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Stability Study of Compounded Ethanol-Free Buprenorphine Oral Syringes for Neonatal Opioid Syndrome

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OBJECTIVE Buprenorphine (BUP) is advantageous in the management of neonatal opioid withdrawal syndrome (NOWS). A widely used BUP formulation for the treatment of NOWS contains 30% ethanol. The objective of this research was to investigate the stability of buprenorphine in compounded ethanol-free oral syringes.

METHODS A 75 µg/mL buprenorphine solution was prepared by diluting the commercial injectable solution with water for irrigation, transferred into amber oral syringes and stored at room temperature (n=3) and refrigerated (n=3) conditions. Microbial testing of the compounded oral syringes was performed to determine growth of microbes for 24 and 48 hours respectively at 37ºC. Assessment of the stability of BUP in the compounded syringes was conducted by pH and liquid chromatography-mass spectrometry (LC-MS) analysis after compounding, at 7, 30, and 60 days of storage.

RESULTS The concentration of BUP at all time points was between 90%-110% of the day 0 baseline. Concentration increased slightly in the oral syringes after day 30, probably because of moisture loss as there were no degradation peaks observed in chromatograms. There was no microbial growth or visual change observed for the compounded ethanol-free buprenorphine oral syringes. The pH of the oral syringes was consistent through 60 days for room temperature and refrigerated samples.

CONCLUSION Buprenorphine in an ethanol-free formulation is stable and can be easily compounded in pediatric pharmacies. This is an appealing formulation for the treatment of NOWS.

ABBREVIATIONS BUP, buprenorphine LC-MS, liquid chromatography-mass spectrometry; NOWS, neonatal opioid withdrawal syndrome; SL, sublingual; USP, united states pharmacopeia

Introduction

Since 2000, there has been an increasing number of infants with a history of in utero exposure to opioids. The sudden cessation of placentally transmitted opioids can cause a constellation of clinical signs of withdrawal that may occur in neonates after in utero exposure to opioids and other substances, called neonatal opioid withdrawal syndrome (NOWS).[1] Symptoms of NOWS include irritability, fussiness, and difficulty in consoling. More severe symptoms impair maternal bonding and weight gain. First line therapies are non-pharmacological such as swaddling, low stimuli environment and mother–to–child bonding. When these do not control symptoms, an opioid is administered to the infant. Buprenorphine (BUP) is a natural semi-synthetic partial μ-opioid receptor agonist used sublingually in the treatment of NOWS.[2] The most commonly used formulation for NOWS is 75 μg/mL buprenorphine in 30% ethanol compounded from buprenorphine injection 0.3 mg/mL administered by sublingual (SL) route.[3] While BUP has excellent efficacy, there is hesitation for use by clinicians due to the presence of ethanol. Hence, the purpose of this study was to assess the stability via physiochemical testing of 75 μg/mL ethanol-free formulation of buprenorphine compounded in oral syringes stored at room temperature and refrigerated conditions for 60 days.

Materials and Methods

Buprenorphine Standard Curve Liquid chromatography-mass spectrometry (LC-MS) calibration was performed by constructing a standard curve using known concentrations (1, 5, 7.5, 15, and 30 μg/mL) of buprenorphine hydrochloride reference standard (Lot no. R09910) purchased from (United States Pharmacopeia, USP. Rockville, MD). This was achieved by preparing a 300 μg/mL standard stock solution that was utilized to prepare the subsequent standard working solutions. These standard solutions were then subjected to LC-MS analysis and all working solutions were analyzed thrice, on the same day (intra-day variation), and on 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% and was defined acceptable if the values were between 80%-120%.[4]

