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Comparative *in vitro* and *in vivo* Bioequivalence Analysis of some Brands of film coated Atorvastatin (a BCS Class II Compound) tablets marketed in Nigeria

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A B S T R A C T

The study is aimed at assessing the bioequivalence/quality of different brands of atorvastatin calcium 10mg tablets marketed in Nigeria.

Physical parameters of the tablets, drug content, dissolution and pharmacokinetics data were assessed. The *in vivo* bioavailability study was carried out using a single dose randomized two period cross-over designs measuring the concentrations in plasma. Plasma samples before dosing and at various time intervals up to 48 hours after dosing were analysed using High-Performance Liquid Chromatography together with UV detector. Pharmacokinetics parameters (C_{max} and AUC) were determined and subjected to statistical analysis.

All brands complied with the official specification for uniformity of weight and disintegration time. Assay of atorvastatin tablets revealed that all samples contained atorvastatin calcium as their active ingredient between 91.4-102.1% (w/w) of labelled potency. The dissolution profiles showed inter brand variability. Four brands attained 70% dissolution within 45 minutes, however, at 60 minutes, all the brands released over 80% of the drug. *In vivo* bioavailability study showed that three out of the four brands were bioequivalent to the innovator brand and can be substituted for each other in their prescription.

Chemical equivalence does not indicate bioequivalence and one brand substituted on assumption of chemical equivalence with another brand may not give the desired onset of action and therapeutic effectiveness. Moreover, dissolution test might not be enough for ascertaining bioequivalence of atorvastatin and *in vivo* tests may be required to ensure the quality of marketed brands of atorvastatin.

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Introduction

Expenditure on medicines accounts for a major proportion of health costs in developing countries, with access to treatment being dependent on the availability of affordable generic medicines. In line with the sixth United Nations millennium development goals which are geared towards combating HIV/AIDS, malaria and other diseases, access to affordable and effective medicines in the form of generic medicine is not only paramount but potentially lifesaving [1]. The therapeutic efficacy of a drug in clinical practice depends on the rate and extent of its availability. The dissolution rate of poorly water-soluble drugs is often a rate-limiting step in their absorption from the GI tract. Such drugs are often associated with high intra subject and inter subject variability and often suffer limited oral bioavailability [2]. Hence, constant surveillance on the marketed poorly water soluble drugs by the government, manufacturers and independent research groups is desirable to ensure availability of quality medicines. Dissolution tests seem to be sensitive and reliable predictors of bioavailability as evaluated *in vivo*, yet *in vitro* testing cannot always predict *in vivo* performance [3].

Among other definitions [4, 5], the United States Food and Drug Administration [6] has defined bioequivalence (BE) as, "the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study". Studies to establish BE between two products are important for certain formulation or manufacturing changes occurring during the drug development and post-approval stages.

Different manufacturers employ different types of excipients. The physicochemical properties of these excipients also vary. In general, the type of excipients as well as their physical and chemical properties, unit operations and formulation processes employed in manufacture of a dosage form affects its quality/bioavailability. When such excipients are used, it is necessary to ensure that they do not adversely affect the stability, dissolution rate and bioavailability of the active ingredient(s) [7].

To evaluate the quality, therapeutic efficacy and safety of commercially available medicine, post market monitoring serves as a confidential tool. Information obtained from such monitoring can accelerate the improvement of existing regulations and product development [8]. The data and information obtained from post-market surveillance could be employed for product improvement, development of standards and regulations [9].

Atorvastatin, a synthetic lipid-lowering agent is currently used as calcium salt for the treatment of hypercholesterolemia, approved for treatment once daily at 10-80 mg [10]. It is a class II compound according to the biopharmaceutical classification system (BCS) [11]. It is insoluble in aqueous solution at pH 4 and below; but it is slightly soluble in water. The intestinal permeability of atorvastatin is high at the physiologically relevant intestinal pH[12]. However, Corsini *et al.* [13] reported that the absolute bioavailability of atorvastatin is only 12% after a 40 mg oral dose. To date several analytical methods for analysis of atorvastatin in biological samples have been developed. These included an enzyme immunoassay [14], gas chromatography/mass spectrometry (GC/MS) [15], High-performance Liquid Chromatography Tandem Mass Spectrometry (HPLC/MS) [16, 17], UPLC-MS/MS [18], RP-UPLC [19] and High-performance Liquid Chromatography with UV detection (HPLC-UV) [20, 21]. Generally, GC/MS and HPLC/MS methods are more sensitive but not readily available in most pharmaceutical laboratories and need highly trained personnel.

