Stability of Oseltamivir Phosphate in SyrSpend SF, Cherry Syrup, and SyrSpend SF (For Reconstitution)

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ABSTRACT

Oseltamivir (Tamiflu) is widely prescribed to treat and prevent influenza virus A, influenza virus B, and H1N1 Flu. It is manufactured by Roche in 75-mg capsules. The need for other administration options for patients who cannot take capsules and cannot use suspending agents which contain alcohol, sorbitol, or preservatives has led to the compounding of oseltamivir into three suspending vehicles that do not contain these chemicals. All three contain neither alcohol nor sorbitol; the third also has no preservatives, a distinct advantage for special patient situations. The objective of this study was to determine the stability of oseltamivir in three different suspending agents: SyrSpend SF, Cherry Syrup, and SyrSpend SF (for reconstitution). The studied samples were compounded into 15-mg/mL suspensions and stored in low actinic plastic prescription bottles at temperatures between 2°C and 8°C. Triplicate samples were assayed at time intervals out to 30 days by a stability-indicating high-performance liquid chromatography method. The method was validated for its specificity through forced degradation studies. All of the samples tested remained within 90% to 110% of their initial concentrations throughout the course of the study. The shelf life of these products is at least 30 days, based on data collected when the aforementioned products are refrigerated and protected from light. Based on the final potency data at day 30, the shelf life may extend past the scope of this study, but 30 days was the limit of this study.

INTRODUCTION

H1N1 flu is a major health concern in the U.S. The sad fact is hundreds of pediatric deaths associated with 2009 pandemic Influenza A H1N1) virus have been reported regularly by the Centers for Disease Control and Prevention (CDC) since April 26, 2009.[1] The situation has become so serious that the CDC now devotes a website to H1N1.[2] Oseltamivir (Tamiflu; manufactured by Roche, Nutley, New Jersey) is an important treatment for this disease, especially for debilitated patients and very young children.

The pandemic created high demand for oseltamivir, resulting in shortages of Tamiflu, particularly of the oral liquid dose form. When shortages of the liquid dose form occur, pharmacists compound oral liquids extemporaneously from Tamiflu capsules. Some compounding vehicles contain sorbitol and alcohol.

Administration of alcohol-containing substances to children, especially infants, has been a concern,[3] and the inclusion of sorbitol in a vehicle for elderly, debilitated, or pediatric patients can pose complications for diet and therapy because introducing high osmolality can create loose stools and diarrhea, leading to dehydration. In fact, a patient with H1N1 may be experiencing diarrhea and dehydration before oseltamivir treatment is begun. High osmolality preparations would exacerbate this condition. Therefore, a suitable vehicle containing neither ethanol nor sorbitol was sought. Pharmacists need access to additional vehicles with documented stability studies involving Tamiflu.

This study used three recognized vehicles:

1. Cherry Syrup (Gallipot, Inc., St. Paul, Minnesota) has a long history as an alcohol-free syrup vehicle; its wide use made it a good candidate for the study.
2. SyrSpend SF (Gallipot, Inc.) is a hydrolyzed starch-based suspending/syrup vehicle. Suspending and sugar-free flavoring components are both combined in one bottle for ease of use. SyrSpend SF provides a suspending vehicle with neither sorbitol (therefore, minimal osmolality issues) nor alcohol. Moreover, it contains no dyes and, in the unflavored version, contains no flavoring agents.
3. SyrSpend SF (for reconstitution) (Gallipot, Inc.) contains neither ethanol nor sorbitol and also contains no preservatives, coloring agents, nor flavoring agents (in the unflavored version.)

Due to ongoing demands for extemporaneous compounding of Tamiflu suspension and shortages of some vehicles in the fall of 2009, stability studies were undertaken using Tamiflu in Cherry Syrup and in SyrSpend SF, both readily available vehicles. The studies were performed by DynaLabs, an independent analytical laboratory in St. Louis, Missouri.

