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Stability study of oseltamivir extemporaneous suspension

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Abstract

Oseltamivir phosphate (OSEL) is prescribed to manage influenza virus A, influenza virus B, and H1N1. OSEL capsule (75mg) was manufactured by GPO, Thailand under the trade name of A-Flu™. Some patients such as children cannot take oral solid dosage forms and need oral liquid preparations. In those cases, pharmacists prepare OSEL extemporaneous suspensions (OSEL ES). However the stability of OSEL ES in a tropical country such as Thailand has not been investigated. Then the stability of OSEL ES used in Queen Sirikit National Institute of Child Health (QSNICH), Bangkok Thailand was investigated. Solution of 10 mg/ml OSEL ES were compounded and stored in three different temperature conditions; room temperature, refrigerated temperature (7 ± 3 °C) and accelerated temperature (45 ± 5 °C). Triplicate samples were periodically taken for 12 weeks and assayed by high-performance liquid chromatography. All of the samples stored under room temperature and refrigerated conditions showed more than 90% of their initial concentrations of OSEL remained throughout the course of the study. For the accelerated temperature 62.91% of the initial concentration of OSEL remained at 12th week, which was significantly lower than the other conditions ($p < 0.05$). In addition, samples stored at 45 ± 5 °C showed changes in color and reductions in pH over the course of the experiment. In conclusion temperature above room temperature should be included for stability testing of extemporaneously prepared suspensions in tropical climates.

Keywords: oseltamivir phosphate, extemporaneous suspension, physicochemical tests, stability test

1. Introduction

During the last 400 years, there has been an influenza epidemic approximately every 3 years and a pandemic about three times every century. In recent times, the Spanish Influenza of 1918-1919 is best remembered, which resulted in a lethality of about 0.5% and up to thirty million deaths worldwide [1]. This unfavorable behavior can be explained by frequent mutation of the viral genome and possible assortment with genetic material from related viruses in animal hosts, which enables evasion of the host immune response [2]. OSEL phosphate is a drug used in the treatment of H1N1 influenza virus. OSEL is manufactured by the Government Pharmaceutical Organization (GPO) in Thailand under the trade name of A-Flu™. In pandemic periods, the demand for OSEL can be high and storages of GPO-A-Flu™ can happened. Limited availability of oral liquid dosage form is another problem which has resulted in preparation of OSEL ES in hospitals. Extemporaneous oral suspensions are usually prepared by dispersing the drug powder in a mixture of water and syrup. The drug powder is obtained from crushing tablets or opening capsules. However, these extemporaneous oral liquid solutions have some problems for diabetes patients owing to having syrup and preservative in the formulation. In Thailand in 2017, at the Queen Sirikit National Institute of Child Health (QSNICH), 8688 30 ml bottles of 10 mg/ml OSEL ES were made by compounding the drug powder from GPO-A-Flu™ capsules with prepared vehicles (syrup and citric acid) [3]. Daytime temperatures in Thailand range from 33-34ºC (91-93ºF) in March raising to 35-

37ºC (95-99ºF) between March and May with some daily maximums exceeding 40ºC (104ºF) [4]. However the stability of OSEL ES in a tropical country such as Thailand has not been examined. Therefor the stability of OSEL ES prepared according to the specification QSNICH, Bangkok, Thailand were investigated.

2. Materials and methods

2.1 Chemicals and Reagents

OSEL capsules (75mg GPO-A-Flu™, Lot No. K580184) were supplied from pharmacy department, Queen Sirikit National Institute of Child Health (QSNICH), Bongkok, Thailand. Capsule were sealed at room temperature. Acetonitrile (Lab-scan, Thailand), methanol (Lab-scan, Pathumwan, Thailand), citric acid anhydrous (Lot no: AA-1710-30731, S. Tong Chemicals Co. Ltd, Thailand), glycerin 99.50 % USP (Lot BGPXWC1126, MANUFACTURER/SUPPLIER, Thailand), Sala cider flavor (SDF 998, Lot no: M600256, MANUFACTURER/SUPPLIER, Thailand), sodium benzoate (Sigma-Aldrich, China), and sucrose (Mitr Phol®, Thailand) were used in this study.

