This result showed that all the brands tested could contain similar quantities of active ingredients and excipients and thus reducing dosage fluctuations when the drugs are administered.

For content uniformity test BP 2016 specifies that amitriptyline tablet should have an active ingredient content ranging from 90-110% and all brands in this study, aside the standard, failed this test (Table 3). The variation in the amount of active ingredients contained in these samples could result in unpredictable treatment outcomes.[12]

Disintegration test was carried out to determine the release of the active ingredient from its dosage form (tablet). Disintegration time is the time required for a dosage form to break up into granules under carefully specified conditions. For disintegration test the USP states that film coated tablets should disintegrate within 15 min.[6] This also applies to hard gelatin capsules. The Amitriptyline tablets used for this research all passed the disintegration test with an average disintegration time ranging from 3.6 to 5.5 min showing that some brands had higher disintegration time than the others (Table 3). For a drug to be absorbed into the systemic circulation it must disintegrate to release its active ingredient. Disintegration is a necessary step in determining the pharmacokinetic properties of a drug. Disintegration gives room for dissolution, so it is subsets of the dissolution process. Thus, the result showed that all the samples are expected to release their active ingredients within specified time limits, having given acceptable disintegration time values. Several factors that affect disintegration process in tablets include effect of fillers, binder lubricants and surfactant.[13]

The effectiveness of solid dosage forms relies on its ability to dissolve in the fluids of the gastrointestinal tract prior to absorption into systemic circulation. Dissolution is therefore a very important factor to consider because without such drugs would not be absorbed. According to the USP, not less than 75% of amitriptyline hydrochloride should be released within 45 min. From the results obtained, all brands of amitriptyline tested passed the dissolution test (Fig 3). This implied that the samples could achieve the desired therapeutic goal when administered, including timely onset of action and attainment of adequate peak plasma concentrations. Factors that affect the dissolution rate of drug ranges from physiochemical properties of drug, drug product formulation factors, processing factors, factors relating to dissolution apparatus and factors related to the dissolution test parameters.[14]

Similarity factor (F2) was carried out to determine the bio-pharmaceutical equivalence between the reference brand and the other samples of amitriptyline using their drug release profiles.[15] The standard specification by the FDA for F2 values ranges from 50 to 100 [16]. Amitriptyline brand A2 and A3 passed the test while A1 failed when compared with R1. Values within the acceptable range indicate the possibility of interchangeability between the tested drug profile and the reference sample.[17]

5.0 CONCLUSION

This study has shown that not all brands of amitriptyline available in Nigerian market meet the stringent quality and safety standards necessary to ensure efficacy and safety of the patient. This also makes outcome unpredictable when brands are switched.

6.0 Conflict of Interests

The authors declare no conflict of interest in the course of this study.

REFERENCES

1. World Health Organization (WHO) model for essential medicines, 2015 pp 1-43.
2. Sarker MM, Rashid MS, Raju AA, Rana M, Karim MF, Akter R, Howlader AN, Ming LC, Ismail NE. Evaluation of the Pharmaceutical Quality of Different Brands of Ranitidine Tablets Manufactured in Bangladesh: A Pharmaceutical and Public Health Prospective. *J. Appl. Pharm. Sci*, 2016; 6: 055–061.
3. Buowari OV. Fake and Counterfeit Drug: A Review. *Afrimedic Journal*, 2012; 3(2): 1-4.

4. Lawson K. A Brief Review of the Pharmacology of Amitriptyline and Clinical Outcomes in Treating Fibromyalgia. *Biomedicines*, 2017; 5(24): 1–12.
5. British Pharmacopoeia. British Pharmacopoeia Commission. The Stationery Office, London, 2016; 2: 2293 – 2296.
6. The United States Pharmacopeia and National Formulary USP29–NF24. Rockville: The United States Pharmacopeial Convention, Inc. 2009; 2778.
7. Hichiya H, Mimura Y, Hanioka N. Evaluation of oral antidepressant drugs for adaptation to the simple suspension method. *Journal of Young Pharmacists*, 2019; 6(4).
8. Ukwueze SE, Ogbokor M, Ezealisiji KM. Quality Assessment of Different Brands of Rabeprazole Tablets Marketed in Some Nigerian Cities. *J Pharm Chem Biol Sci*, 2017; 5(4): 345-353.
9. Yu L. Pharmaceutical quality by design: product and process development, understanding and control. *Pharm Res*, 2008; 25(4): 781-791.
10. Sahle SB, Ayane AT, Wabe NT. Comparative quality evaluation of paracetamol tablet marketed in Somali region of Ethiopia. *International Journal of Pharmaceutical Sciences and Research* 2012, 3(2): 545-550.
11. Kahsay G, G/Egziabher A. Quality Assessment of the Commonly Prescribed Antimicrobial Drug, Ciprofloxacin Tablets, Marketed in Tigray, Ethiopia. *Momona Ethiopian Journal of Science* 2010, 2(1): 93-107.
12. Akgeyik E, Kaynak MS, Çelebier M, Altınöz S, Şahin S. Evaluation of pharmaceutical quality of conventional dosage forms containing paracetamol and caffeine available in the Turkish drug market. *Dissolut. Technol*, 2016; 23 (2): 36-41.
13. Olusola A, Adekoya A, Olanrewaju O. Comparative evaluation of physiochemical properties of some commercially available brands of metformin hydrochloride tablets in Lagos, Nigeria. *J Appl. Pharm Sci*, 2012; 2(2): 41-4.
14. Nayak, AK. Comparative in vitro dissolution assessment of some commercially available paracetamol tablets. *International Journal of Pharmaceutical Sciences Review and Research*, 2010; 2(1): 29–30.
15. Ukwueze SE, Nwachukwu IP, Ezealisiji KM. Ultraviolet Spectrophotometric Evaluation of Different Brands of Azithromycin Dihydrate Tablets and Capsules Sold in Nigeria. *European Journal of Pharmaceutical and Medical Research*, 2018; 5(2): 80-84.
16. Food and Drug administration (FDA). Centre for Drug Evaluation and Research: Guidance for industry: Dissolution testing of immediate release solid oral dosage forms, 1997; pp 6-8.
17. Shah VP, Tsong Y, Sathe P, Liu JP. In vitro dissolution profile comparison--statistics and analysis of the similarity factor, f2. *Pharm Res*, 1998; 15(6): 889-96.