MATERIALS AND METHODS

Chemical Reagents

Oseltamivir phosphate in capsule form (Lot U2082; Tamiflu) was generously provided by Gallipot, Inc. and Bellevue Pharmacy Solutions (St. Louis, Missouri). High-performance liquid chromatographic (HPLC)-grade acetonitrile (Lot CZ629; Burdick & Jackson, Kalamazoo, Michigan), triethylamine (Lot 47270751; EMD, Gibbstown, New Jersey), formic acid (Lot A0266198; Acros Organics, Fair

Lawn, New Jersey), and ammonium formate (Lot 0001422904; Fluka, Milwaukee, Wisconsin) were used in the study. HPLC-grade water was supplied by filtering deionized water from a Millipore Elix through a Millipore Simplicity (Billerica, Massachusetts).

Equipment and Chromatographic Conditions

The HPLC instrument was a Varian Prostar (Palo Alto, California) equipped with a model 230 tertiary gradient solvent delivery system, a model 335 photodiode array (PDA) detector, and a model number 410 programmable autosampler fitted with a 100-mcL sample injection loop and a 250-mcL syringe. The HPLC was operated and data was quantitated using Galaxie software from Varian. The mobile phase for the system was 20 mM ammonium formate pH 3.3 with formic acid, triethylamine, acetonitrile (500:2.8:500) and was delivered at 1.5 mL/minute. Chromatographic separation was achieved using a 150 mm × 4.6 mm Phenomenex (Torrance, California) Gemini C18 column with 5-mcm particle packing. The mobile phase was used as solvent in diluting the standard and assay preparations to 100 mcg/mL. Assay and standard preparations were monitored at 254 nm following 100-mcL full sample loop injections.

Validation of Forced-Degradation Studies to Determine Stability-Indicating Characteristics of the HPLC Method

Oseltamivir samples were stressed and assayed to determine the sensitivity of the HPLC method regarding the analyte of interest and any possible degradant or impurities. Oseltamivir, in capsule form, was diluted to 100 mcg/mL in solutions of base (0.05 N NaOH), acid (0.05 N HCl), and hydrogen peroxide (35%), in addition to exposure to ultraviolet (UV) light at 365 nm and heat at 120°C. Time under these stressors varied due to the relative stability of oseltamivir to each individual condition. Additional peaks found in the chromatograms were labeled and the resolution was determined (based on *United States Pharmacopeia (USP)* guidelines) between the degradant and the oseltamivir; a resolution of 1.5 was considered full separation.

Preparation of Oseltamivir Suspension Samples

The compounding of the samples was based on a study by Winiarski et al.[4] SyrSpend SF (for reconstitution) was reconstituted with 60 mL of HPLC-grade water. The three oseltamivir suspensions (SyrSpend SF, Cherry Syrup, and SyrSpend SF [for reconstitution]) were all compounded in the same manner. Forty Tamiflu capsules were weighed in bulk, opened, their contents poured into a low actinic plastic prescription bottle, and set aside. The empty capsules were cleaned of any residual powder by reaming with cotton swabs and weighed in bulk. From these weights the average capsule fill weight was determined to be 165.28 mg, of which 75 mg (from the label claim), or 45.3775%, was oseltamivir base (as the phosphate). The capsules were then discarded. Approximately 826.4 mg powder was carefully added to a ceramic mortar and ground until a fine uniform particle size was achieved. A 10-mL volume of the appropriate suspending vehicle was added to the mortar and, after thorough mixing, the suspension was poured into a suitable low actinic volumetric flask. Two 5-mL aliquots of suspending vehicle were added to wash thoroughly any residual powder out of the mortar and into the flask. The flask content was brought to volume (25 mL) with suspending vehicle, thoroughly mixed, and were poured into a low actinic plastic prescription bottle for storage at *USP*-controlled refrigerated temperature (2°C to 8°C).

