JPPT | Extemporaneous Compounding

Stability-indicating LC-MS Method for Determination of Stability of Extemporaneously Compounded Buprenorphine Oral Syringes for Neonatal Abstinence Syndrome

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OBJECTIVE In the hospital settings, buprenorphine is used for the treatment of patients with neonatal abstinence syndrome. It is extemporaneously compounded and stored in oral plastic syringes. However, limited information exists about the stability of buprenorphine and its compounded formulations when stored under specific conditions. Hence, we developed a stability-indicating high-performance liquid chromatography–mass spectrometry (LC-MS) method to analyze the stability of buprenorphine over time.

METHODS A stability-indicating LC-MS method was developed to map the potential degradation peaks of buprenorphine when exposed to acidic, basic, and oxidative conditions. This method was used to study the stability of compounded buprenorphine oral syringes stored under refrigeration (2°C–8°C) and room temperature (25°C ± 2°C with 60% relative humidity). Syringes from each storage condition were assessed for stability using pH meter and stability-indicating LC-MS assay for 30 days.

RESULTS Buprenorphine gets completely degraded in the presence of acid at the end of 1 hour of exposure. Various degradation peaks were identified using LC-MS assay for buprenorphine under acidic, basic, and peroxide conditions. Stability study of oral buprenorphine syringes showed no precipitation, cloudiness, or color change during this study at all storage conditions. The LC-MS assay revealed that buprenorphine oral syringes retained greater than 90% of the initial concentrations for 30 days.

CONCLUSIONS Highly sensitive stability-indicating LC-MS method was developed for studying the stability of extemporaneously compounded buprenorphine oral syringes. This study demonstrates that buprenorphine extemporaneous formulation prepared according to the manufacturers' recommendations is stable under refrigerated or room temperature conditions for 30 days in oral plastic syringes.

ABBREVIATIONS FDA, US Food and Drug Administration; LC-MS, liquid chromatography–mass spectrometry; LOD, limit of detection; LOQ, limit of quantitation; NAS, neonatal abstinence syndrome; % CV, percent coefficient of variation; USP, US Pharmacopeia; XIC, extracted ion chromatogram

KEYWORDS buprenorphine; compounding formulation; extemporaneously compounded; LC-MS; oral syringes; stability; stability-indicating

J Pediatr Pharmacol Ther 2021;26(4):395–404

DOI: 10.5863/1551-6776-26.4.395

Introduction

Opioid use has dramatically increased in the United States. In 2017, the Department of Health and Human Services reported that the United States is facing an opioid epidemic. Therapeutically, opioids have been used extensively for acute and chronic pain management, as well as for maintenance therapy for opioid use disorder. Yet opioid exposure during pregnancy can cause neonatal abstinence syndrome (NAS), also known as neonatal opioid withdrawal syndrome.[1-4] Neonatal abstinence syndrome causes neurologic, autonomic, and gastrointestinal symptoms in the newborn.[5,6] The palliative therapy consists of supportive approaches, such as promoting breastfeeding and rooming in with the mother; however, more severe disease requires pharmacologic treatment to ensure proper feeding, development, and infant-maternal bonding. First-line pharmacologic treatment is an opioid such as morphine, methadone, or buprenorphine. Buprenorphine has been identified by meta-analysis to be the most effective opioid for the pharmacologic treatment of NAS.[7]

Clinical trials using buprenorphine for NAS have used buprenorphine hydrochloride at the concentration of 0.3 mg/mL as an oral formulation prepared in 30% ethanol and simple syrup US Pharmacopeia (USP). Anagnostis et al[8] reported that 30% ethanolic buprenorphine solution remains stable in amber glass bottles for up to 30 days.

They found a negligible difference between the concentration of buprenorphine stored at room temperature (20°C–25°C) and under refrigeration (2.2°C–7.8°C). Further, they reported buprenorphine stored in oral syringes maintains potency for up to 7 days. Their study was performed by storing syringes in −70°C until analyzed. Moreover, this formulation was developed in their laboratory without using standard guidelines as a preliminary investigation. Further, although this study used liquid chromatography–mass spectrometry (LC-MS), it did not use a stability-indicating method. Jappinen et al[2] also noted that a mixture of buprenorphine, haloperidol, and glycopyrrolate in 0.9% sodium chloride solution stored in intravenous syringes was stable over 30 days at room temperature or under refrigeration. To date, no study has shown buprenorphine oral syringe formulation stability using LC-MS method for 30 days. Therefore, it is important to check the stability of buprenorphine oral syringes in plastic container for a period of at least 30 days at room temperature and refrigerated conditions.

