IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 12, Issue 9 Series. I (September 2022), PP. 01-06
www.iosrphr.org

Chemical Stability of Compounded Atorvastatin Suspension Over a 5-week Period at Varied Temperature and Storage Conditions

Jace Swingle[1], Madison Grilliot[1], Julie Oestreich[1], Richard Dudley[1]
[1]*Department of Pharmaceutical Sciences, College of Pharmacy, University of Findlay, Findlay, OH, USA*
Received 08 September 2022; Accepted 25 September 2022

Abstract:
Background: Atorvastatin, an inhibitor of 3-hydroxy-3-methylglutarylcoenzyme-A (HMG-CoA) reductase, is widely prescribed in adult and pediatric patients for its potent cholesterol lowering effect. However, atorvastatin is only commercially available in tablet form. Therefore, pharmacists have compounded atorvastatin suspension making the medication available to more pediatric patients and select adults unable to take tablets. Limited reports on the preparation of atorvastatin suspension and lack of chemical stability data has relegated pharmacists to prepare a maximum 2-week supply of suspension per United States Pharmacopeia (USP) guidelines.
Materials and Methods: The present study examined the stability of compounded atorvastatin suspension (80 mg/5 mL) when stored in two different storage containers (plastic and glass) at two different temperatures (4°C and 25°C). Suspension was prepared according to a recipe provided from an area hospital and freely available online. Samples were analyzed weekly using a stability-indicating method on a reverse phase C18 column by high-performance liquid chromatography.
Results: Over the 5-week study, potency remained above 90% (range 93 to 106% across all storage conditions) with the exception of the 35-day refrigerated glass sample (85%).
Conclusion: Our data suggests that the chemical stability of atorvastatin, when compounded as a suspension using Ora-Plus and Ora-Sweet, is well within the USP guidelines–90% to 110% stated potency–for all tested temperatures regardless of the storage vessel for at least a 4-week period.
Key Word: Stability; Chemical stability; HPLC; Suspension; Physicochemical properties; US Pharmacopeia (USP)

I. INTRODUCTION

Clinicians routinely prescribe atorvastatin (Atv), (Figure no 1), for its lipid lowering effects. This agent provides benefit for both primary and secondary prevention of atherosclerotic cardiovascular disease including myocardial infarction and stroke. It is also indicated to reduce low-density lipoprotein (LDL) cholesterol levels in patients with familial hypercholesterolemia, including pediatric patients [1, 2]. Commercial availability of atorvastatin includes tablets of 10, 20, 40 and 80 mg allowing for convenient titration of oral doses and administration. However, a subset of patients, including pediatric patients, may experience difficulty in taking tablets of varying strength. Therefore, pharmacists routinely prepare oral suspensions from solid dosage forms to accommodate diverse patient populations.

At present, there is no standard method for the preparation of Atv suspension nor does a standard formulation or concentration exist. The lack of such important guidance may limit the use of Atv in many patients. Adding to the absenceof standardization in Atv suspension preparation is the significant knowledge gap in stability data relative to other extemporaneously prepared suspensions. To date, few studies describe compounded Atv suspension stability. Zaid and coworkers (2016) demonstrated that compounded atorvastatin suspension made from crushed tablets retained at least 97% of stated potency throughout a 30-day period regardless of temperature [3]. However, their complex formulation is not convenient for a compounding pharmacy, and the study was devoid of forced degradation conditions. A separate study conducted by Goel and coworkers (2013) examined the stability of Atv under a variety of degradation-inducing conditions and showed that Atv is susceptible to acid hydrolysis and slightly susceptible to degradation after exposure to an alkaline environment and oxidizing conditions [4]. Other studies have corroborated these findings [5-7]. Despite this informative literature, complicated formulations and variability in Atv concentrations create gaps in this knowledgebase for practical application. Additional questions, including the impact of the storage vessel (glass vs. plastic) as well as the influence of

storage temperature, remain unanswered. It is also unknown what impact common suspending vehicle(s) have on stability, in particular those with non-neutral pH's.