Compounding Ethanol-Free Buprenorphine Oral Syringes Commercially available Buprenex (Lot no. 67737) was purchased (Par Pharmaceutical. Chestnut Ridge, NY). The oral syringes

were prepared by mixing 1 part Buprenex in 3 parts water for irrigation (Lot no. Y426138; Baxter Healthcare Corporation. Wayne, PA). The oral syringes were compounded by withdrawing 8-mL of 300 µg/mL buprenorphine into a 30-mL syringe (Lot no. 6165975; BD, Franklin Lakes, NJ) through a filter needle. The solution was placed in a 60-mL amber glass bottle, and 24-mL of water for irrigation with no preservatives, was added. The bottle was then mixed thoroughly by inverting it several times. Using 1-mL amber oral dispensers (Lot no. H9387101; Baxter Exactamed, Wayne, PA), 1-mL of the BUP solution (75 µg/mL) was withdrawn and capped. The capped oral syringes were refrigerated (between 2-6ºC) or stored at room temperature (25ºC, 60% relative humidity). Three syringes from each storage condition were assessed for physical and chemical stability on days 0, 7, 30, and 60. The syringes were protected from light exposure and the storage temperatures and humidity conditions were closely monitored throughout the study.

pH Three syringes at each storage condition were assessed for pH changes at each sampling point for the compounded oral syringes using a Mettler Toledo Inlab® Ultra-Microelectrode pH meter (Mettler Toledo Exact Equipment. Washington Crossing, PA). The instrument was calibrated using a three-point standardization, with 3 buffer solutions (pH of 4.0, 7.0, 10.0) as per USP <791> guidelines.[5] After calibration with the standard buffer solutions, an assessment of the pH change of the oral syringes was performed by recording three readings (n=3) of the solution in each oral syringe under each storage condition for each day of the study, and the average of the readings were determined to ascertain if the formulation was stable over 60 days.

Liquid Chromatography Mass Spectroscopy All chemical evaluations were performed using LC-MS, system 1260 Agilent Infinity II and 6545 XT LC/QTOF (Agilent Technologies. Singapore). The mass spectrometry method was established in the scanning mass range of 200−600 *m/z*, using 6545 XT LC/QTOF. All the scans were performed under positive ion mode and electron spray ionization. The sheath gas flow rate was maintained at 12 psi; the auxiliary gas flow was 10 psi; the electron spray voltage used was 2.80kV; and the capillary temperature was 350°C. Capillary, tube lens, and skimmer voltage were kept at 130V, and 65V, 28V respectively. These parameters were optimized using MassHunter Workstation software. LC-MS runs of the calibration standards and subsequent samples were performed using isocratic elution

with 50:50 (water with 0.1% formic acid [FA]: acetonitrile with 0.1% FA). All the standards and samples injection volume were set to 1-µL, and chromatographic separations were performed using Symmetry C18, 3.5 µm; (4.6 × 75.0 mm) column (Waters, Milford, MA) at a flow rate of 0.5-mL/min with a run time of 1.6 minutes. Column compartment and sampler temperature were set to 30°C and 20°C, respectively.

Calibration of the LC-MS method was done by performing a standard curve of buprenorphine. The standard working solutions were then subjected to LC-MS analysis using the devised method parameters (Table 1). A standard curve of BUP was constructed (Figure 1) and representative chromatogram of the drug substance (Figure 2) demonstrated good peak area response at an EIC value of 468.3098. After all the standard working solutions were analyzed, the developed analytical method (Table 2) was used during the 60-day study to determine the stability of buprenorphine in the compounded ethanol-free oral syringes stored under the two different storage conditions (n=6). The concentration of the drug substance was determined by averaging the concentration values of both sets of oral syringes at each storage at each time point of the study.

Table 1. Liquid Chromatography-Mass Spectrometry Method Parameters

Parameter	Condition
Column	Symmetry C18 (3.5µm, 4.6 x 75mm)
Composition of Mobile Phase	50:50; 0.1% FA in water and 0.1% FA in acetonitrile
Flow rate	0.5 mL/min
Run time	1.6 Minutes
Column temperature	30ºC
Sample temperature	20ºC
Injection Volume	1 µl
Retention Time of Analyte	1.2 Minutes

Microbial Testing One mL of the compounded BUP formulation was added to agar petri dishes. The dishes were incubated at 37ºC and observed for the growth of microbes at 24 and 48 hours.