In a continuation of previous studies, we intend to demonstrate the stability of buprenorphine oral syringes (compounded as per USP standards) for a period of 30 days under different temperature and humidity conditions.[9] Here, we report a novel, highly sensitive stability-indicating LC-MS method to determine the stability of buprenorphine syringes, which were compounded in Thomas Jefferson University Hospital pharmacy. This study provides relevant physical and chemical stability data and possible degradation peaks for buprenorphine under acidic, basic, and oxidative stress conditions, which may help hospital pharmacies dispense buprenorphine in single-dose packages for patient administration and decrease waste by storing the unused ready-to-dispense formulations for longer time periods.

Materials and Methods

Materials. All chemicals were of analytical grade. Buprenorphine, sodium phosphate, phosphoric acid, sodium chloride, sodium hydroxide, hydrochloric acid, acetonitrile (LC-MS grade), and all other chemicals were purchased from Fisher Chemicals (Fair Lawn, NJ). Vials containing buprenorphine at 0.3 mg/mL were purchased from Par Pharmaceutical (Chestnut Ridge, NY).

High-Performance LC System. All chromatographic studies were performed on a Dionex UltiMate 3000 high-performance LC system connected with an Exactive mass spectrometer (Thermo Scientific, Waltham, MA). Buprenorphine concentration in all samples was assessed by LC-MS. The separations were performed on a Symmetry C18 column (75 mm length and 4.6 mm internal diameter, Waters Associates, Milford, MA) with 3.5-µm particle diameter. The mobile phase was 50% solvent A (0.1% formic acid in water) and 50% solvent B (0.1% formic acid in acetonitrile). For LC-MS, 3-minute runs were performed at 30°C, with a scanning mass range of 370 to 469 m/z. The mobile phase was filtered and degassed before use. The flow rate was 0.35 mL/min, and the injection volume was 2 µL.

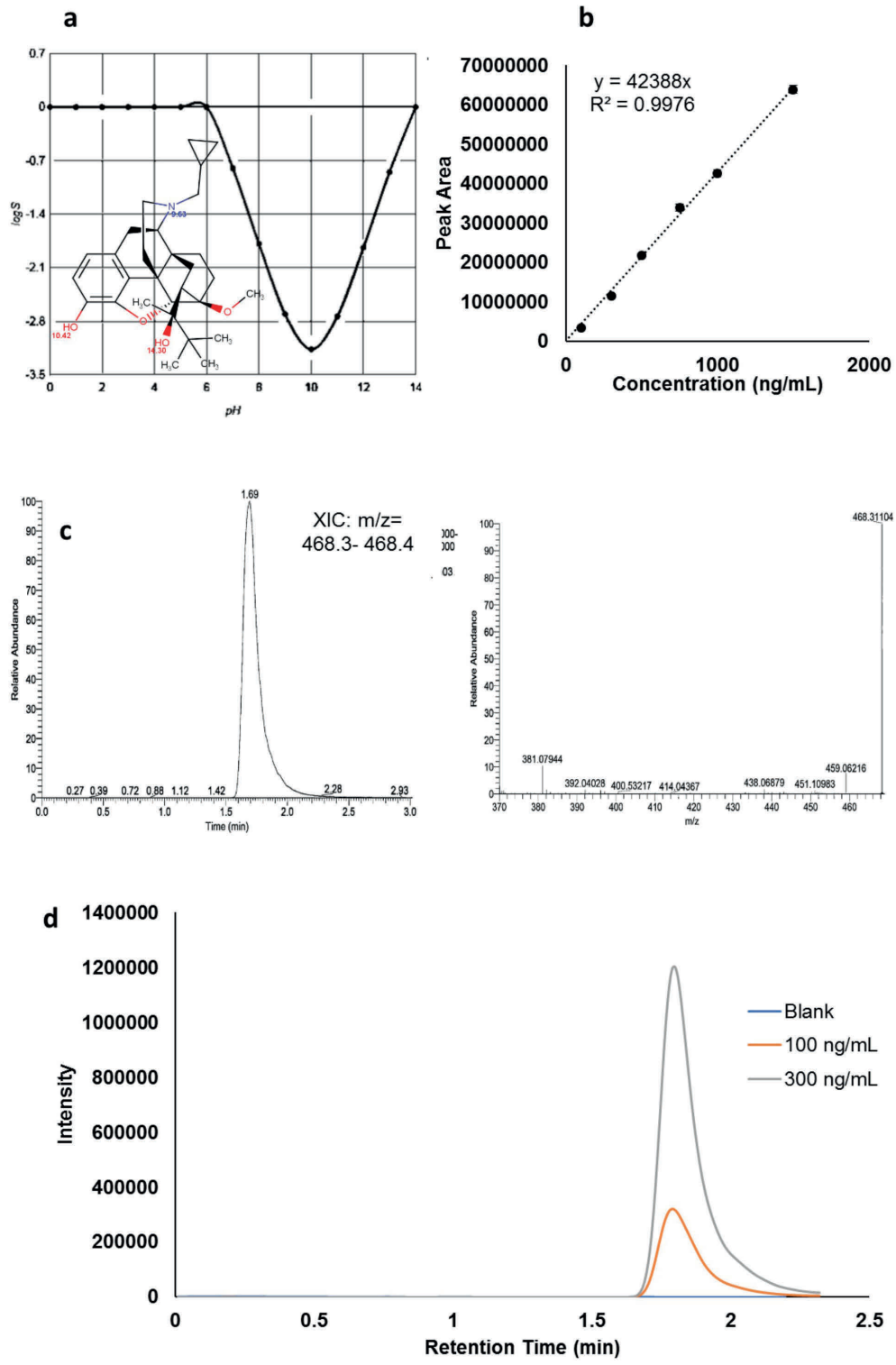
Buprenorphine Standard Curve. Liquid chromatography–mass spectrometry calibration was performed by constructing a standard curve using known concentrations (100, 300, 500, 750, 1000, and 1500 ng/mL) of buprenorphine in deionized water. All standards were analyzed twice (intraday variation) on 2 different days (interday variation), totaling 4 injections. The accuracy was calculated at each concentration as the ratio of the measured concentration to the nominal concentration multiplied by 100%. The limit of quantitation (LOQ) of the method was defined as the lowest concentration of buprenorphine that could be quantitatively determined with acceptable precision and accuracy, defined as 80% to 120% and ≤ 20%, respectively.

