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# Stability Testing of Buprenorphine Oral Syringes

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# Contents

# 1. Conclusions:

The buprenorphine 0.075 mg/mL, 1 mL oral syringes are stable for 60 days when stored at room temperature (25°C and 60% relative humidity) and refrigeration 2-6 °C.

As per the chromatograms of all the samples, the retention times of the peaks in the sample chromatogram correspond to the peak in the reference material chromatogram. As per the % assay of buprenorphine in formulations, all the formulations had 90-110% of the buprenorphine. No microbiological growth was observed when these syringes were assayed on day 60.

# 2. Study Details:

### *Goals and Objectives*

The proposed investigation aims to evaluate the stability of buprenorphine oral syringes over a period of 60 days at two different storage conditions.

Materials

Equivalent chemicals, equipment and supplies may be substituted for the items listed below unless otherwise specified. Solution preparations may be scaled up or down as needed.

REAGENTS

Water, MS-grade

Acetonitrile (ACN), MS-grade

Formic acid, MS Grade

SUPPLIES

The test method uses the supplies listed below.

- Class A volumetric pipette
- Class A volumetric flask
- HPLC standard glass vials
- Sonicator

- 0.45um nylon filter
- Tryptic Soy Agar (TSA) plates

## *Methods*

*Mobile Phase*

Mobile Phase A is water with 0.1% formic acid (FA).

Mobile phase B is acetonitrile (ACN) with 0.1% formic acid.

*To 4 liters of LCMS grade Optima ACN (Fischer Scientific) 4 ml of formic acid was added to give final concentration of 0.1% FA in ACN.*

Mobile phase composition for the LCMS runs was 50% A and 50% B.

*Formulation Preparation*

An initial formulation was made by dilution (1:4) of Buprenex in water of irrigation. This formulation was compounded by withdrawing 12 mL of 0.3 mg/mL buprenorphine into a 20 mL syringe through a filter needle. The buprenorphine was then placed in a 60 mL amber glass bottle. To this amber glass bottle, 36 mL of water for irrigation was added. This bottle was inverted several times to mix the contents. Using Baxter EXACTAMED 1 mL Amber Oral Dispensers, 0.5 mL buprenorphine 0.075 mg/mL was withdrawn into each syringe and capped.

The syringes were stored at the following two conditions:

- 25°C and 60% Relative Humidity in Temperature/Humidity Chamber model LH-1.5 (Associated Environmental Systems, Ayer, MA).
- Refrigeration at 2-6 °C.

Three syringes were withdrawn from both these conditions at the specified time points (0, 7, 15, 30, and 60 days) and subjected to stability testing. Physical, chemical, and microbiological testings were conducted on each sample.

# 3. Testing:

## *3.1. Physical stability.*

pH: pH is defined as an important parameter that governs the stability of the product as the change in pH can cause the precipitation of the product. Thus, pH was measured at each sampling point with pH meter using three-point standardization with buffer solutions (pH of 4.0, 7.0, and 10.0).

## *3.2. Chemical evaluation*

*Chemical evaluation*. Each buprenorphine 0.075 mg/mL 1 mL Oral Syringe was added to 50 mL volumetric flask to which around 30-40 mL of nano pure water was added. This 50 mL volumetric flask was sonicated for 5 minutes and then left for 5 minutes at the room temperature. The assay of buprenorphine was performed by using a calibrated liquid chromatography mass spectrometry (LCMS).

The calibration of HPLC method was performed by constructing a standard curve using known concentrations (1, 5, 7.5, 15 and 30 ug/ml) of buprenorphine. All samples were analyzed three times (intra-day variation) on three different days (inter-day variation). The accuracy was calculated at each concentration as the ratio of the measured concentration to the nominal concentration multiplied by 100%.

**Assay:** All the chromatographic studies were performed on an Agilent HPLC system connected with Exactive mass spectrometer. Buprenorphine concentration in all samples was assessed by liquid chromatography mass spectrophotometry (LCMS). The separations were done on a Symmetry® C18 column (75 x 4.6 mm I.D., Waters Associates, Milford, MA, USA) with the particle diameter of 3.5 µm. The mobile phase was 50% solvent A (0.1% formic acid in water) and 50% solvent B (0.1% formic acid in acetonitrile). Transition (m/z) of 414/400 was used for the detection of peak in MS. The total run time of 1.6 minutes at the temperature of 30°C was used. The mobile phase was filtered and degassed before use. The flow rate was 0.35 mL/min and the injection volume was 1 µL.