Bioavailability assessment of various brands of atorvastatin tablets in different countries has been published [16, 20, 22]. However, the bioavailability studies on the marketed atorvastatin tablets in Nigeria (a country with erratic drug distribution system) is limited to *in vitro* bioavailability [23, 24]. Being a BCS class II compound, there is need for *in vivo* bioavailability study as part of the post monitoring surveillance. Thus, in this study, atorvastatin tablets were selected to evaluate the quality of different brands marketed in Nigeria

with more emphasis on the study of physicochemical tests and *in vivo* bioavailability.

Materials and Methods

Materials

Drug and dosage form samples: standard of atorvastatin calcium crystalline (Batch No. ATI 4112, Mfg. date Sep. 2014, Retest date Aug 2016, Morepen Laboratories Limited, Morepen village, Nalagarh Road, Solan India) was a kind gift from Ranbaxy (India). HPLC grade methanol and acetonitrile were purchased from Fisher Scientific UK, Bishop Meador Road; manufactured by Merck, Germany. Five brands of film coated atorvastatin tablets (10 mg) were randomly purchased from local registered pharmacy shops in Lagos, Nigeria. The samples were properly checked for their National Agency for Food and Drug Administration and Control (NAFDAC) and batch numbers, production and expiry dates and other label information. Some of the information is shown in Table 1. They were randomly coded as AT1, AT2, AT3, AT4 and AT5 and stored properly; AT1 is the innovator or reference brand. All other chemicals were of analytical reagent grade and were used as received.

Table 1. Some label information and codes of evaluated brands of atorvastatin 10 mg tablets.

Brand Code	Mfg. Date	Exp. Date	Cost per 30 tablets ($)	Country of Manufacture
AT1	April 2012	March 2015	24.0	Ireland
AT2	October 2012	September 2015	6.5	India
AT3	June 2013	May 2016	5.0	India
AT4	March 2014	February 2017	2.0	India
AT5	March 2013	March 2016	3.8	Slovenia

Methods

Physicochemical parameters

Uniformity of weight, hardness test, tablet thickness and disintegration test were carried out using procedures detailed in an earlier study [3].

Chemical assay: A simple and selective HPLC method [20] was used to determine the atorvastatin content of the different brands of the tablets. For each atorvastatin brand, ten tablets of same brand were weighed and pulverized to obtain a fine powder used for the assay. The chromatographic system consisted of a pump, Model 1200 Infinity series, Agilent HPLC, equipped with a UV-VIS detector. Injections were carried out using a 20µL loop at room temperature. The limit of detection, limit of confirmation and limit of quantification for this method was recorded as 10, 15 and 30µg/ml.

Dissolution studies

Dissolution studies were conducted to determine the release pattern of the drug from the product. Three tablets from each brand were tested using dissolution medium of 900mL of 0.1N HCl, rotating the paddle at 50rpm at 37±0.5^{0}C by thermostatic setting. An aliquot of 5mL of samples were withdrawn at different time intervals: 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 minutes. These samples were filtered into a pre-labelled sampling bottles using ashless filter paper for each of the sample. The volume of the content of the dissolution beaker was maintained at 900ml. For every 5ml sample withdrawn from the bath, 5ml of the dissolution medium was introduced immediately as a replacement. The samples collected were filtered using membrane filter Z (0.45µm) then diluted 1/100 using 0.1N HCl. The HPLC method [20] was used to determine the atorvastatin content of the samples.

In vivo Bioavailability studies

The protocol for the plasma bioavailability study was conducted in accordance with international conference on harmonization of good clinical practice guidelines [25] and in compliance with the Declaration of Helsinki and its amendments [26]. The single-dose two period cross-over studies of atorvastatin tablets were conducted in healthy, non-smoking volunteers of ages between 18 and 40 years with body mass index (BMI) between 18.5 - 29.9kg/m^2 and normal gastrointestinal functions. All the subjects gave written informed consent and the College of Medicine; University of Lagos ethics committee approved the clinical protocol. The fifteen participants enrolled were randomly assigned and divided into five different groups of three participants and their blood sample collected prior to drug administration.