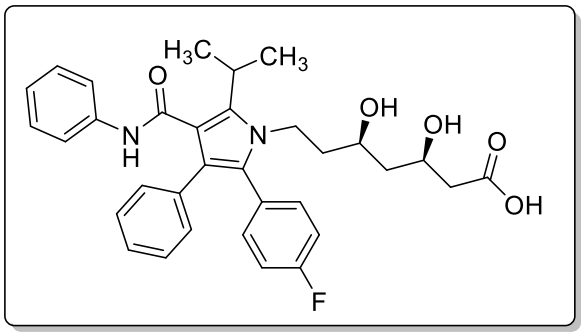

Figure no 1. Chemical Structure of Atorvastatin

The United States Pharmacopeia (USP) states that in the absence of robust stability data, all water-containing formulations for oral administration have a beyond-use date of no more than 14 days when stored at controlled cold temperatures [8]. The formula used to prepare atorvastatin suspension in the present study used a 1:1 mixture of Ora-Plus and Ora-Sweet as the suspending vehicle. This creates an aqueous environment placing it under this stipulation. To assess the stability of this preparation in variable storage conditions, we conducted a 35-day study using a novel stability-indicating HPLC method.

II. MATERIAL AND METHODS

Atorvastatin standard USP (Lot: LRAC0148) was purchased from Sigma (St. Louis, USA), and atorvastatin 80 mg tablets (Apotex Corp. Lot: PZ7272 Exp Date: March 2022), Ora-Plus (Lot: 9248004 Exp Date: May 31, 2021) and Ora-Sweet (Lot: 9237914 Exp Date: May 31, 2022) from a local pharmacy wholesaler using appropriate licenses. Acetonitrile (ACN) (HPLC grade) and trifluoroacetic acid (TFA) were purchased from Fisher Scientific. Water (18 Ω) was readily available in our lab.

Chromatographic Conditions

An Agilent 1100 HPLC system consisting of a binary pump, an autosampler, and a variable wavelength UV detector was used for chemical separation and detection. We collected data with Agilent ChemStation software. The column used was an Agilent Eclipse XDB C18 (4.6 x 150 mm, 3.5 µm). The mobile phase consisted of a mixture of deionized water and acetonitrile 40:60 (v/v). This isocratic mixture was pumped at a flow rate of 1 ml min^{-1} for a 10-minute run at a controlled temperature of 30°C. Detection wavelength for atorvastatin and degradation products as well as UV-active ingredients from the suspending and flavoring agents was set at 254 nm. The injection volume for each sample was 5 µL.

Preparation of Atorvastatin Suspension (16 mg/mL)

A standardized formulation for preparing atorvastatin suspension is lacking. However, protocols for the preparation of other statin suspensions have been developed by various health systems. For this study, atorvastatin suspension was prepared according to a protocol adapted for the preparation of pravastatin suspension [9]. Forty atorvastatin tablets (80 mg each) were placed in a mortar and pulverized using a pestle. Continuous trituration with pestle achieved maximum particle size reduction resulting in a fine powder. The resulting powder was wetted with a minimal amount of suspending vehicle to form a uniform paste. Then, the paste was geometrically diluted with suspending vehicle and brought to a final volume of 200 mL (final atorvastatin concentration = 16 mg mL^{-1}).

Atorvastatin Standard Curve

Prepared atorvastatin suspension (16 mg mL^{-1}) was used for standard curve calculations. Suspension was vigorously shaken, and 1 mL was removed and placed in a separate container. To each 1 mL sample, we added mobile phase to dilute suspension to the following concentrations for analysis: 0.5 mg mL^{-1},0.75 mg mL^{-1}, 1 mg mL^{-1}, and 1.25 mg mL^{-1}. Samples from the prepared dilutions were filtered through a 5-micron filter and placed in marked amber sample vials. Investigators then injected a sample volume of 5 µL using the aforementioned isocratic method. Linear regression analysis performed on triplicate samples and an R^2 value of 0.9958 suggested acceptable linearity. Concentrations of each atorvastatin sample obtained during the study phase were calculated using the equation y = 7553.1x – 258.73 where y = area under the curve (AUC) and x = sample concentration (mg / mL).

