

Research

Simultaneous Assay of Baclofen, Lidocaine, and Ketamine in Compounded Topical Cream

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Abstract

This study was conducted to determine the potency of the compounded topical preparation CPRM1 in Lipoderm base. CPRM1 is a coded proprietary formula developed by the Professional Compounding Centers of America and historically compounded and dispensed by Cavalier Pharmacy, located in Wise, Virginia. CPRM1 is compounded in response to prescriptions for chronic-pain management from local physicians for local patients. The research laboratory of Appalachian College of Pharmacy, located in Oakwood, Virginia, performed an assay of CPRM1 cream, using gradient reversed-phase high-performance liquid chromatographic method. This cream contained Baclofen, Lidocaine, and Ketamine. The assay results of all three drugs in the preparation were within $\pm 10\%$ of the labeled strength and ensured quality per guidelines in *United States Pharmacopeia* (USP) Chapter <795>Pharmaceutical Compounding–Nonsterile Preparations [1]. The investigators developed a simple and reproducible gradient high-performance liquid chromatographic assay method, and performed abbreviated validation to establish the specificity, recovery, accuracy, and precision of the method for quantitative analysis of three active pharmaceutical ingredients–baclofen, lidocaine, and ketamine–in the preparation. The compounded cream, CPRM1, was analyzed in quadruplicate. All three drugs were found to be within the limit of acceptance as set forth in the *United States Pharmacopeia* Chapter <795>. The chromatographic condition of this simultaneous analysis was simple. The elution solvent contained 12% acetonitrile and 88% phosphate buffer with pH adjusted to 3.6. All three drugs were eluted as sharp, symmetrical peaks, with the elution being completed within 15 minutes. The isocratic elution mode, after elution of the last focused compound, was changed to gradient mode to wash out the residuals, and the run was completed in 30 minutes. CPRM1 was stored at room temperature to verify stability of these three drugs in the compounded cream. The study continued for 12 weeks (i.e., approximately 3 months), with weekly sampling. Assay results of all three drugs demonstrated >90% of the label claim during the 12-week stability study period.

Introduction

A survey shows about 30.7% of adults in the U.S. suffer from pain that occurs from various origins [2]. Neuropathic pain is one of the most critical pain. This condition creates complex challenges for effected individuals because of its impact on quality of life, in addition to its treatment costs. Neuropathic pain refers to a painful disorder characterized by dysfunction or disease of the nervous system at a peripheral level, or central level, or both. Research shows neuropathic pain may result from any of the following causes, diabetic neuropathy, chemotherapy-induced neuropathy, postherpetic neuralgia, etc. [3]. Currently clinicians combine *N*-methyl-D-aspartate (NMDA) receptor blockers (for example, ketamine), glutamate and/or α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) antagonists (for example gabapentin, carbamazepine, valproic acid, or phenytoin), gamma-aminobutyric acid (GABA) agonist (e.g., baclofen), and other receptor agonists or antagonists for treatment [4]. Commercial products of these drugs are mostly in oral-dosage form, which create a number of adverse effects.

Contemporary trends for chronic pain management also include personalized compounded topical preparations [5,6]. These preparations contain combinations of active pharmaceutical ingredients with the objective of providing synergistic effect in pain

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management. The *Globe Newswire* conducted a national survey in 2013, addressing the use of "Prescription Topical Creams" in chronic pain management. More than 83% of about 3,600 respondents reported a significant reduction in their pain after using custom compounded prescription pain creams [7]. The survey respondents reported that the creams reduced their pain levels by more than half, on average. More than 5% of respondents reported that the creams eliminated their pain. In addition, 38% reported reducing other oral-pain medications while using the creams. A placebo-controlled clinical trial concluded that transdermal cream containing ketamine and lidocaine was effective in 73% of patients with acute neuropathic pain and may be a good alternative to oral medications [8]. Topical pain preparations currently approved in the U.S. are:

Diclofenac sodium 1.5% topical solution

Diclofenac sodium gel 1%

Diclofenac hydroxyethylpyrrolidine 1.3% patch

Each of these topical nonsteroidal anti-inflammatory drug (NSAID) products delivers drugs to subcutaneous tissues for the management of pain associated with osteoarthritis or soft-tissue injuries. Topical NSAID preparations approved in the European Union include ibuprofen creams and gels, ketoprofen gel, felbinac gel and cutaneous foam, and piroxicam gel. Meta-analyses have confirmed the efficacy and safety of these preparations [9]. The Professional Compounding Centers of America (PCCA) conducted a transdermal penetration study of lidocaine, ketamine, baclofen, and gabapentin that included their proprietary vehicle Lipoderm, using a Franz cell model and human skin as the penetration barrier. The study data demonstrated that all four drugs penetrated through the excised skin and reached their peak level at various time points, confirming the usefulness of Lipoderm as a vehicle for extemporaneous compounding [10].