After overnight fasting, all the volunteers were given 60mg (6 tablets of 10mg Atorvastatin) dose of either formulation (reference and test) along with 500ml of distilled water, no food was allowed until five hours after dose administration. The blood collection were carried out in the following schedule, 15, 30, 60, 120, 240, 480, 720 and 1440 minutes after dosing, and kept in refrigerator before analysis at -4^{o}C. Acetonitrile (1.0mL) was added to 0.5ml human plasma which contained standard solutions of atorvastatin and internal standard (RS)-2-(4-(2-methylpropyl) phenyl) propanoic acid. To prevents atorvastatin binding to proteins and coagulate plasma proteins acetonitrile was added. The mixtures were then vortexed for 10minutes, to ensure deproteinization and centrifugation of samples for 20 minutes at 6000 rpm, 0.5ml of supernatant was taken and analysed via HPLC.

With reference to calibration standard curve, the amounts of atorvastatin in the samples were determined using HPLC method [20]. Pharmacokinetics parameters were determined. The AUC was estimated via linear trapezoidal method, under the assumption that a single dose was given. AUC was extrapolated to infinity (AUC $0 \rightarrow \infty$) and C_{max} and T_{max} were obtained from extrapolated data from the plasma sampling time's data.

Statistical analysis

ANOVA was employed for the physicochemical parameters while *in vitro* dissolution profiles was statistically compared using a model-independent approach [27,28,29], two fit factors [difference factor (*f*1) and similarity factor (*f*2)] that compare the dissolution profiles of a pair of drug products were applied to the dissolution data. *f*1 is the percentage difference between two curves at each point and is a measurement of the relative error between the two curves while *f*2 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 two curves. Equations 1 and 2 below were used to calculate *f*1 and *f*2.

$$f_1 = \left\{ \frac{\sum_{1} |R \quad t|}{\sum_{t}^{n}} \right\}$$

Equation 1

$$f_2 = 50 \log \left\{ (\quad - \sum \qquad\qquad 100 \right\}$$

Equation 2

Where n is the number of time points, R_t is the dissolution value of reference product at time t and T_t is the dissolution value for the test product at time t.

Pharmacokinetics parameters (C_{max} and AUC) were subjected to statistical analysis using SPSS version 13 SPSS Inc. Chicago Illinois. The pharmacokinetic parameters obtained for atorvastatin were expressed as the mean values ±SD, a 90%confidence interval at a 5% level of significance was applied to the data.

Results and Discussion

Physicochemical Parameters

The five brands examined complied with the compendial specification for uniformity of weight which states that for tablets having 80-250 mg weight (Table 2), not more than 2 tablets should differ from the average weight by more than 7.5% and none will deviate by 15% of average weight. It was observed that the mean weight of various brands varied widely, the difference was highly

significant (P<0.05), except for the AT1 and AT3. Generally, excessive weight variation is attributable to such factors as type of excipients, tooling of the compression machine, flow properties of the powder, improper die filling or presence of air in the powder or granular bed and inconsistent powder or granule density due to wide range of particle size.

Tablet hardness for all the brands ranged from 4.66 to 9.07kgf. A force of 4 kgf is the minimum requirement for a satisfactory tablet [30]. Hence the tablets of all brands were satisfactory for hardness. The knowledge of tablet hardness is useful in gauging the tablet's resistance to damage that might occur during production handling, packaging, and storage. It is also useful for quantifying the internal bonding strength of powder, which will help to achieve compatibility of formulation with performance specifications.

The tablet thickness ranged from 2.73 to 3.90mm. Tablets with uniform thickness indicates consistency in the compression process [30]. The wide differences might be attributed to differences in excipients employed by different manufacturers.

Table 2. Physicochemical parameters of the five different brands of atorvastatin tablets.