Stability-indicating LC-MS Assay. The suitability of the present LC-MS conditions to be used as a stability-indicating method was tested by studying the accelerated degradation of buprenorphine. The stability-indicating capability of the assay was examined by accelerating the degradation of standard 500 ng/mL buprenorphine in 1 M HCl,1 M NaOH, and 30% H_2O_2 solutions, and these solutions were heated at 90°C for 1 hour. Samples were withdrawn before and after heating each of the solutions. Each sample was analyzed by LC-MS using the conditions explained above.

Preparation of Buprenorphine Syringes. A stock solution of buprenorphine was prepared by the Thomas Jefferson University Hospital pharmacy to a concentration of 0.075 mg/mL, based on the standard regimen for treatment of NAS.[10, 11] The solution 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, 15.12 mL of 95% ethanol USP and 20.88 mL of simple syrup USP was added. This bottle was inverted several times to mix the contents. Using ExactaMed 1 mL amber oral dispensers (no. H9388501; Baxter, Deerfield, IL), 0.5 mL buprenorphine 0.075 mg/mL was drawn into each syringe and capped. The amber-colored syringes were refrigerated (2°C–8°C) or stored at room temperature (25°C ± 2°C with 60% relative humidity). Three syringes at each storage condition were assessed for physical and chemical stability over 30 days. Stability was assessed on days 0, 3, 7, 15, 22, and 30. The storage temperatures were closely monitored throughout the study.

Physical Evaluation. The pH is an important parameter that governs the stability of the product as changes in pH can cause precipitation. Thus, pH was measured at each sampling point, beginning at day 0 (initial). The SevenEasy pH meter (AG 8603, Mettler Toledo, Columbus, OH) attached with pH electrode (InLab Routine Pro-ISM, Mettler Toledo) was used after 3-point standardization with standard buffer solutions (pH 4.0, 7.0, and 10.0).

Figure 1. (a) Simulated pH-dependent solubility profile for buprenorphine; (b) Standard curve of buprenorphine assay; (c) Representative extracted ion (XIC) spectra for buprenorphine with mass range $(M+H)^+$ 468.3–468.4; and (d) Representative chromatograms along with mass spectra for the LOD (S/N: 2119) and LOQ (S/N: 5298).



LOD, limit of detection; LOQ, limit of quantitation

Table 1. Percent CV and Percent Accuracy for the Calibration Standards for Interday and Intraday Runs

Concentration (ng/mL)	% CV		% Accuracy	
	Interday	Intraday	Interday	Intraday
100	0.020	0.082	81.025	81.948
300	3.096	0.749	89.286	88.338
500	0.513	5.330	101.318	102.859
750	0.531	2.532	105.312	106.927
1000	2.041	2.559	99.310	99.006
1500	0.853	2.756	99.349	99.889

%CV (percent coefficient of variation) and % accuracy

Buprenorphine Stability Assay. Each buprenorphine 0.5-mL oral syringe (0.075 mg/mL) was added to a 50-mL volumetric flask to which around 30 to 40 mL of nanopure water was added. This flask was sonicated for 5 minutes and then left for 5 minutes at room temperature. After 5 minutes the volume was made up to 50 mL using nanopure water. The assay of buprenorphine was performed using calibrated LC-MS.

Chromatogram peak areas were used to determine buprenorphine concentrations in the syringes. Three samples were withdrawn on each sampling day for each storage temperature. The percentage of buprenorphine remaining in each infusion was calculated based on the buprenorphine content at day 0. The drug concentration was considered stable if its concentration was more than 90% of the initial concentration.