Results

Standard Curve and Method Development An LC-MS method developed for this study which was determined to be reproducible and consistent with the acceptance criteria described in the literature.[6] The standard curve generated by analyzing the standard samples within the concentration range using the developed analytical method demonstrated good linearity (Figure

1), with the average coefficient of determination (r^2) of 0.99. The slope of the standard curve illustrated an exceptional agreement with the coefficient of variation with intra-day and inter-day studies, with variation less than 10%. The acceptable recovery values of the standard samples for intra-day and inter-day were between 80%-120% (Table 2).

Figure 1. Standard Curve of Buprenorphine

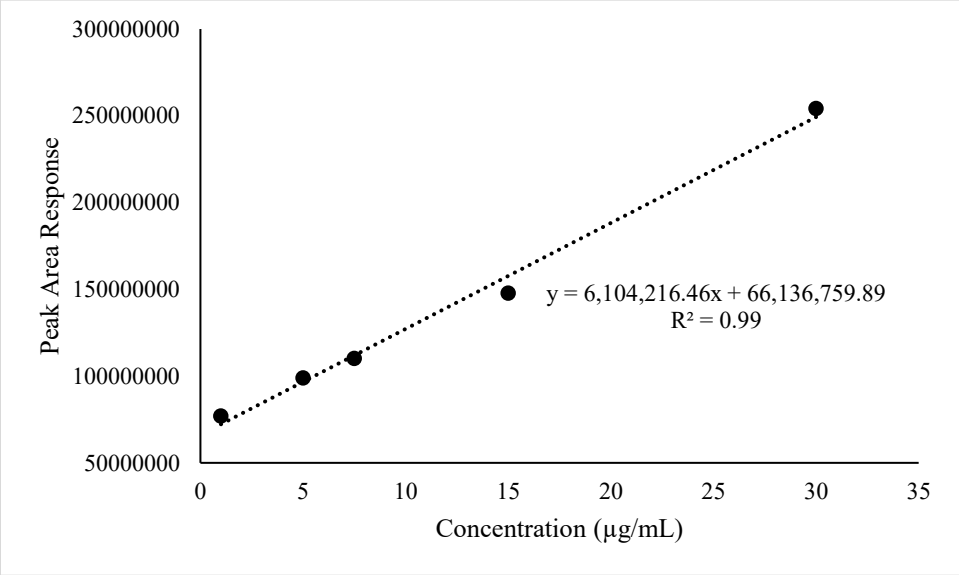
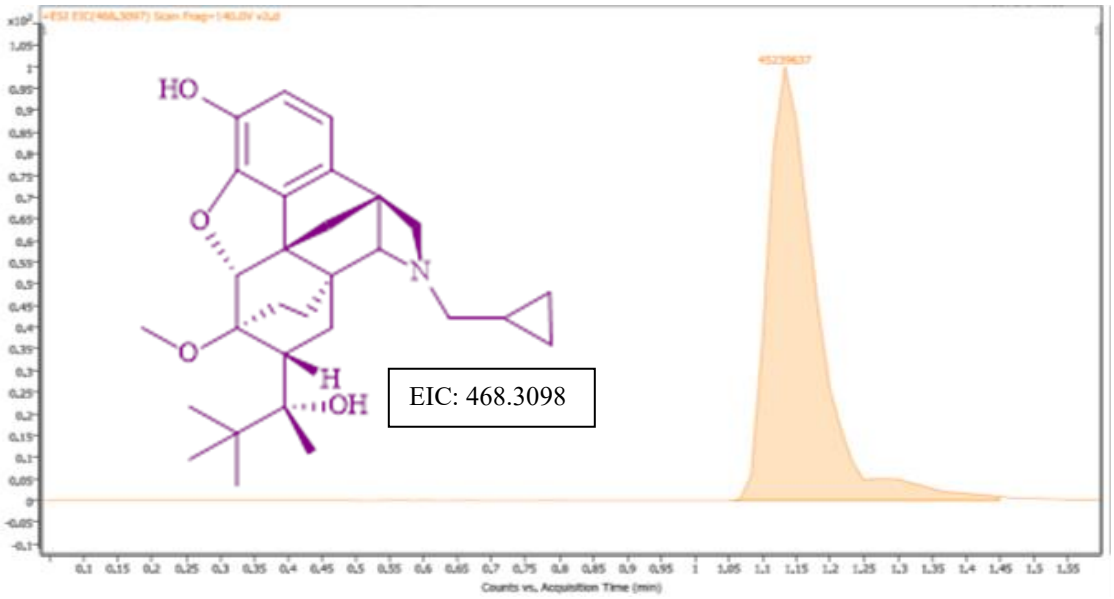