2.2 Instruments

Automatic balance (Model PL-402-L, Mettle Toledo, Switzerland), brookfield viscometer (Model DV-III programmable rheometer, LabX, USA), filtration (40/38 Favoirt®, China), high performance liquid chromatography (HPLC Auto: Agilent 1100 series, Japan), hot air oven (HW-HP01, Thailand), magnetic stirrer (MANUFACTURER/SUPPLIER, Taiwan), micropipette (MANUFACTURER/SUPPLIER, Thailand), refrigerated centrifuge (Model 6200-Kubota, Kubota Laboratory Centrifuges, Japan), thermometer (PKC Instruments, Thailand), UV-spectrophotometer (Model UV1201 series, Shimadzu, Japan), Vortex mixer (Model S0100-220, National Labnet Co. Inc, USA) were used in this study.

2.3 pH determination of suspension

The pH of all developed formulations was measured using digital pH Meter (Ezedo/PL600, Taiwan). Each experiment was performed in triplicate.

2.4 Method of analysis by UV–VIS spectrophotometer (Standard curve)

OSEL Phosphate 75 mg was dissolved with distilled water and make up to 25 ml with distilled water. Five concentrations of working solution were prepared by diluting 1, 2, 3, 4 and 5 ml of standard solution with distilled water to 10 ml. The resulting five concentrations were measured spectrophotometrically at 230 nm using a UV–VIS spectrophotometer.

2.5 Method of analysis by HPLC

Method: HPLC wavelength 230 nm solvent delivery module was used for method development and validation. Column: C18 (4.6 x 250 mm) column with a mobile phase of phosphate buffer (pH 6; 0.05 M), methanol and acetonitrile (570:255:175, v/v) was used at a flow rate of 1.0 mL/min, injection volume: 20 µl at room temperature, [5]. Standard solution of OSEL was prepared by dissolving 75 mg of drug with distilled water and made up to volume with distilled water. And then, the five concentrations of working solution were prepared by diluting 1, 2, 3, 4, 5 ml of standard solution with distilled water 1.5 ml of OSEL solution:mobile phase (500µl:500 µl) were kept in micro tube size and mixed by centrifuge (10.000 rpm for 20 min). The resulting solutions were kept in HPLC vial for analysis. For analysis of drug suspension, OSEL suspension (10 mg/ml): distilled water (1:9) was prepared by mixing 50µl:450µl of drug suspension and distilled water respectively. And then, 1.5 ml of OSEL suspension:DI (500µl:500µl) were kept in micro tube size and mixed by centrifuge (10.000 rpm for 20 min). The resulting solutions were kept in high performance liquid chromatography (HPLC) vial for analysis.

2.6 Production target

The QSNICH master formulations OSEL phosphate suspension, vehicle syrup are shown in Tables 1, 2 and 3, respectively.

Table 1 Formulation of OSEL phosphate suspension 10 mg/ml by QSNICH [6]

Ingredient	Master formula	Function
OSEL phosphate (g)	0.3	API
vehicle qs.to (ml)	30	vehicle

Table 2 Vehicle for OSEL phosphate by QSNICH [6]

Ingredient	Master formula	Function
citric acid (g)	10	chelating agents
distilled water (ml)	200	vehicle
sala cider (ml)	0.06	flavoring agent
syrup qs.to (ml)	10,000	sweetening agent

Table 3 Syrup for vehicle by QSNICH [6]

Ingredient	Master formula	Function
glycerin (g)	0.1	wetting agent
Sodium benzoate (g)	0.1	preservative
sucrose (g)	80	sweetening agent
distilled water qs.to (ml)	100	vehicle

2.7 Statistical analysis of results

All data are expressed as mean ± standard deviation (SD). The statistical analysis was evaluated using two-way analysis of variance (ANOVA) with Bonferroni post-tests were performed to determine the differences among groups. The significance of difference was determined at 95% confident interval ($p < 0.05$).

2.7.1 Preparation of OSEL suspension (10 mg/ml)

2.7.1.1 Formulation of OSEL suspension (10 mg/ml) QSNICH

Formulation of OSEL suspension (10 mg/ml) according to QSNICH specifications. About 50 ml of purified water was added to the process vessel. Sucrose was added with stirring. Sodium benzoate was dissolved with the syrup in a water bath at a temperature of 100 °C for 15 min and the volume adjusted to 100 ml with purified water. OSEL phosphate 7200 mg (powder) was mixed with 20 ml premixed glycerin and vehicle for 20 min. This was added to the bulk preparation with stirring. The sala cider flavor was added and the volume was adjusted to 720 ml with vehicle. The product was logged for quality control analysis. For packaging the bottles were loaded into the filling machine and four bottles to be tested filled (3 for stability testing and 1 for control). The labelled products were packed into jackets, shrink wrapped and put into corrugated cardboard boxes.