Data Analysis. A 2-tailed Student *t* test was used to compare the differences between the data of interest, where $p < 0.05$ was considered as statistically significant in nature. Wherever possible, data are presented as mean ± standard deviation. All the statistical evaluations were done using Excel, version 1808 (Microsoft Corp, Redmond, WA).

Results

Standard Curve and Method Validation. Our theoretical simulation using solubility predictor function (ChemAxon, Budapest, Hungary) showed the pH-dependent solubility of buprenorphine. The pKa of buprenorphine is > 9 (Figure 1a), which indicates that the drug is a weak base. This also suggests that acidic conditions can result in ionization and increased aqueous solubility, which can help in better elution profiles. The chromatogram of buprenorphine standards shows a peak at the retention time of 1.8 ± 0.4 minutes (Figure 1c). As a control, a blank sample was also injected into the high-performance LC system and no peak was observed. As shown in Figure 1b, a good linearity was exhibited in the concentration (range, 100–1500 ng/mL) by using the presently developed LC-MS method. The average coefficient of determination of $r^2 > 0.99$ was observed for the standard curve. The slopes of the curves illustrated an excellent agreement with coefficient of variability.

Table 2. Low, Medium, and High Reference Concentrations Used for Validation of the Buprenorphine Standard Curve

Concentration (ng/mL)	% CV		% Accuracy	
	Interday	Intraday	Interday	Intraday
250	9.106	0.843	102.283	102.324
900	1.953	4.962	103.617	95.825
1200	6.761	5.388	93.850	109.576

% CV, percent coefficient of variation

The interday and intraday relative standard deviations (or percent coefficient of variation [% CV]) were found to be 1.175% ± 1.160% and 2.335% ± 1.835%, respectively. For each concentration studied, a relative error of less than 10% was obtained, whereas the average percent accuracy for the interday and intraday was found to be 95.933% ± 9.016% and 96.495% ± 9.437%, respectively. An acceptable precision and accuracy were acquired by this method for all the standards and quality controls based on the recommended criteria.[12] As shown in Table 1, an acceptable value for percent accuracy (100% ± 20%) and % CV (<10%) was accomplished at each of the added concentrations. Moreover, in accordance with the requirements of the stability-indicating assay, the LOQ was 300 ng/mL and limit of detection (LOD) was 100 ng/mL. We used manual method of peak detection, signal-to-noise ratio, and reduced % CV at lower concentrations of standard buprenorphine to determine the LOD and LOQ for this method. Peak overlay for LOD and LOQ is shown in Figure 1d.

The present LC-MS method was validated by taking 3 random concentrations of buprenorphine (low, medium, and high) and subjecting them to LC-MS as shown in Table 2. Data for validation runs for interday and intraday precision and accuracy were per the FDA guidelines of bioanalytical method validation.[13,14]

Stability-indicating LC-MS Method Characterization. The current LC-MS method met all acceptance criteria and was reproducible for the study of buprenorphine in unknown samples. The failure to recognize the degradation peaks is the most common point that leads to erroneous reporting of the data on the stability studies.[15] The degradation profiles of buprenorphine in acid, base, and peroxide are shown in Figure 2a. Complete degradation of buprenorphine was observed under acidic conditions, whereas in basic conditions, there was significant decrease in the peak area. Under both conditions, degradation peaks were observed and these peaks did not overlap with the peak of interest. It

Figure 2. (a) Degradation time profile for buprenorphine in presence of acid, base, and peroxide; (b, c, and d) Representative extracted ion chromatogram (XICs) (degradation) of buprenorphine at 0, 1, and 2 hr in acid (1N HCl), base (1N NaOH), and peroxide (H_2O_2), respectively.