Brand Code	Average weight (mg)	% Deviation from Ave wt.	Hardness (kgf) ± SD	Thickness (mm) ± SD	Disintegration Time (minutes)	Chemical assay (%)
AT1	155	1.20	6.05±0.15	3.75±0.09	0.83±0.05	99.5
AT2	185	2.61	7.04±0.10	2.93±0.09	1.23±0.09	92.7
AT3	154	1.23	4.66±0.31	2.73±0.20	0.50±0.06	91.4
AT4	122	1.27	5.32±0.11	3.11±0.22	5.47±0.10	96.1
AT5	256	1.08	9.07±0.11	3.90±0.18	0.30±0.03	102.1

Disintegration time (Table 2) of all the brands was within limit. British Pharmacopeia [31] specifies that uncoated tablets should disintegrate within 15 min and film coated tablets in 30 min. All atorvastatin tablets were film coated and maximum time for disintegration was found 1.23min in case of brand AT2 while minimum AT5 disintegrated in 0.30 min. It should be noted that brand AT5 with hardness of 9.07kgf had the lowest disintegration time of 0.30 min. This indicates that apart from the hardness of tablet other parameters like micromeritics properties play important role in tablet disintegration.

Potency of all the brands was found within 91.4-102.1%. Atorvastatin is an International Nonproprietary Names (INN) drug, no official specification is available. But by comparing with the USP specification of another brand, simvastatin (potency limit: 90-110%), the potency of all the brands was within limit (Table 2).

Dissolution studies

Inter-brand variations in dissolution profiles were observed. Brands ATI, AT3 and AT5 released less than 50% drug within 15 minutes while brands AT2 and AT4 released 65.24% and 62.45% of atorvastatin within 15 minutes respectively (Figure 1). However, at 60minutes, all the brands released over 80% of the atorvastatin drug. From these data it is clear that although potency and disintegration times were almost similar within different brands, the brands differ in case of drug release. The differences in the patterns of release must have been caused by the manufacturer's choice of formulation method /design or manufacturing process most especially in the composition of excipient used causing alteration in drug performance. To this extent, manufacturing methods coupled with excipients used in the production processes, could contribute to the overall quality and release proficiency of medication. According to FDA's guides [32] for industry guides, two dissolution profiles are considered similar and bioequivalent, if $f1$ is between 0 and 15 and $f2$ is between 50 and 100.

From the results presented in table 3, it can be inferred that only AT3 and AT5 are bioequivalent to the reference drug, AT1.

Table 3. *f*1 and *f*2 values of the Atorvastatin brands.

Pair Comparison	*f*2	*f*1
AT2 vs AT1	42	12
AT3 vs AT1	74	3
AT4 vs AT1	47	10
AT5 vs AT1	56	7

In vivo Bioavailability studies

The parameters from the bioavailability study (Table 4 and Fig. 2) suggest that the test formulation AT2, AT3 and AT5 were bio-equivalent in terms of C_{max} and AUC to the reference formulation, AT1 based on the values obtained for C_{max} and T_{max} utilizing a 90% confidence interval at p value < 0.05. This was based on the regulatory bio-equivalent criteria range of 80 -125% interval of the FDA guidelines thus establishing bio-equivalence [33]. The four brands (AT1, AT2, AT3 and AT5) were bio-equivalent and can be substituted for each other in their prescription and use.

An *in vitro-in vivo* correlation (*IVIV*C) can impart *in vivo* meaning to the *in vitro* dissolution test and can be useful as surrogate for bioequivalence. AT3 had the highest *f*2 value and lowest *f*1 value with AUC and C_{max} being closest to the originator product thus AT3 was subjected to establish an *IVIV*C. A level A *IVIV*C between fraction dissolved (FD) and fraction absorbed) FA for the formulation was investigated using linear–nonlinear regression and depicted in Figure 3. Fairly good point-to-point relationship was observed for the formulation with regression coefficient of 0.9459 indicating a close correlation between the *in vitro* release rates with their *in vivo* absorption.