3. Results and Discussions

The ultraviolet spectra of OSEL showed the maximum absorption wavelength at 230 nm. Therefore, 230 nm wavelength was selected for HPLC determination to achieve the highest sensitivity for the study.

The standard curve was plotted by peak area for the different concentrations (Figure 1). The linear reguations of standard curves was $y = 2.826x + 27.324$. The correlation coefficients (r^2) was 0.9999 (n=3). The peak areas of samples were measured and the concentrations of samples were calculated from the standard curve.

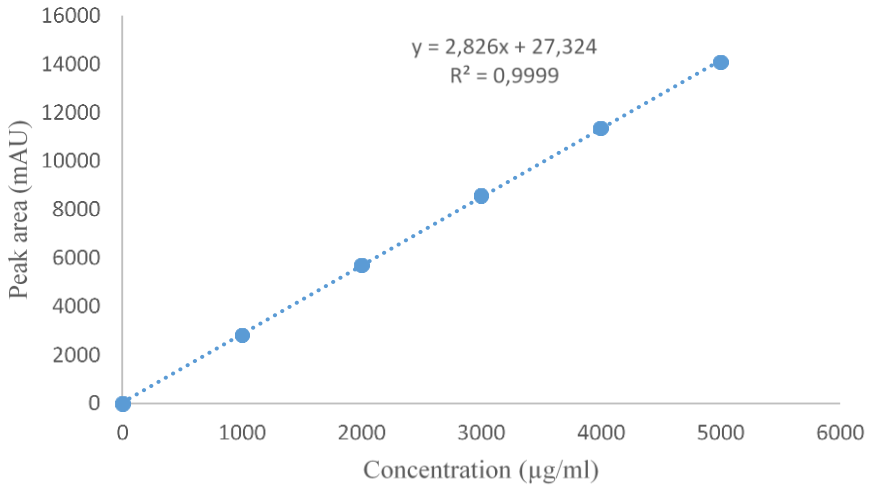

Figure 1 Standard curve of OSEL (n=3)

HPLC chromatograms of OSEL standard, OSEL ES and vehicle used in QSNICH are shown in Figures 2, 3 and 4, respectively.

Figure 2 HPLC chromatogram of OSEL. Column: C18 (4.6 x 250 mm) column with a mobile phase of phosphate buffer (pH 6; 0.05 M), methanol and acetonitrile (570:255:175, v/v) UV detector wavelength 230 nm; flow rate of 1.0 ml/min, injection volume: 20 µl and room temperature

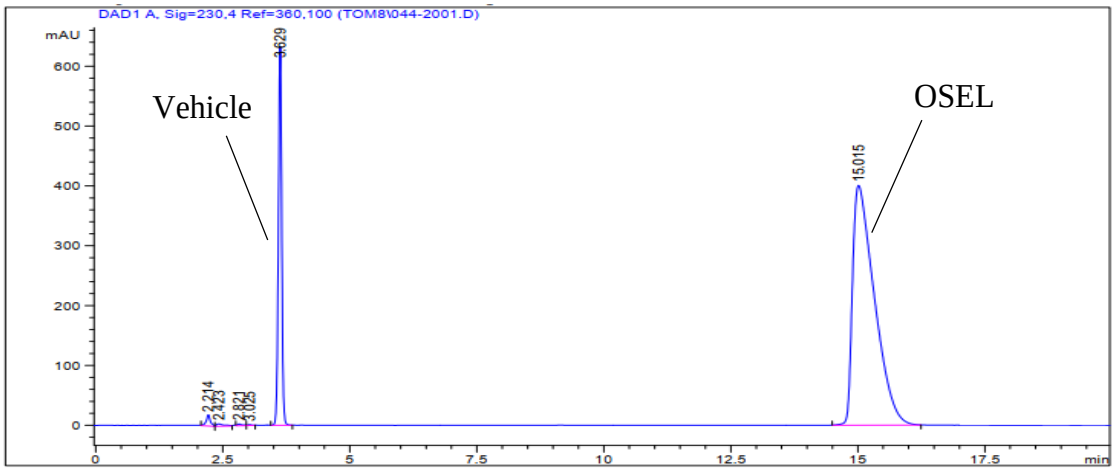

Figure 3 HPLC chromatogram of OSEL ES. Column: C18 (4.6 x 250 mm) column with a mobile phase of phosphate buffer (pH 6; 0.05 M), methanol and acetonitrile (570:255:175, v/v) UV detector wavelength 230 nm; flow rate of 1.0 ml/min, injection volume: 20 µl and room temperature