Atorvastatin Storage Conditions at room temperature and under refrigeration

After preparation, atorvastatin suspension was vigorously agitated and aliquoted into three separate plastic and three separate glass containers. The containers were labeled and placed in dark storage at controlled room temperature. Similarly, prepared atorvastatin suspension was vigorously agitated and aliquoted into three separate plastic and three separate glass containers for storage in a scientific refrigerator maintained at 4℃ for the duration of the study.

Sample Preparation and Analysis

At time zero, and on day 7, 14, 21, 28, and 35, we accurately measured 1 mL, after agitation, of the 16 mg mL^{-1} atorvastatin suspension from each tested storage container and temperature with a one mL oral syringe and transferred to a 20 mL sample vial. Given that a 30-day supply is usual and customary, the present study examined the degradation of Atv for up to 35 days. To each vial, we added fifteen mL of mobile phase to the vial and vortexed the mixture to ensure complete Atv extraction. Approximately 1.5 mL of the mixture was filtered and transferred to a labeled vial and loaded into the autosampler. Each sample from the study period was prepared in triplicate and each peak in the resulting chromatogram was compared to that of standards and subsequently identified based on retention time. We performed 2-way ANOVA, with Bonferroni post-hoc test, on time and temperature with alpha set at 0.05.

Forced Degradation Studies

To ensure adequate separation and detection of possible degradation products, atorvastatin suspension was subjected to forced degradation. Consistent with other studies and guidelines, suspension was combined with an equivalent volume of each of the following and incubated for 48 hours: hydrochloric acid (1M), sodium hydroxide (1M), or hydrogen peroxide (3%). Suspension was also placed in an oven and incubated at 100℃ for 48 hours [10].

III. RESULT

Atorvastatin suspension, tested each week for a total of 5 weeks, retained well above the 90% potency threshold required for stability as stipulated by USP in both glass and plastic containers at both room temperature and refrigeration (Table 1 and Table 2) with one exception. The week 5 refrigerated sample stored in glass reached the lowest level of potency measured during the entire study (85%). The 2-way ANOVA did not demonstrate statistically significant Atv degradation over time ($p = 0.16$ for glass and $p = 0.46$ for plastic) or with temperature ($p = 0.09$ for glass and $p = 0.20$ for plastic). In addition, there was no statistically significant interaction between time and temperature ($p = 0.38$ for glass and $p = 0.35$ for plastic).

A consistent separation was noted between the suspending agents and the active ingredient as illustrated in the time zero (0) chromatogram (Figure 2a). This level of separation was maintained throughout the entire 5-week period. No acid-induced degradation products were noted as a result of the suspending vehicle. Treatment with acid resulted in the formation of an atorvastatin degradation product with a corresponding peak retention time of 5.1 min as shown in Figure 2b. Treatment with base had little impact on the overall stability of atorvastatin (Figure 2c). Likewise, peroxide did not impact the overall stability of atorvastatin (Figure 2d). Heat led to a significant degradation of atorvastatin resulting in numerous chromatographic signals during the 10-minute run (data not shown).

Table no 1: Average stability (percent of labeled amount) and coefficient of variation of triplicate samples from glass and plastic storage containers over the 5-week sampling period at room temperature storage.

Atorvastatin Suspension Room Temperature - Glass	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**
Mean API (% of labeled amount)	101%	100%	106%	106%	97%	104%
Number of replicates	3	3	3	3	3	3
Coefficient of Variation	7.8%	7.7%	0.29%	4.4%	1.1%	1.2%

Atorvastatin Suspension Room Temperature - Plastic	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**
Mean API (% of labeled amount)	101%	101%	102%	100%	93%	96%
Number of replicates	3	3	3	3	3	3
Coefficient of Variation	7.8%	1.9%	1.1%	4.0%	2.1%	1.8%

Table no 2: Average stability (percent of labeled amount) of triplicate samples from glass and plastic storage containers over the 5-week sampling period at 4°C storage as well as calculated coefficient of variation.