Table 4. Pharmacokinetic data for the five brands of Atorvastatin tablets

Atorvastatin	AUC ng/ml.hr	C_{max} ± S.D	T_{max} ± S.D
AT1	907.21 ± 104.32 (115.3 – 327.2)	42.8 ± 18.37 (20.3 – 39.9)	2.04 ± 1.32 (0.9-1.4)
AT2	777.85 ± 128.03 (194.1 – 428.6)	47.7 ± 16.89 (25.2 – 38.1)	2.02 ± 1.39 (1.1-2.1)
AT3	909.79 ±132.01 (166.2 – 397.8)	43.1± 20.09 (19.32 –28.1)	2.04 ± 1.31 (0.7-1.3)
AT4	738.33 ± 111.81 (111.9 – 308.2)	39.1 ± 19.66 (22.4-29.5)	2.05 ± 1.82(0.9 – 1.6)
AT5	858.55 ±139.27 (140.5 – 326.4)	44.0 ± 16.32 (25.7-36.4)	2.01 ±1.30 (1.3 – 1.9)

Values are expressed as mean ± SD (90% confidence interval)

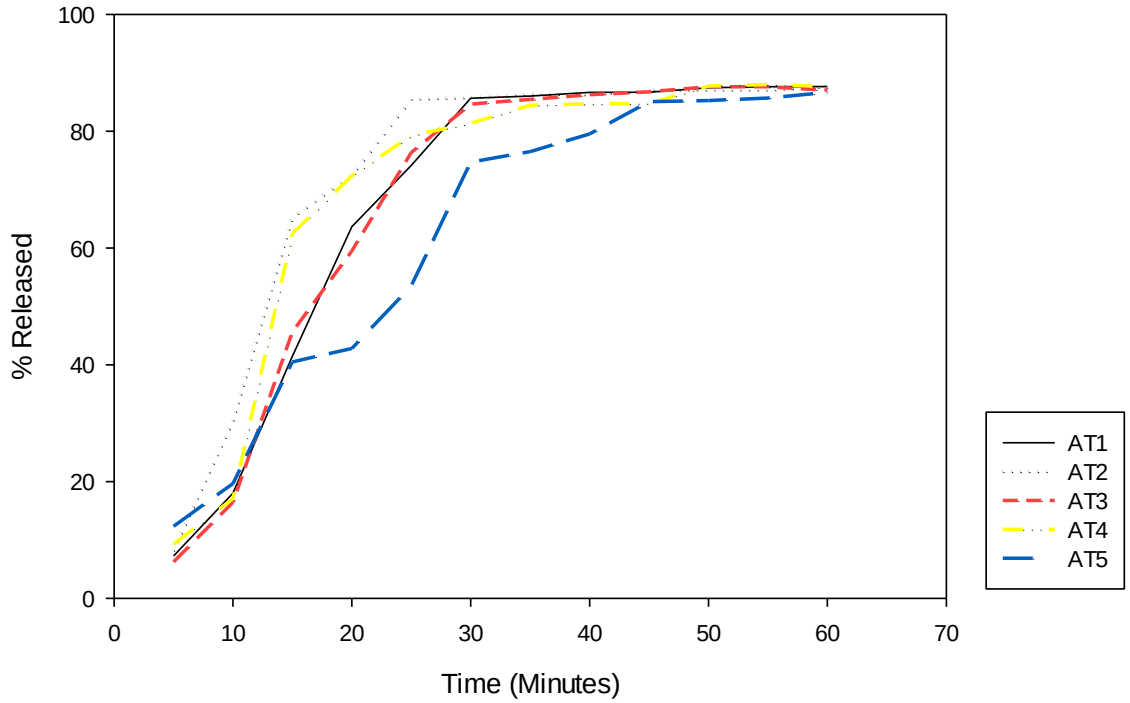

Fig. 1. Dissolution profiles of different brands (AT1-AT5) of atorvastatin tablets.