The suspension was composed of commonly used excipients found in many suspensions. In the case of the sweeteners, sucrose was combined with saccharin sodium to enhance the sweetening ability leading to a decreased amount of the individual sweeteners being used. However since it was a pediatric formulation an alternative sweetener like sorbitol or glycerin could have been used in place of the sucrose to reduce the chance of dental caries [7] . Inclusion of liquid glycerin would enhances the viscosity of the suspension. The combination of the preservatives paraben concentrate leads to synergistic effect between the two resulting in a lesser quantity of each preservative being used. The preservative action of the parabens. Noticeably there was no buffering agent in the formulation to control pH [8].

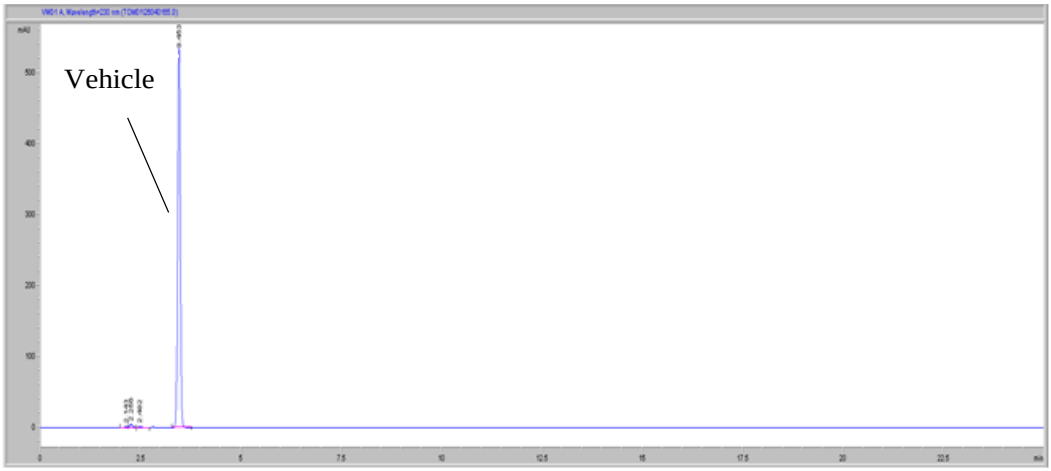

Figure 4 HPLC chromatogram of vehicle. Column: C18 (4. 6 x 250 mm) column with a mobile phase of phosphate buffer (pH 6; 0.05 M), methanol and acetonitrile (570:255:175, v/v) UV-detector wavelength 230 nm; flow rate of 1.0 ml/min, injection volume: 20 µl and room temperature