Atorvastatin Suspension 4°C - Glass	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**
Mean API (% of labeled amount)	101%	96%	101%	102%	96%	***85%***
Number of replicates	3	3	3	3	3	3
Coefficient of Variation	7.8%	1.1%	1.8%	1.4%	1.6%	12%

Atorvastatin Suspension 4°C - Plastic	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**
Mean API (% of labeled amount)	101%	96%	97%	96%	94%	98%
Number of replicates	3	3	3	3	3	3
Coefficient of Variation	7.8%	4.3%	3.8%	2.3%	1.0%	2.0%

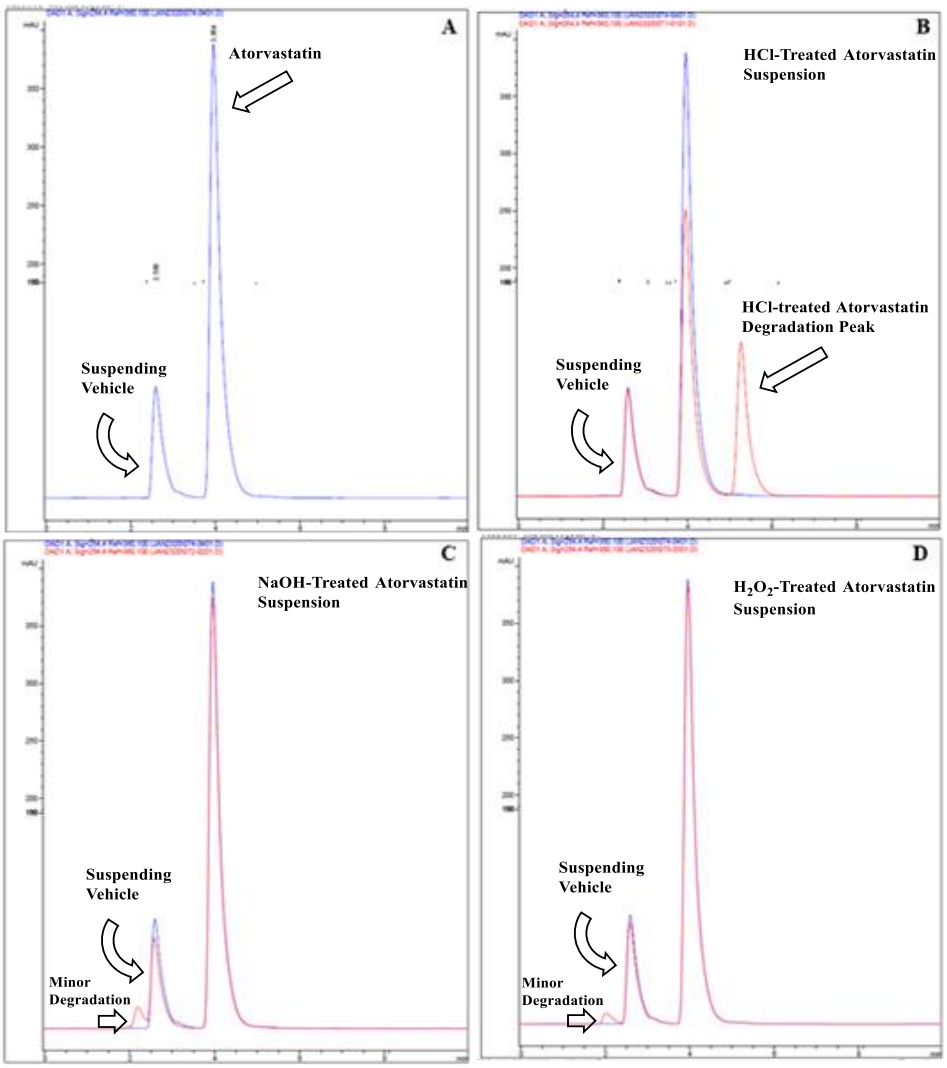

Figure no 2:Forced Degradation of Atorvastatin. (A) Time 0 Chromatogram. Using an equivalent amount of Oral-Plus and Ora-Sweet, atorvastatin had a consistent retention time of 3.9 minutes and the suspending vehicle mixture had a retention time of 2.5 minutes. Freshly prepared atorvastatin suspension was treated with an equivalent amount of 1 M HCl (B), 1 M NaOH (C), and 3% H_2O_2 (D) for 48 hours at room temperature. Treatment with acid (B) resulted in significant degradation of atorvastatin as demonstrated by a decrease in the parent molecule peak height and AUC relative to untreated samples (blue) and the appearance of a new degradation peak (red overlay) which is shown with a retention time of 5 minutes. Treatment with base (C) or peroxide (D) had little impact on the overall stability of atorvastatin.