*Data analysis*. The stability of buprenorphine was determined by calculating the percentage of the initial amount at each sampling point. The drug was considered stable only if the concentration exceeds

90% of the initial concentration. Student's t-test was used to compare the difference between the data of interest with $\alpha = 0.05$ as the minimal level of significance. Wherever possible, the data was presented as mean ± standard deviation.

**Table 1.** HPLC Method Parameters

| Parameter | Condition |
|---|---|
| Flow rate | 0.3 mL/min |
| Run Time | 1.6 minutes |
| Sample Temp | Ambient |
| Column temp | 30°C |
| Injection Volume | 1 µL |

**Table 2.** MS Method Parameters

| Parameter | Condition |
|---|---|
| Ionization Type | Positive |
| Scanning Mass range | 400 to 414 m/z |
| Sheath Gas flow | 30 |
| Ionization Voltage | 5.00 |
| Capillary Temperature | 375°C |

## *3.3. Microbiological evaluation*

In order to determine whether the current formulation met the criteria for acceptance of microbiological quality, the microbiological testing for performed as per the USP guidelines for the non-sterile products. Three syringes were withdrawn from room temperature at day 60 and spread on TSA plate. Three plates were incubated for positive control by plating tap water. All the plates were incubated at 35 °C for 3 days. The plates were observed every day for any growth.

# 4. Results

Calibration curve

All the standard concentrations (1, 5, 7.5, 15 and 30 ug/ml) of buprenorphine were analysed by using the standardized HPLC conditions as outlined above. A good linearity was exhibited in this concentration range by using this HPLC method. The average coefficient of determination of 0.99 was observed. The slopes of the curves illustrated an excellent agreement with coefficient of variability.

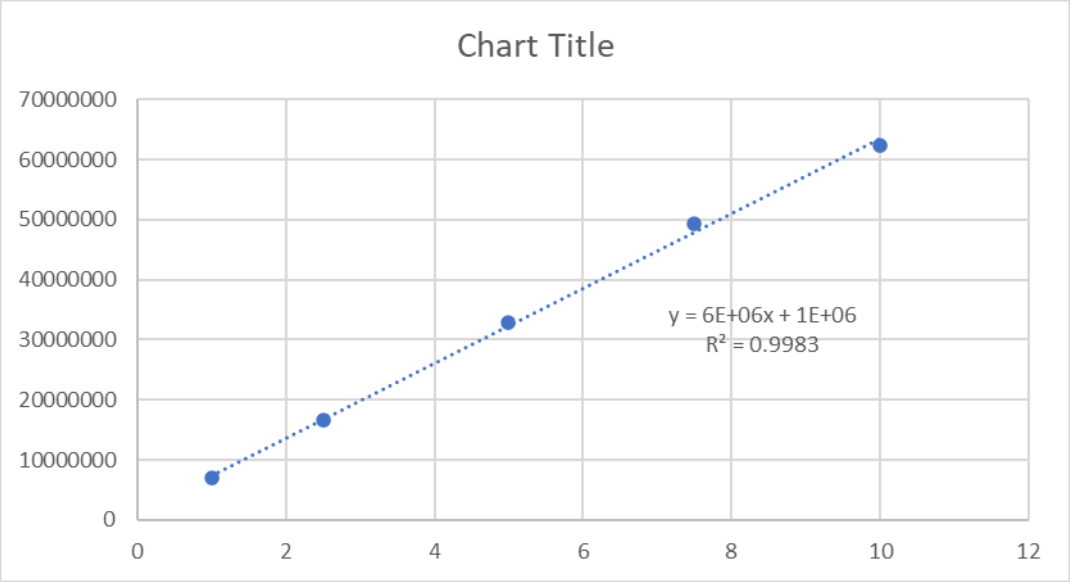


**Figure. 2.** Standard curve of buprenorphine assay.

pH

There was no significant increase or decrease in the pH values for all the solutions over a period of 60 days.