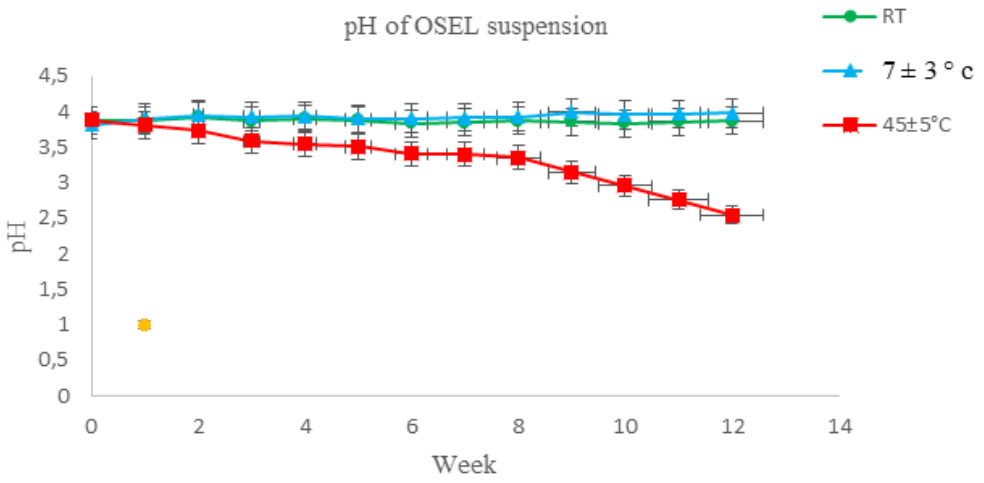

Figure 5 Changed in pH of OSEL ES (QSNICH formulation) after storage at 7 ± 3 °C (blue line) , room temperature (green line), and 45 ± 5 °C (red line) (n=3)

OSEL ES 10 mg/ml. samples were packaged in 30 ml plastic bottles and stored at refrigerated temperature (7 ± 3 °C), room temperature and 45 ± 5 °C for 12 weeks. The acceptance criteria for stability of the suspensions were the retention of the white color. OSEL ES samples stored at 7 ± 3 °C and room temperature showed little change in color over 12 weeks (Figure 6, columns 1 and 2). OSEL ES samples stored at 45 ± 5 °C were not stable, showing unacceptable changes in color after 1, 2 and 12 weeks storage (Figure 6, columns 3). OSEL ES samples stored at refrigerated temperature (7 ± 3 °C) showed no significant in pH over the course of the experiment (range 3.84 - 3.94, p > 0.05; Figure 5, blue line). Similarly, there was no significant change in pH for OSEL ES samples stored at room temperature (range 3. 86 - 3. 93, p > 0. 05; Figure 5, green line) . The

suspension stored at 45 ± 5 °C showed a reduction in pH from 3.87 to 2.56 over 12 weeks (Figure 5, red line), which. Indicates that the formulation is not stable at this temperature. The suspension did not contain a buffering agent and this made them vulnerable to variations in pH and this might explain the recorded pH of the suspension. Hydrolysis is one of the important pathways of drug degradation [9] and hydrolysis may be dependent on the pH of the medium. The pH of the medium also affect the growth the of microorganisms and the efficacy of preservative [10] . Hence, the pH of a suspension pH is one of the important factors to be considered in product stability. In order to test the effect of pH on the stability of the product, samples of the suspension with varying pHs were analysed over a time period. The suspension appeared as a viscous brown to black brown suspension which was readily redispersed. (Figure 5) . At the end of 2 months of storage the appearance and ease of redispersibility of all the suspensions remained unchanged implying that the suspension pH probably does not have an effect on the appearance and dispersibility.

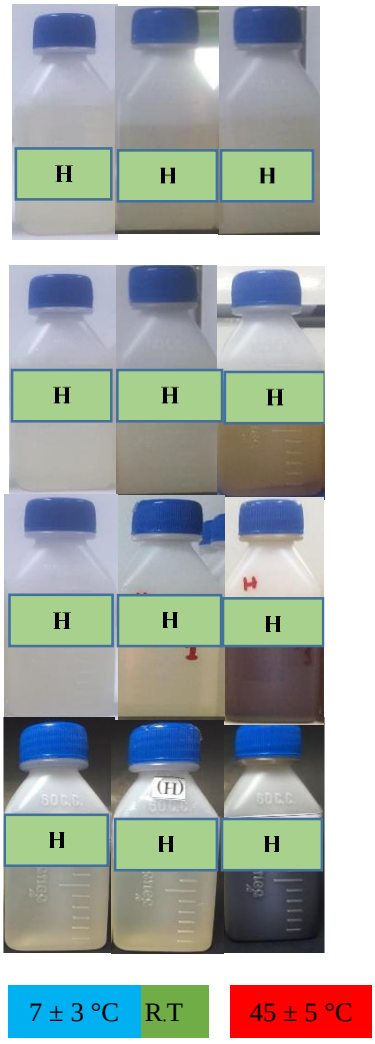

Figure 6 Change of colour in OSEL ES (QSNICH formulation) after storage at 7 ± 3 °C (blue) , room temperature (RT, green), and 45 ± 5 °C (red). First row: week 0, second row: week 2, third row: week 8, fourth row: week 12