Baclofen (Figure 1), molecular weight 213.7, is an agonist at the gamma-aminobutyric acid type B (GABAB) receptor. Studies have reported its efficacy in trigeminal neuralgia. It is often used in trials on any type of neuropathic pain. The effective dose range is very wide (20 mg/day to >200 mg/day orally) and titration from a low initial dose is necessary. Baclofen is a muscle relaxant that, when used topically, is effective in doses with minimal systemic absorption [11].

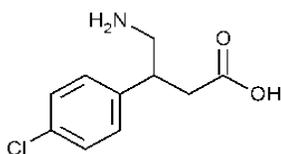


Figure 1: Structural formula of Baclofen.

Lidocaine (Figure 2) is a local anesthetic. It has a molecular weight of 234.34 and pK_a of 8.01. A 2004 study, published in *The Oncology*,

showed that a lidocaine transdermal patch provides analgesia in postherpetic neuralgia by reducing aberrant firing of sodium channels on damaged pain fibers located directly under the patch [12]. Amount of lidocaine absorbed from the patch is insufficient to cause systemic effects or local anesthesia. Other laboratory studies of compounded topical creams support their use for other neuropathic pain [13]. Topical lidocaine gel is also thought to reduce discharges of small afferent nerve fibers by blocking voltage-gated sodium channels [14].

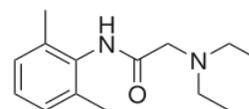


Figure 2: Structural formula of Lidocaine.

Ketamine (Figure 3) is an arylcyclohexylamine derivative, an NSAID. Its molecular weight is 237.7. A low dose of ketamine produces strong analgesia in neuropathic pain, most likely by inhibition of the NMDA receptor, although other mechanisms are possibly involved, including enhancement of descending inhibition and anti-inflammatory effects at central sites [15-17].

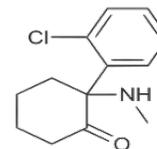


Figure 3: Structural formula of Ketamine.

The physicians these days often manage chronic pain of various origins, such as fibromyalgia, reflex sympathetic dystrophy, arthropathies, painful neuropathies, etc. using personalized topical compounded preparations, like gels, creams, and solutions of the above mentioned drugs. These preparations demonstrated site-specific treatment while decreasing or eliminating systemic side effects [18,19].

The CPRM1 cream is a compounded preparation, containing multiple drugs. This combination has been prescribed by local physicians to manage pain in diabetic neuropathy, postherpetic pain, and pain of complex origin. The purpose of this study was to determine the strength of baclofen, lidocaine, and ketamine, in this CPRM1 compounded preparation using HPLC method. Our study, conducted at the Appalachian College of Pharmacy, reports the strengths of lidocaine, ketamine, and baclofen in CPRM1 cream. This study also reports the stability of these three drugs in the preparation for a period of 12 weeks.

Materials and Methods

Chemicals, Reagents, and Equipment

The compounded preparation CPRM1 in Lipoderm cream was provided by Cavalier Pharmacy, Wise, Virginia.

Ketamine hydrochloride (HCl) USP (Lot 106286/E), and Baclofen USP (Lot 104470/E) were purchased from Medisca Inc. in Plattsburgh, New York. Lidocaine HCl monohydrate USP (Lot 1AL0451) was purchased from Spectrum Chemical Mfg. Corp. Chromatographic mobile phase modifier trifluoroacetic acid (Lot A14365-18), buffer salt monobasic potassium phosphate (Lot 90870), and polyether sulfone syringe filter, 25mm with a 0.4- μ m pore size (Lot 12463112), were purchased from VWR International LLC in Radnor, Pennsylvania. Nitrocellulose filters with 0.45- μ m pore size (Lot R2EA164961), and the vacuum pump Maxima Dry, Model PU1306-N820-9.01, were purchased from Fisher Scientific Company LLC, Hanover Park, Illinois.

Double-distilled water was prepared in-house using the all-glass Accumax India (Model AI-179) distiller. Methanol (Lot 51104) was purchased from EMD, Darmstadt, Germany, and HPLC-grade acetonitrile was purchased from VWR International.