Stability Study

Three different samples were submitted for stability: (1) oseltamivir 15-mg/mL suspension in Gallipot, Inc. Cherry Syrup (Lot 0909326J11), (2) oseltamivir 15-mg/mL suspension in Gallipot, Inc. SyrSpend SF (Lot 0909135J12), and (3) oseltamivir 15-mg/mL suspension in Gallipot, Inc. SyrSpend SF (for reconstitution) (Lot 0812299). The samples were packaged in 60-mL low actinic plastic prescription bottles, each containing 25 mL of suspension, and stored at *USP*-controlled refrigerated temperature (2°C to 8°C) using a digitally controlled laboratory refrigerator from Forma Scientific (Edison, New Jersey). Time points for the study were initial (T=0), 3 days (T=3), 7 days (T=7), 14 days (T=14), and 30 days (T=30). The evaluation parameters were appearance and percent recovery assays. Appearance was evaluated by visual inspection of both the suspension and the container. The acceptance criteria for the Cherry Syrup suspension were the retention of the pinkish-red color, the homogeneity of the suspension, and the intact container closure system. The acceptance criteria for the SyrSpend SF and the SyrSpend SF (for reconstitution) were the retention of the opaque color, the homogeneity of the suspension, and the intact container closure system. The stability of oseltamivir in each of the three suspensions was defined by the percent recovery in respect to T=0 using the validated HPLC method. The samples were prepared in triplicate by adding 0.5 mL of suspension with a volumetric pipette to 75 mL mobile phase for a 1:151 total dilution and calculating the averages and standard deviations for all replicate injections.

RESULTS

The stability of oseltamivir in Cherry Syrup, SyrSpend SF (for reconstitution), and SyrSpend SF are shown in Tables 1, 2, and 3, respectively. The results of 14.42 mg/mL for Cherry Syrup, 14.83 mg/mL for SyrSpend SF (for reconstitution), and 14.11 mg/mL for SyrSpend SF at T=0 were set as the initial concentration of the study, and all subsequent time points were compared to these values. Figures 1, 2, and 3 show the data in terms of concentration and show that all three suspensions remained within specifications (90% < [oseltamivir] < 110%) throughout the duration of the study.

DISCUSSION

The HPLC method was shown to be stability indicating by forcibly degrading oseltamivir and separating the degradant peaks from that of the main analyte. Oseltamivir was relatively stable to heat, UV light, and acid; however, base and oxidizer created an initial degradant peak in 30 minutes. The chromatograms of the base (0.05M NaOH), oxidizer (35% H_2O_2), and standard are shown in Figure 4. Figure 5 shows the PDA purity channel of the oseltamivir peak for the base, oxidizer, and standard as well. Table 4 shows the purity calculations Galaxie performed on the oseltamivir peak for all three chromatograms listed. Additionally, validation parameters listed in Table 5 show that all system suitability results met acceptance criteria.

Cherry Syrup (Lot 0909326J11)

Table 1 and Figure 1 show the stability data for an oseltamivir Cherry Syrup suspension stored refrigerated and light protected for 30 days. The sample's potency began at 14.42 mg/mL which was 96.13% of the compounding target of 15 mg/mL. This value was set as the baseline for all the subsequent time points. The

sample's potency increased slowly for the first 14 days, reaching a maximum at 15.12 mg/mL, which was 100.8% of the compounding target and 104.9% recovery of the T=0 concentration. The next time point, T=30, gave a 5.5% decrease in potency from T=14; however, this was still 99.4% of the compounding target. No other time points were tested between T=14 and T=30, therefore, no current estimation of the time in which the sample would become subpotent could be hypothesized; however, the data did start to take on a parabolic shape. The sample showed no discoloration, and no degradant peaks were seen in any time point's chromatograms.

TABLE 1. Stability of Oseltamivir in Gallipot, Inc. Cherry Syrup Refrigerated (2°C to 8°C) for 30 Days.