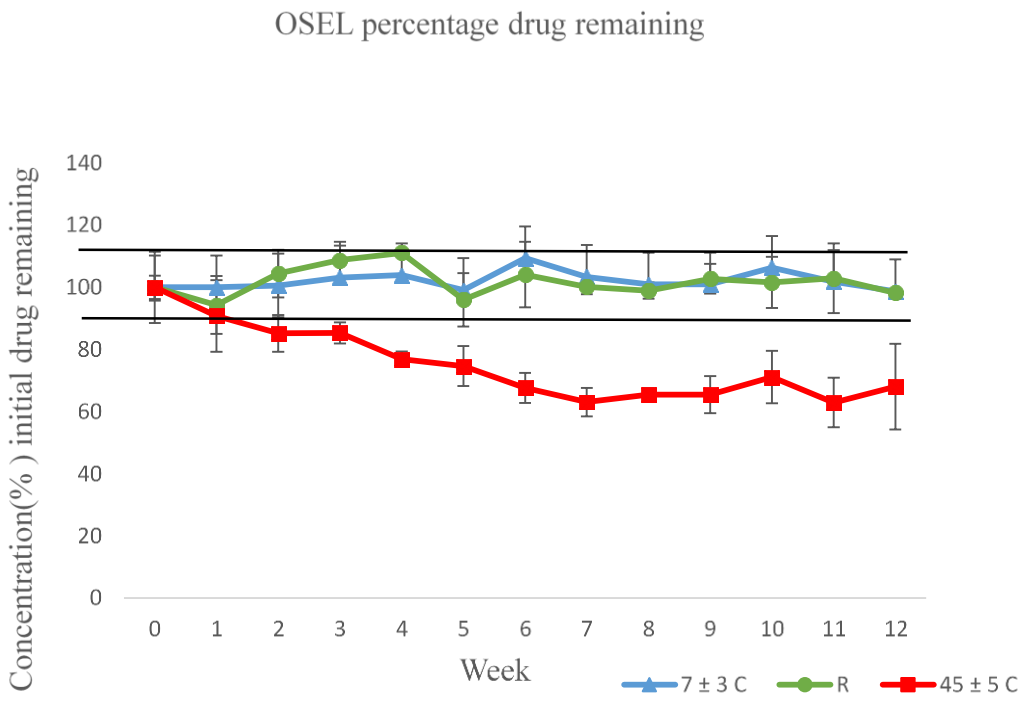

Figure 7 Percentage of drug remaining in OSEL ES (QSNICH formulation) after storage at 7 ± 3 °C (blue line), room temperature (green line), and 45 ± 5 °C (red line) (n=3)

The initial content of drug in the OSEL ES was 100% . The results indicated a small reduction in the drug content of OSEL ES in the suspensions stored at the temperature ranges after 1 week (Figure 3) After 12 weeks of storage, each OSEL ES suspension showed a reduced level of drug (OSEL ES), ranging from 98.24 % for refrigerated temperature (7 ± 3 °C), 98.17% for room temperature, and 62.91 % for 45 ± 5 °C ($p < 0.05$). This is the first suggestion that high storage temperatures have an effect on the drug content of extemporaneously prepared OSEL suspensions. Previous studies [11-13] were carried out at only 3 temperature at refrigerated temperatures (2-8 °C), room temperature and 30 °C. These studies did not determined at 45 ± 5 °C. For studies [13] the Cherry Syrup preparation, in either bottle type, was stable for up to 35 days under refrigeration (5°C) and up to 5 days at room temperature (25°C). It was not stable when stored at 30°C for 5 days. The Ora-Sweet SF preparation was stable for up to 35 days at 5°C or 25°C and for up to 13 days at 30°C in either bottle type. Both preparations maintained microbiologic stability for 35 days. The upper limit for temperature to be kept the OSEL ES safely is room temperature, for the other studies [14] the laboratory analyses showed that only amoxicillin suspensions stored between 2 and 8 °C for seven days showed the lowest level of degradation for 14 days.

4. Conclusion

Extemporaneously prepared OSEL suspensions should be put on long term stability studies to provide data for a definitive decision to be made on their stability OSEL ES can be stored at 7 ± 3 °C and room temperature because OSEL ES drug content was more than 90 percent at up to 12 weeks storage. This assay content is within the acceptable criteria. However, the same formulation was unstable when stored at 45 ± 5 °C. OSEL ES stored at 45 ± 5 °C was only stable for one week, showing color changes, pH change, and drug content below 90% after 1 week storage.

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