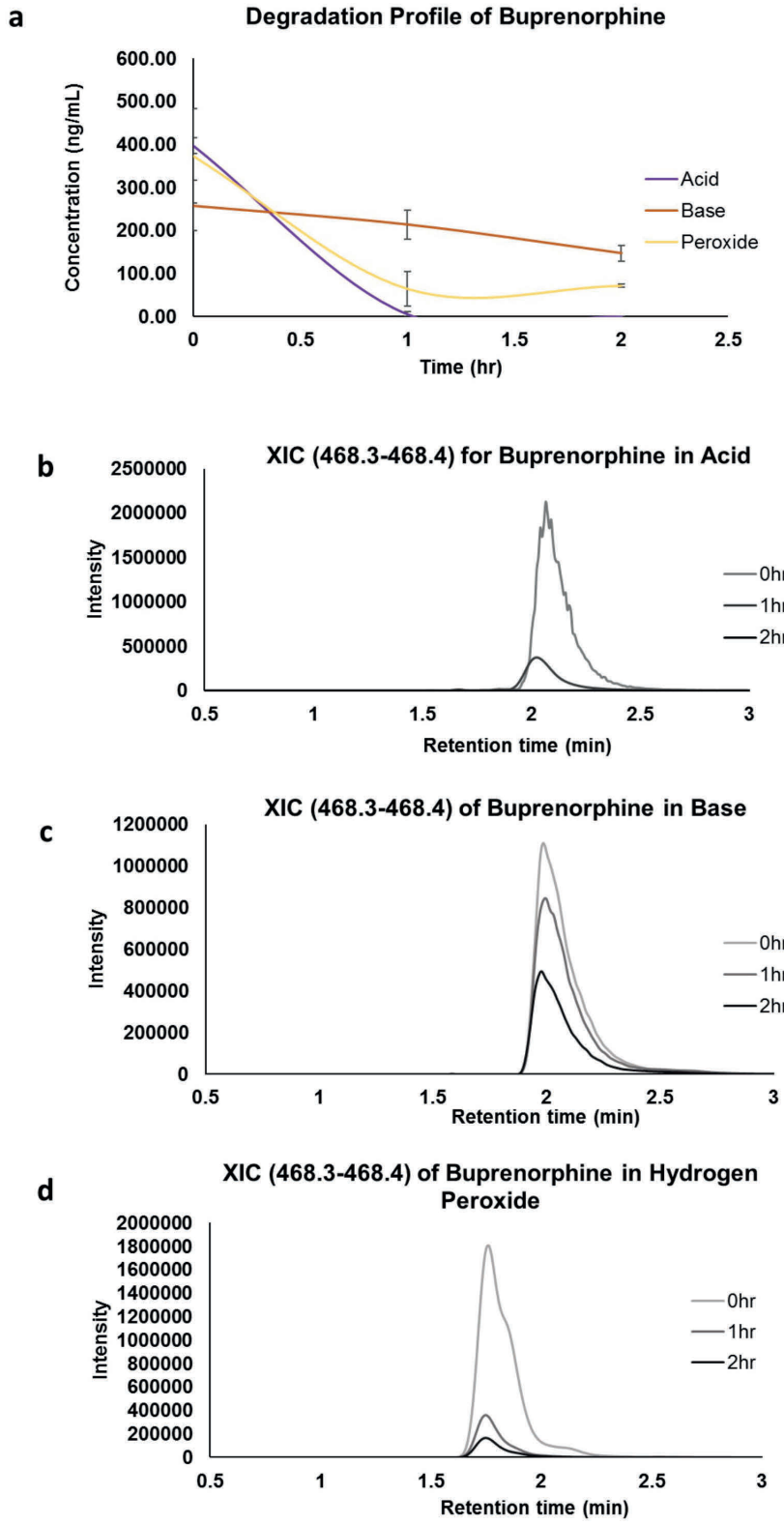


Figure 3. (a) Extracted ion chromatograms (XICs) of the probable degradation peaks of buprenorphine treated with acid (Peak 1 (437-438 m/z), Peak 2 (384-385 m/z), Peak 3 (408-409 m/z), and Peak 4 (370- 371 m/z)); (b) XIC of the degradation peaks of buprenorphine treated with base (Peak 1 (410-411 m/z), Peak 2 (454-455 m/z), and Peak 3 (396-397 m/z)); and (c) XIC for oxidative degradation peaks of buprenorphine (Peak 1 (429-430 m/z) and Peak 2 (445-446 m/z)). All the chromatograms are produced by subtracting blank (acid, base, and peroxide) background from the degradation chromatogram.