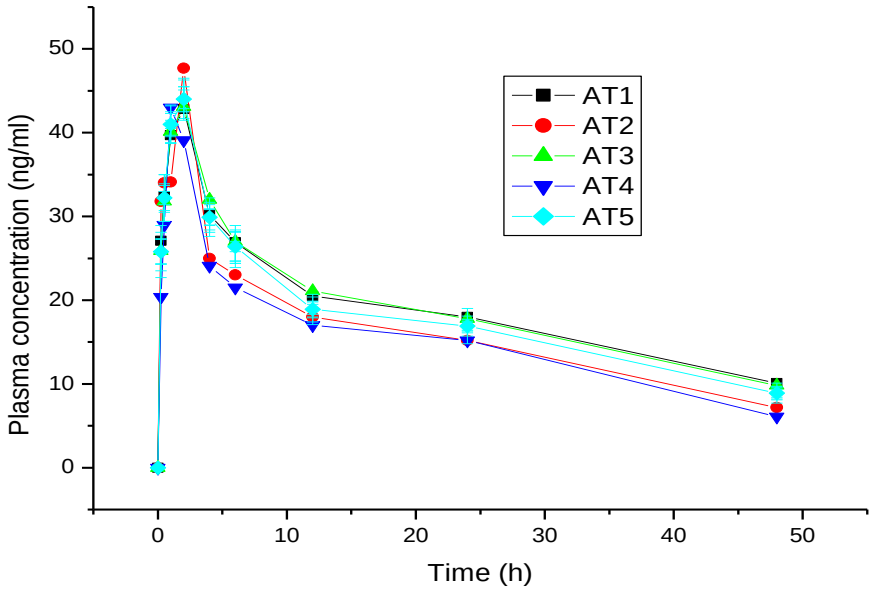

Fig. 2. Mean plasma concentrations vs. time profiles of the different brands (AT1-AT5) of atorvastatin tablets,

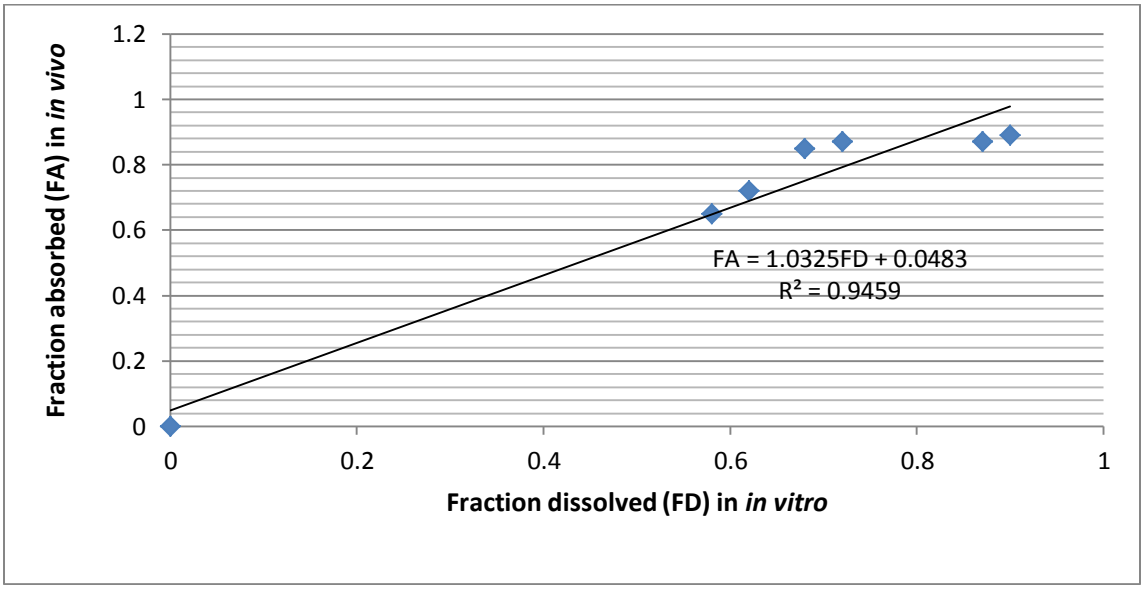

Fig. 3. Establishment of the level A *in vitro* - *in vivo* correlation (*IVIV*C).

Conclusions

The oral delivery of poorly soluble drugs like atorvastatin tablets is frequently associated with low bioavailability and high intra- and inters subject variability. This study has also emphasized that chemical equivalence does not indicate bioequivalence and one brand substituted on assumption of chemical equivalence with another brand may not give the desired onset of action and subsequent therapeutic effectiveness. *In vitro* dissolution test might not be enough to predict *in vivo* bioavailability and probably *in vivo* test may be required for final comments regarding the quality of marketed brands of atorvastatin.

Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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