Chromatographic Instrumentation

Waters Alliance 2695 Separation Module was used for the reverse-phase chromatography with Symmetry C-18, 5- μ m (4.6 \times 150mm) column. Analytes were detected and quantified using a Waters 2996 Photodiode Array (PDA) detector. Post-run analyses were performed at 220nm wavelength in the PDA. The separation system also had built-in degassing equipment. All chromatographic data were processed using Waters Empower 2 software, database version 6.10.00.00. The chromatographic separation assembly and the column were purchased from Waters Corporation, Milford, Massachusetts.

Mobile Phase and Sample Preparation

Preparation of Buffer

Stock buffer solution, 1M, was prepared by dissolving 68.04 g of monobasic potassium phosphate (molecular weight 136.09) in 500 mL of freshly prepared double-distilled water. The stock buffer was diluted to 25 mM with distilled water, and its pH was adjusted to 3.6 with 1M HCl. A few drops of trifluoroacetic acid were added to this buffer solution, as a solvent modifier. The buffer solution was filtered through a vacuum filtration unit, using a 0.4- μ m filter. Acetonitrile and the freshly prepared buffer solution were mixed online at 12:88% v/v ratio for the isocratic elution part of the analyses. The solvent flow rate was maintained at 1 mL/min.

CPRM1 Samples and Standard Solution

Sample: Topical Cream CPRM1

About 500mg of the sample cream was transferred to a 10-mL glass beaker. About 5mL of acetonitrile was added to the beaker, and the cream was stirred with a glass rod. While the drugs were dissolved in acetonitrile, the insoluble cream matrix formed agglomerates. The supernatant liquid was transferred to a 50-mL volumetric flask

and 5mL of fresh acetonitrile was added to the agglomerate, mixed briefly, and transferred carefully into the volumetric flask. The process was repeated again. One drop of 1M HCl was added to the flask in order to facilitate dissolution of baclofen. The flask was then sonicated for 5 minutes and set on cooling for about 10 minutes before adjusting the volume to 50mL. This formed the stock sample solution. The stock sample solution was filtered through a 0.45- μ m syringe filter. Methanol, 10% v/v in water, was used as a diluent to prepare all working sample solutions. The stock filtrate was diluted to various extents to prepare a working sample solution, based on the concentration of the drug of interest.

Six milliliter (6 mL) of the filtrate was diluted to 100mL to determine the nominal concentration of ketamine; 2 mL of the stock filtrate was diluted to 10 mL to determine the concentration of lidocaine; and 5mL of the stock filtrate was diluted to 10mL to determine the concentration of baclofen. These final sample solutions were again filtered through syringe filters prior to filling the HPLC vials.

Preparation of the Stock Standard Solution of Drugs

USP reference standards of ketamine, lidocaine, and baclofen, 25mg each, were accurately weighed and transferred into a 50-mL volumetric flask. The drugs were dissolved in 50% methanol in water, with intermittent sonication. One drop of 1M HCl was added to the solution to facilitate dissolution of the baclofen standard. This primary stock solution contained 500 μ g/mL of each of the standards.

Preparation of the Working Standard Solution of Drugs

The primary standard solution, 25mL, was transferred to a 100-mL flask and made up to the final volume with 10% methanol in water. This combined standard solution contained 125 μ g/mL of each of baclofen, lidocaine, and ketamine, and was used as 125% strength of the calibration curve. This strength of standard solution was diluted to prepare 100 μ g/mL, 75 μ g/mL, 50 μ g/mL, and 25 μ g/mL by diluting each of 8mL, 6 mL, 4 mL, and 2 mL to a final 10-mL volume with 10% methanol in water. These five concentrations were always used to develop the standard curve with freshly prepared stock and working standard solutions.

Results and Discussion

Method Development and Optimization

A sensitive RP-HPLC method has been developed for the simultaneous analysis of baclofen, lidocaine, and ketamine in a compounded CPRM1 preparation. The mobile phase of the method was composed of 12% acetonitrile and of 88% 25mM buffer (pH 3.6), with a few drops of solvent modifier TFA. At this composition, the peaks of baclofen, lidocaine, and ketamine were separated from the solvent front and from any other eluents. Since the cream contains a number of other ingredients, a gradual

gradient was used to wash out the residual chemicals from the column. Therefore, the mobile phase remained isocratic (12% acetonitrile and 88% buffer) for the first 15 minutes and then gradually increased acetonitrile concentration to 80% and buffer concentration was gradually decreased to 20%. After 25 minutes, the mobile phase composition was changed back to the original form (12% acetonitrile and 88% buffer). Hence the total run-time was set at 30 minutes.