IV. DISCUSSION

The present study evaluated the stability of atorvastatin suspension at two temperatures, stored in different containers over a period of up to 5 weeks. Our data suggests that the adapted formulation for preparing suspension resulted in a stable suspension for up to 4 weeks when stored in glass at both temperatures and up to 5 weeks when stored in plastic containers. Since a value below 90% stated potency is below the USP threshold, the week 5 refrigerated suspension in glass should be considered not stable and unsuitable for patient use. Interestingly, the suspension stored at the same temperature in plastic bottles reflected a potency of 99.1% at the same timepoint (week 5). While not impossible, it is difficult to explain such a significant drop in potency from the previous week for this sample (96% to 85% stated potency). One potential explanation is that adequate agitation of the suspension was not achieved for fear of cracking the glass bottle. Despite this outlier, atorvastatin remained remarkably stable at room temperature regardless of storage container composition for 5 weeks.

Our novel HPLC method provided optimal separation of Atv from chromophore-containing suspending agents as well as degradation products seen after forced degradation studies. When a sample of atorvastatin suspension was exposed to degrading environments, degradation peaks could be detected. The most caustic environment for atorvastatin was exposure to acid which induced a rapid degradation consistent with other published reports [4, 11]. The described HPLC method allowed for identification of the acid-degraded peak which eluted at approximately 5 minutes, well separated from the suspending vehicle and the parent drug (Figure 2b). Prior literature has suggested that alkaline and oxidizing conditions induce minor degradation of atorvastatin [4]. In our analysis, we observed similar results. Incubation of pure atorvastatin standard or the compounded suspension with 1 M NaOH or 3% H_2O_2 resulted in negligible degradation. Minor degradation peaks appeared much sooner in the chromatogram as compared to the acid degraded peak (Figure 2c and 2d) suggesting the formation of a more water-soluble product. Heat exposure led to near complete degradation, consistent with previous reports on the heat-labile nature of atorvastatin (data not shown) [4].

It is important to note that we also extracted vehicle (Ora-Plus and Ora-Sweet 1:1) alone to determine if the suspending or flavoring agent would interfere with atorvastatin identification or forced degradation peak identification. Indeed, the mixture of the suspending and flavoring agent had no impact on Atv peak identification, and base and peroxide-induced products did not interfere with identification of Ora-Plus/Ora-Sweet used in the preparation. Based on our ability to separate and detect forced degradation products, we concluded this new method is stability indicating.

Previous studies have demonstrated the acid-labile nature of atorvastatin [3, 4]. Interestingly, the 1:1 mixture of Ora-Plus and Ora-Sweet is slightly acidic (measured pH = 4) while the pH of the compounded atorvastatin suspension was less acidic with a measured pH of 5. Despite the pH of the suspension, no acid-induced degradation products were noted indicating that the citric acid used in Ora-Sweet and Ora-Plus has little impact on atorvastatin stability. Forced degradation afforded the ability to detect degradation products and ensure stability measurements were not adversely affected from their presence.

Our study on the stability of compounded atorvastatin has limitations. First, it is important to note that the adapted suspension becomes very thick and viscous under refrigeration, making adequate agitation for resuspension challenging. Inadequate shaking during the course of the week may have led to difficulty in proper distribution of atorvastatin and may explain the aberrant result at 5 weeks at 4°C. In clinical practice, atorvastatin is taken on a daily basis, and therefore patients prescribed atorvastatin suspension would be instructed to shake the bottle every day thus preventing any profound settling. Plastic bottles are less expensive and much easier to agitate for resuspending compared to the glass containers. As our results indicated no statistically significant difference between glass and plastic containers on chemical stability, we recommend plastic based on ease of resuspension.