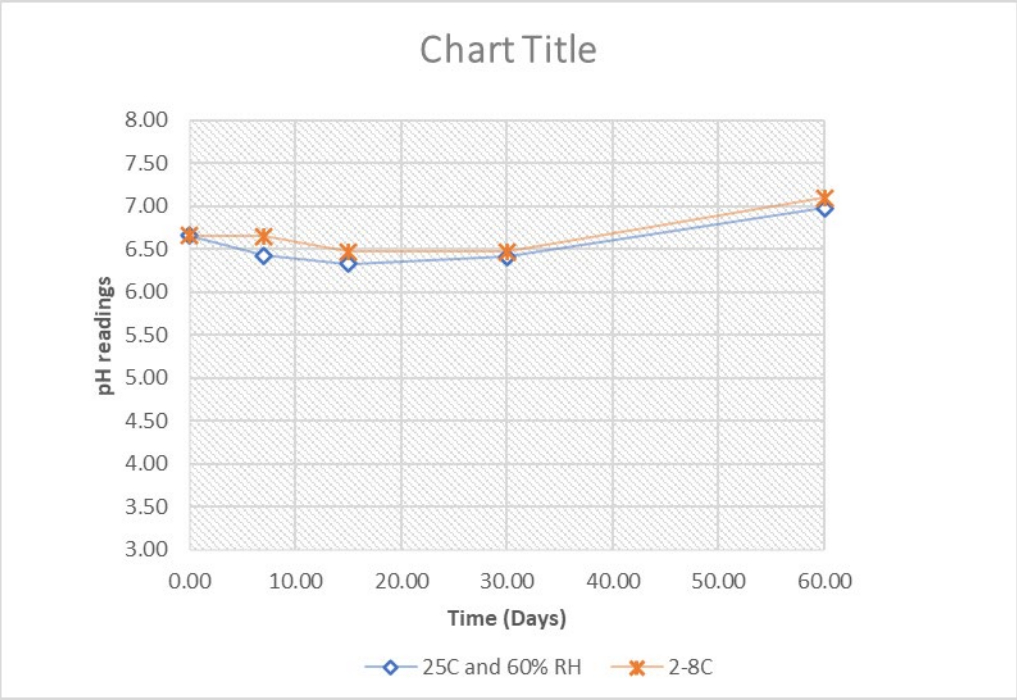


**Figure 2.** pH versus time profile for the buperinorphine oral syringes.

Drug Content

All the preparations were in the range of 90-110% of the labeled amount for buprenorphine 0.075mg/mL of 1 mL oral syringes stored under refrigeration (2-6°C) and controlled room temperature conditions (25°C and 60% Relative Humidity).

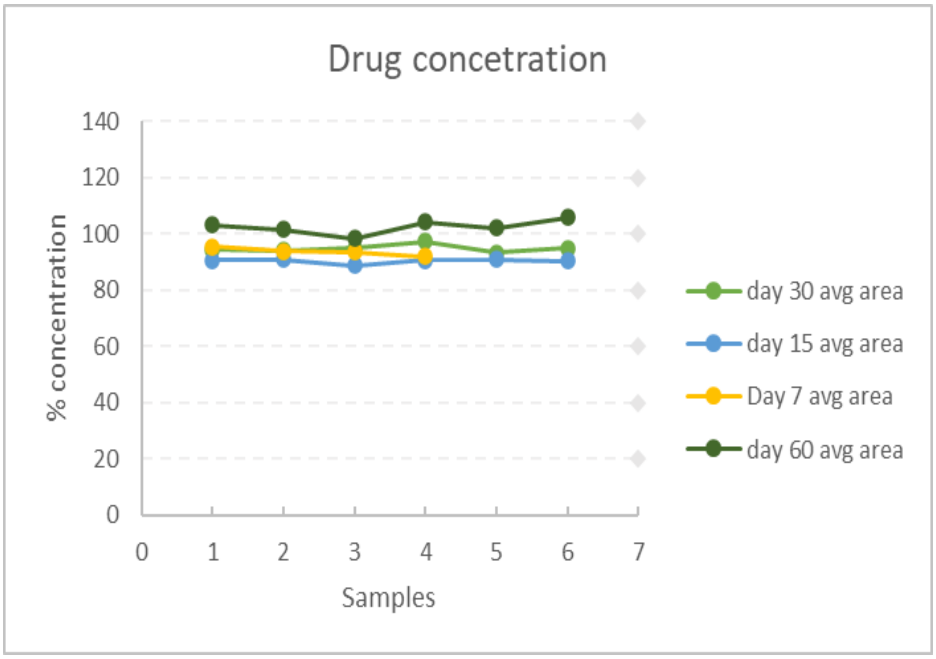


**Figure 3.** Percentage of initial concentration versus time profile for the buperinorphine oral syringes.

Microbiological assay:

No microbiological growth was observed after 48 and 72 hours of plating the buprenorphine solution on the plates that were used for 1 ml Buprenorphine syringes. Lot of colonies were observed on the plates with tap water (positive control) after 48 hours of plating.