The method was optimized with emphasis on specificity, linearity, accuracy, reproducibility, and adaptability for routine use in the laboratory.

Typical Chromatographic Profiles

Figure 4 shows the simultaneous separation of the three drug substances, baclofen, lidocaine, and ketamine, as these are combined in the reference standard solution and in the extracted solution of CPRM1 cream (Figure 5). All three peaks were sharp, symmetrical, and eluted with baseline resolution. Although peak responses are intense at 193 nm, we have chosen to use 220 nm to improve the signal-to-noise ratio. Under the established chromatographic condition, retention time of baclofen, lidocaine, and ketamine was 7.2, 10.2, and 11.8 minutes, respectively.

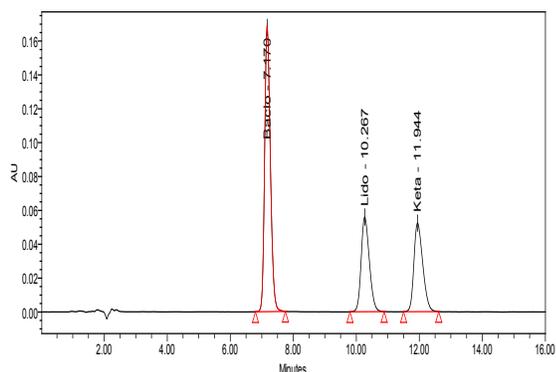


Figure 4: A typical chromatogram of baclofen, lidocaine, and ketamine in standard solution.

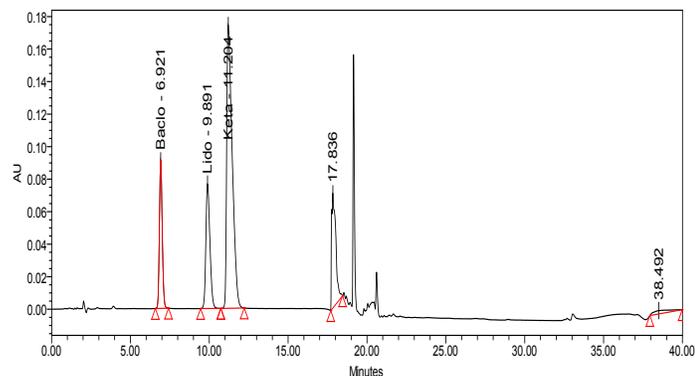


Figure 5: A typical chromatogram of baclofen, lidocaine, and ketamine in CPRM1 sample solution.

Specificity of the Method

The peak purity indices of all three drugs in standard and in sample solutions were determined with a PDA detector, under optimized chromatographic conditions. All three drugs demonstrated a smaller purity angle than the purity threshold (Figure 6), indicating that no additional peaks were co-eluted with any of these three drugs. Table 1 summarizes the results of specificity and peak purity.

Table 1. Specificity of the chromatographic method determined by Peak Purity Test during Simultaneous Analysis of Baclofen, Lidocaine, and Ketamine.

Component	Purity Angle	Purity Threshold	Comment
Baclofen	0.126	0.387	No co-elution
Lidocaine	0.190	0.441	
Ketamine	0.246	0.515	

Linearity and Range

The nominal expected concentration of the test solution for each of the three drugs was 100µg/mL. The calibration curves were constructed with five standard solutions, covering from 25% to 125% of the nominal concentrations, inclusive. The peak-responses of each of the three drugs were plotted against the corresponding drug concentrations in the linear regression analysis model. The correlation co-efficient of all three “line of best fit” are summarized in Table 2, together with the slope and the intercept of each line. The close approximation of the R² value to “1.0” (c.f. Table 2) demonstrates superimposition of the experimental values to the “Least Square Analysis” model. The linearity plots are in Figure 7.

Table 2. Calibration Data Showing Linearity of the High-performance Liquid Chromatographic Method for Simultaneous Analyses of Baclofen, Lidocaine, and Ketamine.