Table 2. Method Development of Buprenorphine Standard Curve

Concentration (µg/mL)	% CV		% Recovery	
	Intra-day	Inter-day	Intra-day	Inter-day
1	5.8	5.0	115.6	118.7
5	8.1	6.2	97.6	102.5
7.5	5.8	4.4	93.8	91.8
15	3.5	6.7	98.3	92.2
30	7.1	6.7	97.4	101.6

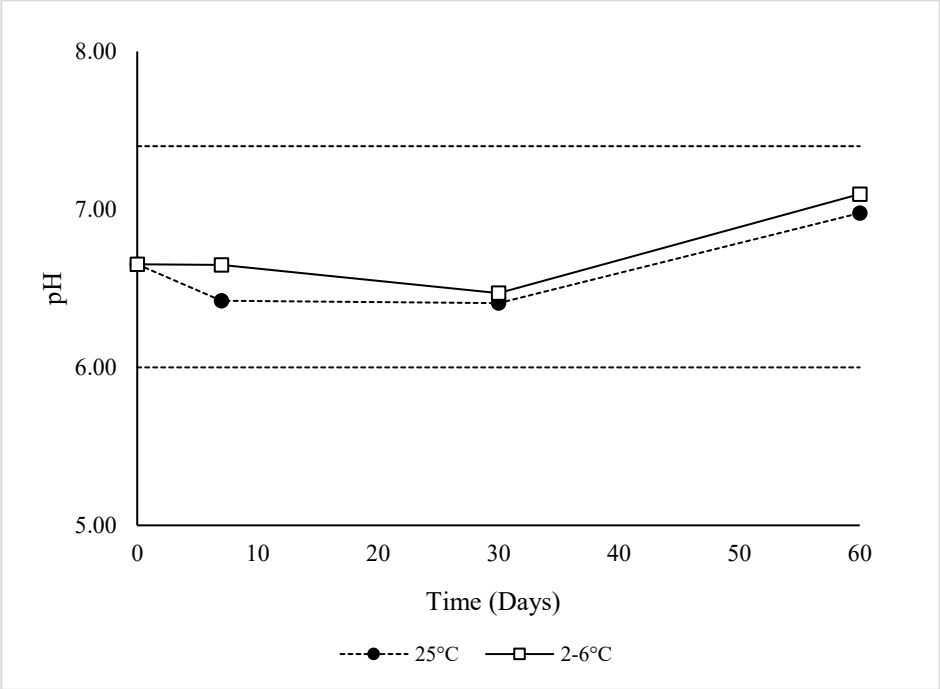
*Intra-day and Inter-day study of calibration standards of buprenorphine performed to determine percent coefficient of variation(%CV) and percent recovery at different concentrations in establishing a standard curve of the drug substance in the analytical method development process.

Figure 2. Representative Chromatogram of Buprenorphine Assay



Physical Evaluation and Drug Content Analysis All syringes were clear throughout the study and no particulate matter was observed upon visual inspection. The pH range of the samples were determined to be 6.28-7.03 at room temperature with an average pH of 6.98±0.06 at day 60. For refrigerated samples, the pH range was determined to be 6.38-7.14 with an average pH of 7.10±0.08 at 60 days. Hence, there was no significant change in the pH of the oral syringes throughout the study period as the pH of the solutions were in the desired range of 6.0-7.4 under both temperature conditions (Figure 3).