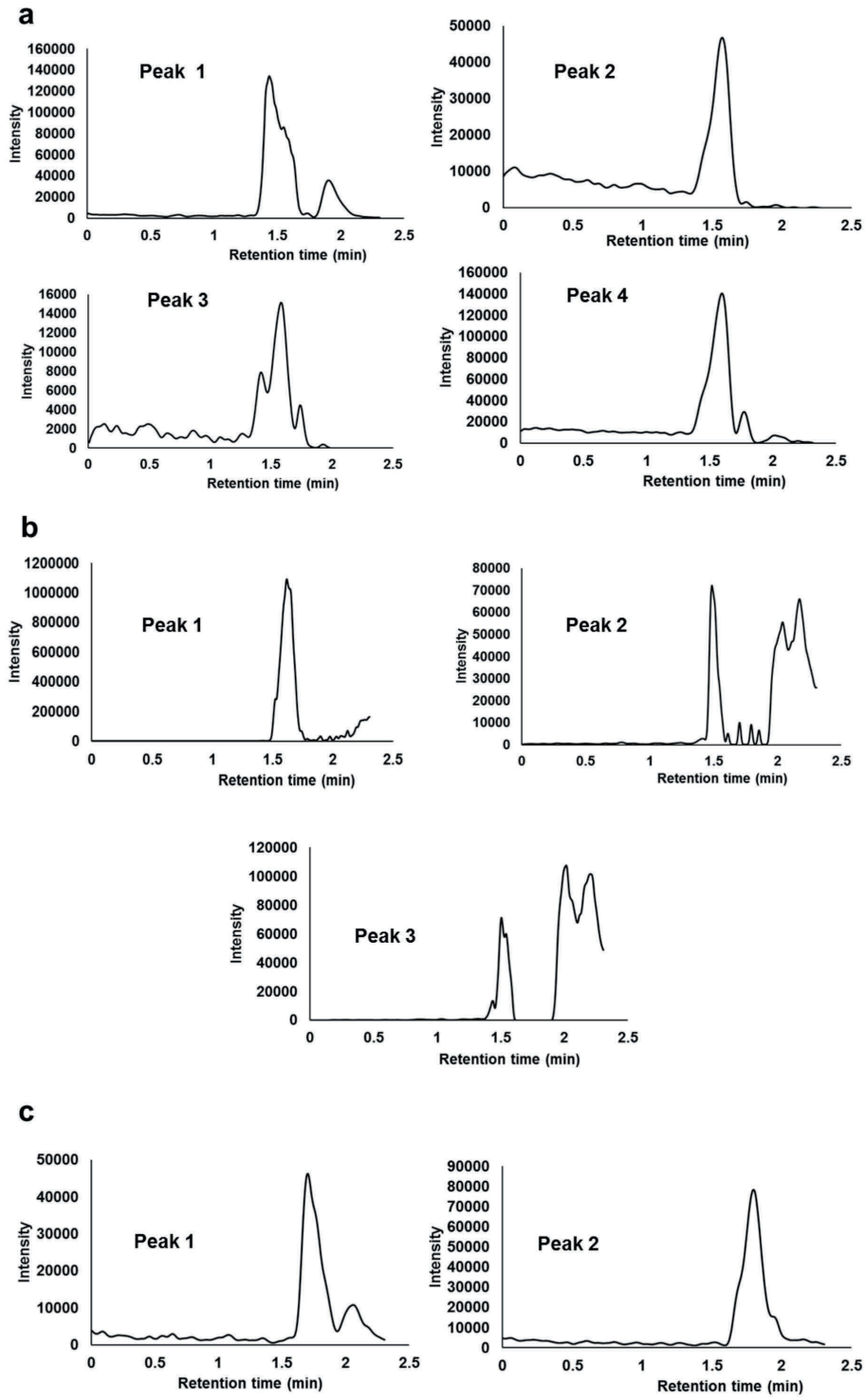


Table 3. Possible Degradation Peaks of Buprenorphine When Exposed to Acidic, Basic, and Oxidative Conditions

Peak No.	Possible Degradation Peaks	XIC Mass-Range	Retention Time (min)
Acid degradation peaks			
1	437.62	437–438	1.44
2	383.53	384–385	1.58
3	407.64	408–409	1.59
4	369.54	370–371	1.60
Base degradation peaks			
1	409.52	410–411	1.63
2	453.62	454–455	1.49
3	395.49	396–397	1.51
Oxidative degradation peaks			
1	429.55	429–430	1.58
2	445.55	445–446	1.81

XIC, extracted ion chromatogram

is evident from the chromatograms of buprenorphine (Figure 2, b–d) that the degradation of the drug was occurring in acidic, basic, and peroxide conditions.

The total ion chromatogram of buprenorphine samples (treated with acid, base, and peroxides) showed an evident degradation pattern. Few degradation peaks with their extracted ion chromatograms (XICs; Figure 3, a–c; Table 3) were identified. All the XICs presented were obtained by subtracting the blank backgrounds of acids, bases and peroxides from test degradation chromatograms. Since MS was used for the analysis, the identification of unknown degradation peaks could be resolved on a mass spectrometer with error of < 0.5 ppm. Hence, this stability-indicating method can simultaneously analyze buprenorphine and its degradation peaks in a sample, and it is sufficiently specific to the drug.

Physical Evaluation and Drug Content Analysis. The oral syringes remained clear throughout the study when kept under refrigeration or at room temperature. No precipitation was observed. The initial pH value of the buprenorphine oral syringes was 5.90. There was no significant change in pH under the 2 storage conditions used in this study over a period of 30 days ($p > 0.05$; Figure 4a). The concentrations of buprenorphine in all oral syringes were in the range of 90% to 110% of the labeled drug amount under all temperature conditions studied for at least 30 days (Figure 4b). Minimal to no degradation and no new degradation peaks were observed. The representative XIC chromatogram of buprenorphine for sample under stability study is shown in Figure 5 (a–b).