Concentration (mcg/mL)	Absorbance Unit at 220 nm		
	Baclofen	Lidocaine	Ketamine
25	854555	321808	354878
50	1734184	653298	719189
75	2605007	976416	1088915
100	3483853	1310750	1451755
125	4341482	1646469	1814570
R ²	0.99998	0.999907	0.999898
Intercept	-13240.7	-5232.19	1707.459
Slope	34894.1	0.379051	1.10431

Accuracy and Precision

The CPRM1 cream was extracted in four replicates. Strength of each of the three drugs baclofen, lidocaine, and ketamine, were determined in each replicate of sample. The assay results, together with the average strength, standard deviation, and the coefficient of variation (%RSD), are shown in Table 3. The percent relative

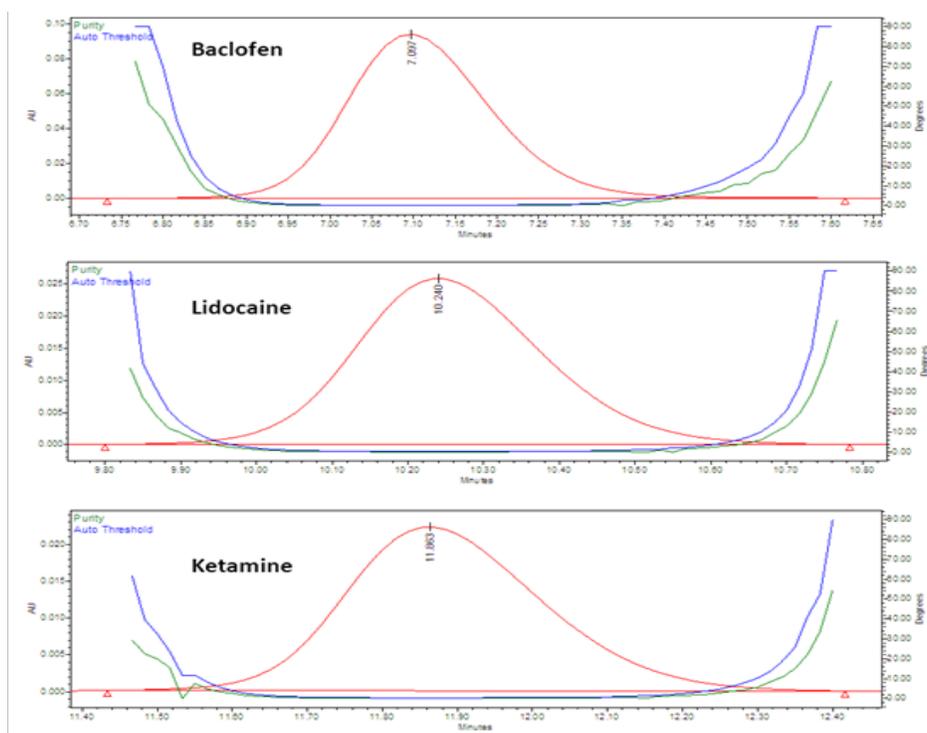


Figure 6: Peak purity (specificity) profile of baclofen, lidocaine, and ketamine in CPRM1 sample solution.

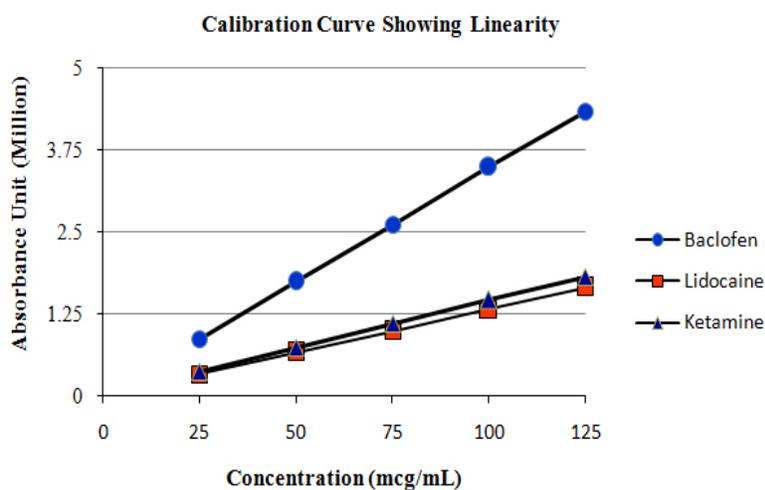


Figure 7: Calibration curves, demonstrating linearity of responses of baclofen, lidocaine, and ketamine.

standard deviations of the quadruplicate assay results are <2, indicating reproducibility and precision of the of the method and simultaneous analysis of CPRM1 topical cream.