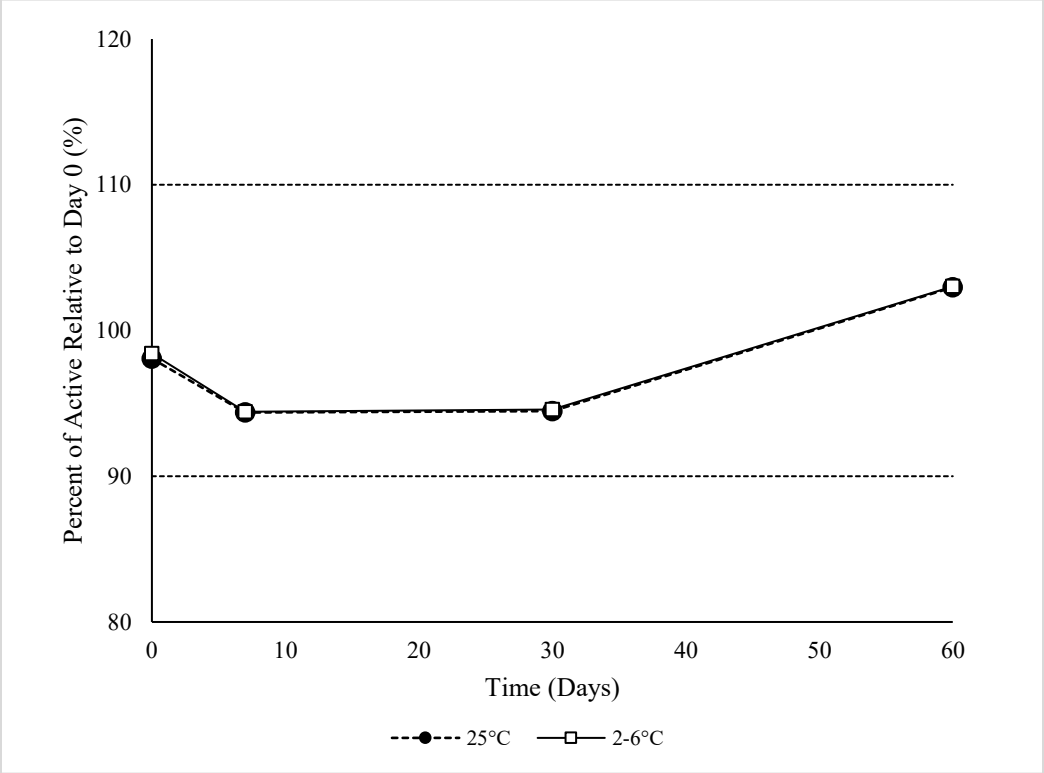
Figure 3. pH Results of the Ethanol-Free Buprenorphine Oral Solution Stability Samples Over 60 days



All the ethanol-free oral syringes at all the time points contained BUP above the 90% threshold range (91.95%-104.18%) for room temperature samples and (91.96%-104.24%) for refrigerated samples. The average concentration relative to the initial concentration was 102.97±1.36 at day 60 for room temperature samples and 103.02±1.36 for refrigerated samples respectively (Figure 4).

Microbial Testing There was no microbial growth noted at 24 or 48 hours.