Discussion

This study is a continuation of our previously published work on the stability of buprenorphine in oral syringes.[8] Our previous work was based on the MS method that was trained to quantitate buprenorphine in plasma by Moody et al.[16] The present method helps in identification of possible degradation peaks of buprenorphine in the presence of various stress conditions with high sensitivity and specificity. As a result, we developed a simple, time-effective, stability-indicating LC-MS method to study the stability of buprenorphine syringes that are compounded (per USP guidelines) in the Thomas Jefferson University Hospital pharmacy.[9] This is the only stability-indicating method for studying

Figure 4. (a) pH versus time profile for the buprenorphine oral syringes in different temperature conditions; and (b) percentage of initial concentration versus time profile for the buprenorphine oral syringes.

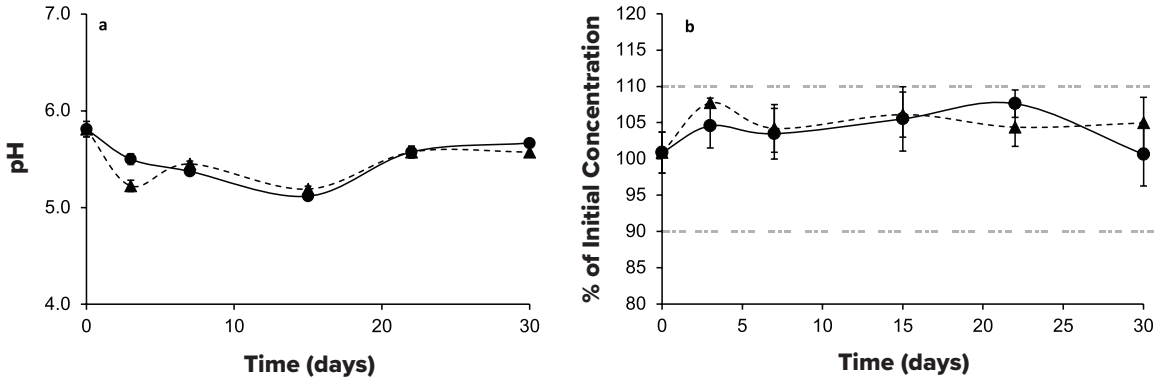
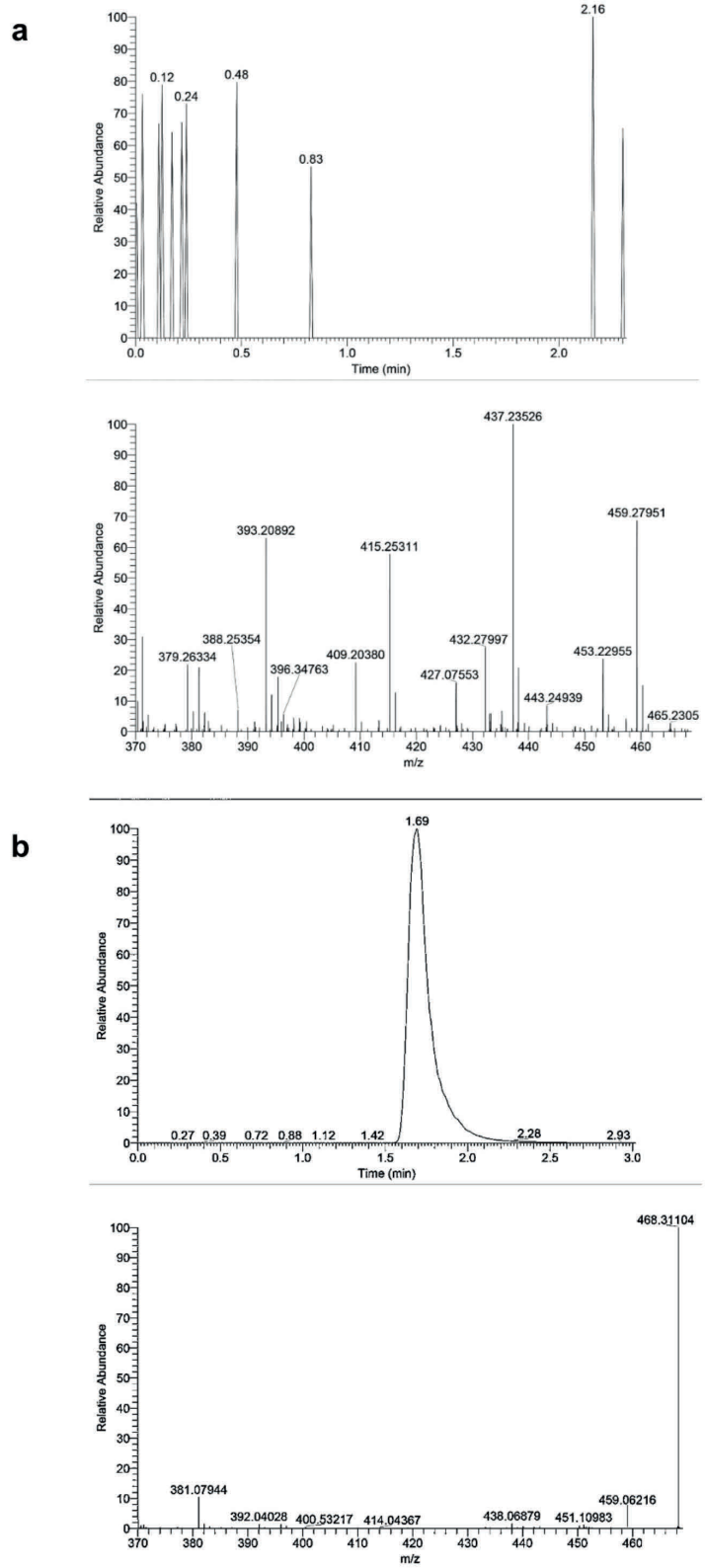