Elapsed Time	Appearance	Recovery (%)
T=0	Pinkish Red Homogenous Container Closure Intact	100.0
T=3	Pinkish Red Homogenous Container Closure Intact	101.7
T=7	Pinkish Red Homogenous Container Closure Intact	103.3
T=14	Pinkish Red Homogenous Container Closure Intact	104.9
T=30	Pinkish Red Homogenous Container Closure Intact	99.4

TABLE 2. Stability of Oseltamivir in Gallipot, Inc. SyrSpend SF (For Reconstitution) Refrigerated (2°C to 8°C) for 30 Days.

Elapsed Time	Appearance	Recovery (%)
T=0	Opaque Homogenous Container Closure Intact	100.0
T=3	Opaque Homogenous Container Closure Intact	98.4
T=7	Opaque Homogenous Container Closure Intact	100.6
T=14	Opaque Homogenous Container Closure Intact	100.1
T=30	Opaque	99.7

TABLE 3. Stability of Oseltamivir in Gallipot, Inc. SyrSpend SF Refrigerated (2°C to 8°C) for 30 Days.

Elapsed Time	Appearance	Recovery (%)
T=0	Opaque Homogenous Container Closure Intact	100.0
T=3	Opaque Homogenous Container Closure Intact	102.6
T=7	Opaque Homogenous Container Closure Intact	104.4
T=14	Opaque Homogenous Container Closure Intact	105.8
T=30	Opaque Homogenous Container Closure Intact	102.6

SyrSpend SF (For Reconstitution) (Lot 0812299)

The initial potency for the oseltamivir SyrSpend SF (for reconstitution) suspension was 14.83 mg/mL, which is shown in Figure 2. This concentration was 98.7% of the compounding target of 15 mg/mL. The accurate compounding of this preparation was related to the reconstituted solution being less dense than the other suspensions tested, therefore measurements were easier. The T=0 result was set as the baseline for all other time points tested. The assay results varied up and down with no general discernible trend. The lowest result was 14.60 mg/mL, which was 97.3% of the compounding target and 98.4% of the baseline value. The percent recoveries for all of the time points can be seen in Table 2. The T=30 assay result had a 99.7% recovery which shows the oseltamivir was stable throughout the entire study. Again, the color, homogeneity, and chromatograms showed no signs of instability.

SyrSpend SF (Lot 0909135J12)

The initial potency for the oseltamivir SyrSpend SF suspension was 14.11 mg/mL, which is shown in Figure 3. Of all three suspensions tested, this was the least accurately compounded preparation, being 94.1% the compounding target of 15 mg/mL. The T=0 assay result was set as the baseline for all other time points. The assay results followed the same trend as the Cherry Syrup previously mentioned. The concentration slowly increased to a maximum of 14.93 mg/mL at T=14 and lost 3% potency from T=14 to T=30. With a percent recovery of 102.6% at T=30, the assay result was still higher than T=0. The data,

TABLE 4. Purity Percentages and Purity Values for Coordinates of Oseltamivir Peak.

Sample	Standard	Base Stressed	Oxidizer Stressed
Area Impure [%]	0.386	0.807	0.672
Area Medium [%]	0.432	0.425	0.240
Area Pure [%]	99.182	98.768	99.088
Purity [APEX]	1000.000	1000.000	1000.000
Purity [FBL]	632.483	719.950	449.828
Purity [FH]	999.689	999.297	999.007
Purity [FL]	998.636	994.276	995.759
Purity [RBL]	632.566	719.856	449.851
Purity [RH]	999.536	997.747	998.827
Purity [RL]	997.046	998.763	996.474

APEX = apex of peak
FBL = front baseline
FH = front (high)
FL = front (low)
RBL = rear baseline
RH = rear (high)
RL = rear (low)