Figure 4. Liquid Chromatography-Mass Spectrometry Results of the Ethanol-Free Buprenorphine Oral Solution Stability Samples Over 60 days



Discussion

We describe a simple to prepare BUP formulation that is shelf stable and ethanol free. This is advantageous in the goal to use excipients which are non-toxic and not themselves pharmacologically active. We did not conduct human testing to see if the formulation altered the absorption kinetics compared to the reference ethanol containing formulation. However, the formulation described in this report has been used for over a year at Thomas Jefferson University Hospital without any observed change in efficacy or safety compared to the previously used ethanolic formulation. This suggests, but does not prove, that the formulations have similar absorption kinetics. Another ethanol-free formulation developed for NOWS had 80% of the bioavailability of the reference formulation.[7] However, this formulation is much more complex with a citric buffer and hydroxyethyl cellulose thickener and the bioequivalence testing was in healthy adults.

Based on the previous work mentioned regarding the development of an ethanol-free formulation of buprenorphine, [8,9] Ahmadi et. al devised a formulation to be administered sublingually

without containing dextrose, buffers, or preservatives. They evaluated the compounded oral syringes for 14 days under refrigerated conditions via pH and assay, using high-performance liquid chromatography (HPLC).[10] The results demonstrated from their assessment of the oral syringes of BUP when refrigerated were stable throughout the time course of the study.

We further explored on the research established in the literature by compounding ethanol-free buprenorphine oral syringes (75 µg/mL) and this formulation was evaluated for 60 days. All oral syringes, when stored at room temperature and refrigerated conditions remained clear, colorless and no particulate matter was observed. These results are consistent with findings of Ahmadi et. al, when their sublingual solution was compounded but, we also assessed microbial growth of our compounded oral syringes and there was no growth of microbes observed. As shown in Figure 3, the pH ranged from 6.3 to 7.0, which was consistent with physiological conditions and these results differed slightly from the pH range found in the literature as the pH values reported were between 5.9 to 6.4.[10] Moreover, the consistent pH of the oral syringes indicates that buprenorphine was stable in this dosage form at either storage condition with no precipitation of the drug substance and was stable in the ethanol-free environment.

The results from the LC-MS analysis of the compounded oral syringes under each storage condition demonstrated that buprenorphine was stable in the formulation throughout the study with minimally observed degradation products as exhibited in Figure 2. However, there was a minor increase in the samples' concentration between days 0 and 60 in the compounded oral syringes as demonstrated in Figure 4, which could be brought on by moisture evaporating through the syringe barrel. This finding was also observed in the work published by Ahmad et. al, when their oral syringes were stored under refrigerated conditions for 14 days and observed an increase between days 0 and 14 with the same rationale. Overall, the results from the stability study by evaluating the newly compounded oral syringes for their physiochemical properties reveal that the compounded ethanol-free oral syringes are stable for 60 days, making it a preferred formulation of buprenorphine as it was determined to be suitable for administration to neonates with NOWS.

Limitations

This study provides strong evidence supporting the physical and chemical stability of compounded ethanol-free buprenorphine oral syringes for 60 days with no microbial growth

observed, however, certain areas warrant further exploration. Although, *in vivo* pharmacokinetic testing was not performed, the formulation has been used clinically without observed differences in efficacy or safety, suggesting comparable performance to the formulation used in the current standard of care. This study focused on two storage conditions, room temperature and refrigeration however, additional testing under stress conditions could further demonstrate the compounded oral syringes robustness. Microbial testing was used to confirm the presence of microbes at the selected time periods and incubation temperature, which provide assurance of safety, though future studies could have more sensitive detection methods. Finally, this study evaluated a 75 µg/mL concentration of buprenorphine in the compounded ethanol-free oral syringes, which serves a proof of concept and a foundation for further work for the development of future formulations. These considerations present avenues for continued research to build upon the encouraging findings from this study.

Conclusion

This study assessed newly devised ethanol-free buprenorphine oral syringes (75 µg/mL), which was compounded by diluting 1 part buprenorphine hydrochloride 300 µg/mL with 3 parts water for irrigation (a 1 in 4 ratio). The buprenorphine oral solution was found to be congenial with the amber oral syringes, and BUP showed physical and chemical stability up to 60 days under room temperature and refrigerated conditions. The formulation is easy to prepare and appears safe and effective in clinical use at a busy neonatal unit.

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