Figure 5. (a) Blank (acetonitrile with 0.1% formic acid) liquid chromatography–mass spectrometry (LC-MS) background spectra; and (b) extracted ion chromatogram (XIC) along with mass spectra for the buprenorphine sample under stability study.



the forced degradation peaks and stability of buprenorphine syringes at different storage conditions at a concentration of 0.075 mg/mL in ethanol for a period of 30 days. This concentration (0.075 mg/mL) is currently being used by Thomas Jefferson University Hospital for the treatment of NAS. Similar to the Anagnostis et al[8] report that buprenorphine at this concentration is chemically stable for 30 days under refrigeration and room temperature when stored in glass bottles, our present LC-MS data for this concentration of buprenorphine also show that buprenorphine oral syringes are stable for 30 days. Because light and temperature have an impact on the degradation of buprenorphine,[8] we have used amber-colored oral syringes stored at 2 different temperatures with 60% relative humidity.

Many previous studies have shown that degradation could be mapped by looking at the active concentration of the drug along with variation in the formulation's pH over time.[17,18] Our study indicates that there was no significant change in the pH value throughout the study period ($p > 0.05$). This indicates that the formulation is stable over time under both room and refrigerated conditions for 30 days stored in an oral amber syringe.

The parent drug stability guideline (Q1A [R2]) issued by the International Conference on Harmonization suggests that stress studies should be carried out on a drug to ascertain its inherent stability characteristics.[19] Understanding the rate of degradation by quantitative mapping could aid in the identification of degradation peaks, which could support the suitability of the proposed analytical procedures. It also requires that the analytical test procedures for stability samples should be validated to be stability-indicating. In order to analyze the concentration of buprenorphine, we developed and validated a stability-indicating LC-MS method. The present LC-MS method met all the acceptance criteria for providing reproducible results with acceptable specificity and sensitivity for quantitation of buprenorphine from unknown samples.

It is reported that failure to determine degradation of parent molecules can lead to errors in presenting data from stability studies of formulations.[15,20] Previously published work on the stability of buprenorphine has ignored this point. As a step forward, in our study of buprenorphine oral syringes, decomposition was < 10% by the end of 30 days under all conditions studied. There are a number of studies that have developed extrapolation models for stability over time and storage conditions. Caution should be exercised when extrapolating data outside the results of the study and needs to be confirmed by actual measurements, as additional variability could be introduced due to differences in storage or dispensing methods.

Based on the present study, we can say that buprenorphine oral plastic syringes are stable for 30 days under 2 different temperature conditions. These recommendations are considerably longer than the present recommendation by the manufacturers. Using our study, efficiencies within the pharmacy can be gained and improve turnaround time for dispensing buprenorphine for patient administration. The results of this study show 30-day stability for buprenorphine oral syringes beyond the manufacturer guidelines of 7 days.

Conclusion

We determined the forced degradation rate and degradation peaks of buprenorphine under acid, base, and peroxide using high resolution LC-MS. The LC-MS method can map the degradation profile of buprenorphine oral syringes with sensitivity up to 100 ng/mL. Buprenorphine 0.075 mg/mL oral syringes are stable under the storage conditions studied and can be used in a clinical setting. The extended stability of this preparation can improve operational efficiency, reduce waste, and improve medication turnaround time.

Article Information

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Disclosures. The authors declare no conflicts or financial interest in any product or service mentioned in the manuscript, including grants, equipment, medications, employment, gifts, and honoraria. The authors had full access to all data and take responsibility for the integrity and accuracy of the data analysis.

Ethical Approval and Informed Consent. Given the nature of this study, institution review board/ethics committee review was not required.

Acknowledgments. The authors thank Thomas Jefferson University Hospital for providing necessary funding for this work. The authors also thank Pamela Walter, Office of Professional Writing, Publishing and Communication, Thomas Jefferson University for carefully reading this work, and Nikita Nikita and Lauren Langbein of Postdoctoral Scientific Editing and Review Team (PSERT), Thomas Jefferson University for carefully reading and editing this work.

Submitted. July 14, 2020

Accepted. September 22, 2020

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