The present study examined the impact of temperature and storage container on the stability of Atv suspension. Atorvastatin suspension was prepared with common ingredients and a simple formula adapted from local health systems to a final concentration of 16 mg mL^{-1}. Each sample retained well above 90% potency throughout the study period regardless of temperature or container with the exception of the 5-week refrigerated glass sample. The process of preparation was straightforward and did not require sophisticated compounding instrumentation. The use of relatively inexpensive Ora-Plus and Ora-Sweet as suspending vehicles precludes the need for more elaborate and expensive agents available in many compounding pharmacies. The novel forced-degradation aspect of our study ensures proper identification of atorvastatin as well as atorvastatin degradation products that could arise from acid, base, temperature, or peroxide exposure.

V. CONCLUSION

In conclusion, this study outlines a novel HPLC method capable of optimal separation of Atv from its degradation products and suspending vehicles. To date, few studies have reported stability data on atorvastatin suspension. Our stability-indicating study adds to the literature and supports that a simple atorvastatin suspension, compounded as described, retains chemical stability for at least 28 days and perhaps longer. Additional studies to

corroborate our results may further afford confidence in preparing a usual and customary 30-day supply of atorvastatin suspension providing both a time and cost-savings benefit for both the patient and the pharmacy.

REFERENCES

[1]. Lexicomp Online L-DO: Wolters Kluwer Health, Inc. Atorvastatin 2021. Available at: http://online.lexi.com/lco/action/doc/retrieve/docid/patch_f/6396?cesid=5X8q1ar9aaE&searchUrl=%2Flco%2Faction%2Fsearch%3Fq%3Datrovastatin%26t%3Dname%26va%3Datrovastatin. Accessed February 15, 2021.

[2]. Rollins DE, Blumenthal DK. Drug Therapy of Dyslipidemias. Workbook and Casebook for Goodman and Gilman's The Pharmacological Basis of Therapeutics. New York, NY: McGraw-Hill Education; 2016:948-53.

[3]. Zaid AN, Assali M, Zalmout S, Basheer A. Compounding and stability evaluation of atorvastatin extemporaneous oral suspension using tablets or pure powder. Eur J Hosp Pharm. 2017;24(3):157-61.

[4]. Goel A, Baboota S, Sahni JK, Srinivas KS, Gupta RS, Gupta A, et al. Development and validation of stability-indicating assay method by UPLC for a fixed dose combination of atorvastatin and ezetimibe. J Chromatogr Sci. 2013;51(3):222-8.

[5]. Oliveira MA, Yoshida MI, Belinelo VJ, Valotto RS. Degradation kinetics of atorvastatin under stress conditions and chemical analysis by HPLC. Molecules. 2013;18(2):1447-56.

[6]. Ramesh D, Habibuddin M. Stability indicating RP-HPLC method for the simultaneous determination of atorvastatin calcium, metformin hydrochloride, and glimepiride in bulk and combined tablet dosage form. Int Sch Res Notices. 2014;754695.

[7]. Shah RP, Kumar V, Singh S. Liquid chromatography/mass spectrometric studies on atorvastatin and its stress degradation products. Rapid Commun Mass Spectrom. 2008;22(5):613-22.

[8]. United States Pharmacopeia and National Formulary (USP 30-NF25) Rockville, MD 2007;1(27th ed.).

[9]. Nationwide Children's Hospital. Pravastatin Oral Suspension 10mg/mL 2010. Available at: https://www.nationwidechildrens.org/-/media/nch/specialties/pharmacy/compounding-formulas/pravastatin-oral.ashx. Accessed January 6, 2020.

[10]. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs-A review. J Pharm Anal. 2014;4(3):159-65.

[11]. Kumar SA, Debnath M, Rao JV, Sankar DG. New validated stability-indicating RP-HPLC method for simultaneous estimation of atorvastatin and ezetimibe in human plasma by using PDA detector. Adv Pharm Bull. 2015;5(3):385-91.

Jace Swingle, et. al. “Chemical Stability of Compounded Atorvastatin Suspension Over a 5-week Period at Varied Temperature and Storage Conditions.” *IOSR Journal of Pharmacy (IOSRPHR),* 12(9), 2022, pp. 01-06.