Table 3. High-performance Liquid Chromatographic Assay of CPRM1 Cream in Replicate Analyses, Showing Accuracy and Precision of the Analytical Method.

Parameters	Recovery (% Label Claim) of Drugs		
	Baclofen	Lidocaine	Ketamine
CPRM1 Sample 1	100.87	98.91372	105.1
CPRM1 Sample 2	100.57	100.426	103.01
CPRM1 Sample 3	100.93	100.8135	105.03
CPRM1 Sample 4	100.82	99.57151	105.21
Average (n = 4)	100.80	99.93	104.59
Standard Deviation	0.158	0.854	1.054
%RSD	0.157	0.855	1.008

Assay of Ketamine, Baclofen, and Lidocaine

The investigators extracted freshly prepared CPRM1 cream, received from Cavalier Pharmacy, and determined the percent label claim of each of the three drugs in the sample. The recovery profiles are in Figure 8 at initial assay in the time-dependent assay profile. The recovery of all three drugs was within the limits of acceptance, defined by USP Chapter <795>.

Stability of Baclofen, Lidocaine, and Ketamine in CPRM1

The CPRM1 cream was stored at room temperature in the laboratory, with the inter- and intra-day excursion of the laboratory temperature 23°C±3°C. A sample of the CPRM1 cream was extracted to perform the stability study of the cream, every week for 12 weeks, with the exceptions of week 4 and week 6 due to unavailability of the HPLC equipment. The stability sample was analyzed for drug contents. All data were within the limits defined

by the USP, confirming stability of all three drugs in CPRM1 cream. The assay profiles of each of these three drugs are shown in Figure 8.

Conclusion

Quality-control guidelines in compounding processes have progressed considerably in the recent past, since the first edition of the USP, with regulations in Chapter <795>. Personalized compounded preparations require skill, specialized training, and expertise. The re-emergence of compounding preparations as a personalized pharmacy service warrants close attention to the quality of the preparation and the service. The compounding pharmacist is responsible for making preparations of acceptable strength, quality, and purity, with appropriate packaging and labeling in accordance with USP guidelines. Most of the compounding pharmacies do not have the infrastructure to determine the “strength” and the “beyond-use date” of their compounded preparations. Many commercial resources are becoming available to the compounding pharmacist, from third-party quality-control testing laboratories to extensive training programs. Appalachian College of Pharmacy scientists provide quality verification, compounding preparation development, and regulatory services to the neighborhood and regional compounding pharmacies, not for profit, but as a part of “community pharmacy support services” program of the College.

The current analysis demonstrated that the compounded CPRM1 cream in Lipoderm complied with USP Chapter <795> regulations based on its drug strengths. Baclofen, lidocaine, and ketamine were stable over a 12-week (3-month) period. Amounts of ketamine, lidocaine, and baclofen were verified to be >90% of strengths stated on the preparation label. Since these are prescription-driven compounded preparations, we decided not to continue the stability studies longer than three months.

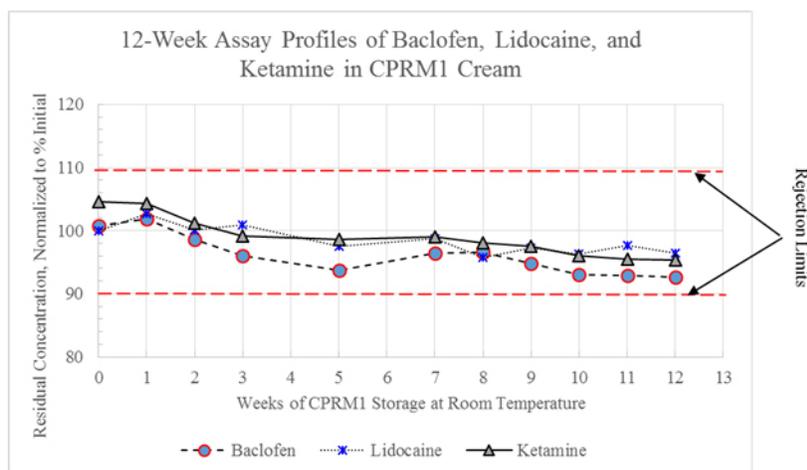


Figure 8: Twelve-week Assay profile of baclofen, lidocaine, and ketamine in CPRM1 cream, prepared in Lipoderm cream base

Acknowledgment

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