FIGURE 1. Plot of Oseltamivir Concentration in Cherry Syrup Suspension

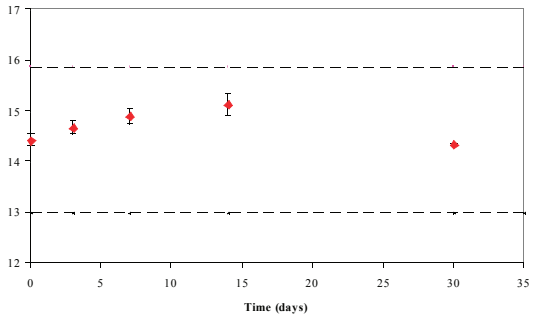
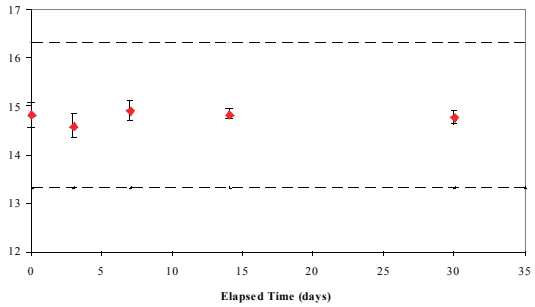


FIGURE 2. Plot of Oseltamivir Concentration in SyrSpend SF (For Reconstitution) Suspension

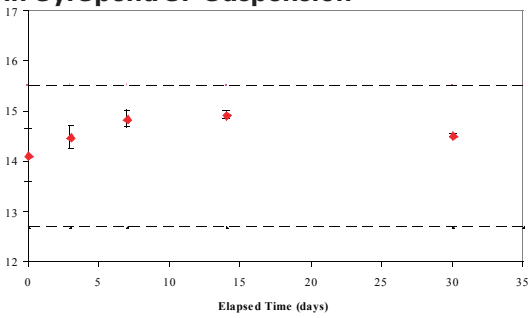


much like what was seen in the cherry syrup, began to take on a parabolic shape. Throughout the study, the sample retained its opaque appearance and no degradants appeared in the chromatograms.

CONCLUSION

This study showed that oseltamivir (as the phosphate) compounded from Tamiflu capsules into sorbitol-free and alcohol-free suspensions is stable for 30 days when stored in low actinic plastic prescription bottles and refrigerated between 2°C and 8°C. No sample fell below 98.4% of the initial concentration throughout the 30 days. Two of the samples tested, the Cherry Syrup and the SyrSpend SF, appeared to have a parabolic assay shape with slight increases in concentration between T=0 and T=14 and then decreasing to T=30. Extrapolation of the data to future time points is challenging due to the lack of time points between 14 days and 30 days; however, the data suggest that the suspensions would be stable past the 30 days; however, this study was limited to 30 days.

FIGURE 3. Plot of Oseltamivir Concentration in SyrSpend SF Suspension



(The dashed lines in Figures 1, 2, and 3, represent upper and lower limits of oseltamivir specifications.)

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TABLE 5. Summary of the Validation Parameters for the High-Performance Liquid Chromatographic Method Used in the Stability Study of Oseltamivir in Gallipot, Inc. Suspension Vehicles.

Validation Parameter	Results
Peak Tailing	1.39 % RSD = 0.54
Theoretical Plates	3758.8 % RSD = 1.35
Linear Range (254 nm)	50 to 200 mcg/mL R^2 = 0.9994
Extraction Precision (Cherry) *n* = 6	% RSD = 1.59
Extraction Precision (SyrSpend SF (For Reconstitution) *n* = 6	% RSD = 1.54
Extraction Precision (SyrSpend SF) *n* = 6	% RSD = 0.37
Accuracy (70.53, 122.88, 199.38 mcg/mL, respectively)	% Target = 103.7%, 100.5%, 100.6%
Ruggedness (2 days, 2 analysts, 2 instruments)	% RSD = 0.98 % Target = 100.5%
Robustness (10% change in % organic and pH)	% RSD = 3.1 % Target = 97.8%
Specificity (Resolution between main degradant peaks)	RT = 2.00 Res (*USP*) = 11.71 RT = 5.12 Res (*USP*) = 3.0

RSD = relative standard deviation, SF = sugar free, *USP* = *United States Pharmacopeia*